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Reviews in ethnopharmacology 2023

Edited by
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Published in
Frontiers in Pharmacology

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ISSN 1664-8714
ISBN 978-2-8325-6238-3
DOI 10.3389/978-2-8325-6238-3

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Reviews in ethnopharmacology: 2023

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Citation

Ielciu, I., Singla, R. K., Daniela, H., Frederich, M., eds. (2025). *Reviews in ethnopharmacology: 2023*. Lausanne: Frontiers Media SA.
doi: 10.3389/978-2-8325-6238-3

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TYPE Editorial
PUBLISHED 01 April 2025
DOI 10.3389/fphar.2025.1589249

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OPEN ACCESS

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RECEIVED 07 March 2025
ACCEPTED 18 March 2025
PUBLISHED 01 April 2025

CITATION
Singla RK, Ielciu I, Hanganu D and Frederich M (2025) Editorial: Reviews in ethnopharmacology: 2023.
Front. Pharmacol. 16:1589249.
doi: 10.3389/fphar.2025.1589249

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Editorial: Reviews in ethnopharmacology: 2023

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KEYWORDS

natural products, medicinal plants, bioactive agents, traditional medicine, ethnomedicine

Editorial on the Research Topic
Reviews in Ethnopharmacology: 2023

Since centuries, natural products have long been used for the treatment and management of various diseases and disorders (Dubey and Singla, 2019; Singla et al., 2019a; Singla et al., 2023b). Natural products have been the goldmine of bioactive compounds that hold great significance clinically (Singla et al., 2019b; Wang et al., 2024). Considering the strong role of natural products in the field of drug discover, we have proposed the Research Topic "Reviews in Ethnopharmacology: 2023" in order to collate the literature in the field of ethnopharmacology. We have received a total of 65 manuscripts, out of which, 30 manuscripts have been successfully published in our Research Topic. A total of 232 authors have contributed in this Research Topic. This Research Topic has garnered significant attention from the readers and viewers, with 83K topic views, 56K article views, and 24K article downloads, as on 5th March, 2025.

The study of Wang et al. proposed the combination therapy of *Rehmannia glutinosa* DC. and *Lilium lancifolium* Thunb. for the treatment and management of depression. This combination is also associated with the traditional Chinese medicine, Baihe Dihuang Decoction. Bioactive compounds from this combination reported to inhibit serotonin or 5-hydroxytryptamine (5-HT) reuptake in various nerve endings and cell bodies, along with modulation of brain-derived neurotrophic factor (BDNF),Tyrosine kinase receptor B (TrkB), Neurotrophin P75 receptor (P75NRT), Phospholipase Cγ (PLCγ), Phosphatidylinositol 3 kinase (PI3K), Raf kinase (Raf), and other targeted genes for depression as described in this article. Orozco-Barocio et al. focused on the cancer prevention and treatment using phytochemicals derived from medicinal plants belonging to *Cactaceae* family. *Opuntia* genera is the most significant genera of this family. This article tends to give an overview on various genera of *Cactaceae* family and their therapeutic potential especially in relation to cancer. Rahman et al. focused on the bioactive metabolites derived from soybean and discussed their medicinal and pharmaceutical properties in their study. Among the valuable metabolites they have highlighted were genistein, phytic acid, dietary fibers, conjugated linoleic acid, pinitol,

and others. Soybean-derived compounds have broad-spectrum therapeutic properties, including but not limited to anticancer, antidiabetic, antiviral, anti-bacterial, antihypertensive, anti-obesity. Kuang et al. focused on *Polygalae Radix* and discussed its botany, metabolites, pharmacological properties, toxicity, and other applications, reporting that more than 320 compounds have been isolated and characterized from this source and saponins, xanthones, and oligosaccharide esters were the major fraction out of all. Apart from the major role in neurodegenerative disorders, *Polygalae Radix* and its metabolites also possess the functionality as anti-oxidant, anti-cancer, anti-inflammatory, along with others. Chen et al. demonstrated that the extract from *Cynomorium songaricum* Rupr. which was enriched with flavonoids, has the capability to improve the physiological conditions occurred due to the cyclophosphamide-induced reproductive function damage in mice, and bisphenol A-induced TM3 cell damage. They have indicated that the mechanism of action is regulation of testosterone synthesis pathway. Li et al. focused on alkaloids derived from *Chelidonium majus* L. (regionally commonly known as bai qu cai, tuhuanglian (土黄连)). They provided information about 94 alkaloids in this manuscript and discussed their therapeutic potential, including but not limited to anti-microbial, anti-viral, anti-tumor, and others. Sun et al. focuses on the polysaccharides derived from *Eucommia ulmoides* and discussed their extraction, purification, structural characterization, and therapeutic applications. Over the years, patents associated with the *E. ulmoides*-derived polysaccharides have been receiving gradually increasing interest, indicating its market potential. Liu et al. focused on *Phyllanthus urinaria* L., its metabolites, and their therapeutic potential for the treatment and management of liver-associated diseases, including viral hepatitis and liver fibrosis. Authors have discussed various metabolites of *P. urinaria* L. like flavonoids, lignans, tannins, etc., and thoroughly discussed the mechanism of action associated with their therapeutic potential. Cai et al. demonstrated in a retrospective cohort study that the combinatorial effect of Fu Zheng Jie Du formula (FZJDF) combined with prone ventilation in the patients suffering from severe pneumonia. Adjuvant effect of FZJDF with prone ventilation has been well-documented through this study with the significant improvement in oxygenation. Qian et al. studied syringin, which is a phenylpropanoid glycoside, and widely occurring in various plants (23 families, 60 genera, 100 plant species), including *Acanthopanax senticosus* (Rupr. et Maxim.) Harms. It has also been a key component in various Chinese herbal medicines, including but not limited to Aidi injection, compound cantharidin capsule, and Tanreqing injection, which are being used clinically. It has broad-spectrum of therapeutic properties. Sadek et al. focused on the bee venom and elaborated as how it is a potential source with therapeutic and regenerative medical applications. This is a comprehensive review which discusses throughly about the bee venom, its metabolites, therapeutic potential, its related safety concerns, and nanoformulations for the mitigation of adverse effects and improving its clinical potential. Fu et al. highlighted the role of the traditional Chinese medicine-based extracts in the treatment and management of sepsis. This review article comprehensively covered the mechanisms associated with the anti-sepsis properties of traditional Chinese medicine. Qingwenbaidu decoction, Dachengqi decoction, and Xuebijing injection are among the traditional Chinese medicine being discussed in this manuscript. Ain et al. focused on neglected tropical disease, malaria and how the natural remedies can be used to treat and manage this protozoal disease. Although most of the clinically used medicine for malaria are either natural products or derived from natural products, but there seems to have emerging resistance. This encourages researchers to explore more natural resources like *Helianthus annuus* L., and compounds like cryptolepine and isoliquiritigenin to combat drug resistance issues. Zhao et al. performed a systematic review and metanalysis of Zao Ren An Shen prescription, a commercially utilized Chinese polyherbal formulation for the treatment and management of primary insomnia. Authors have adopted a new meta-analysis methodology, trial sequential analysis (TSA) for analysing the data. Although there is promising efficacy of this Zao Ren An Shen prescription in primary insomnia, the quality of evidence was limited. Thus, authors have recommended clinical researchers to conduct rigorously-designed randomized control trials, so as to validate and get quality evident data. Gao et al. studied the natural products which elicits protective effect against rheumatoid arthritis, primarily through their inhibitory effect on synovial angiogenesis. They have further discussed various pathways associated with synovial angiogenesis that have been impacted by natural products, including but not limited to *Tripterygium wilfordii* Hook. f.

Yu et al. focused on Traditional Chinese Medicine affecting age-related macular degeneration, with primary focus on mitophagy modulators. Various linked pathways like phosphatase and tensin homolog-induced kinase 1/Parkin, and others have also been discussed. Ma et al. performed a comprehensive review on Chinese botanical drugs which specifically target mitophagy for the treatment and management of diabetic kidney disease. Various linked pathways like ubiquitination-dependent mitophagy pathway, receptor-mediated mitophagy pathways, and membrane lipid-mediated signaling pathways have also been comprehensively discussed. Some of the Chinese botanical drugs discussed in that manuscript include Tangshen formula, Huangkui capsule, Esculetin, along with many others. Zhang et al. discussed various metabolites like nicotine, solanesol and cembranoid diterpenes, which were isolated from *Nicotiana tabacum* L. (tobacco). Such metabolites have been experimentally validated to potentially alleviate neurological disorders, inflammatory disorders, metabolic disorders, as explained in this article. The article covered the English and Chinese literature, spanning over 2 decades. Mottaghipisheh et al. focused on *Vitex trifolia* L. and discussed its ethnomedicinal values, phytochemistry, and therapeutic potential. Out of all the discussed metabolites, metabolites from terpenoids and flavonoids classes so far the most characterized from this plant. *Vitex trifolia* L. holds great potential and broad spectrum of pharmacological properties, including but not limited to hepatoprotective, anticancer and antiviral properties. Li et al. comprehensively discussed traditional Tibetan medicines and their therapeutic potential in treating and managing various lung diseases. In this review articles, they have compiled information about 586 Tibetan medicines, out of which, 33 have been

FIGURE 1
Frequency mapping of various frequently used terminologies in the abstracts of these 30 documents.

experimentally studied for their effectiveness in lung diseases. Rhodiola, gentian, sea buckthorn, and liexiang dujuan, are some of the most important Tibetan medicines in treating lung diseases. Cui et al. performed Bayesian network meta-analysis and systematic review analysis to understand the efficacy of the combination therapy of Chinese patent medicine with calcium channel blockers for the treatment and management of essential hypertension. Some of the Chinese patent medicine discussed in this work are Tianma Gouteng Granule, Songling Xuemaikang Capsule, Qinggan Jiangya Capsule, along with others. Wang et al. prepared a systematic review article and also performed meta-analysis to understand the effectiveness of the protective potential of ligustrazine while treating ischemic stroke. Based on the 32 included studies, they have confirmed that ligustrazine has protective effect in cerebral ischemic injury-based animal models. They have also discussed possible mechanisms behind this protective effect. Cao et al. provided a scientific metrology study to understand Traditional Chinese Medicine targeting ferroptosis. In the last 1 decade, Frontiers in Pharmacology has documented the maximum number of publications in this field. Chen et al. discussed the pharmacological and clinical potential of *Brucea javanica* (L.) Merr. and its metabolites. They have mentioned that around 200 metabolites have so far been characterized from this plant source and the most studied pharmacological application is its utility as anti-tumor agent. Clinical studies have also been recorded, which are mostly observational studies. Yu et al. explored the role of Traditional Chinese Medicine in treating and managing age-related macular degeneration and how the gut microbiota influences. This review examined the mechanistic interplay between gut microbiota dysbiosis and age-related macular degeneration (AMD), focusing on emerging evidence of bidirectional gut-retina axis signaling and its implications for AMD pathogenesis. Tang et al. focused on Armeniacae semen amarum—seeds of *Prunus armeniaca* L., and discussed its botany, phytochemistry, pharmacological and toxicological properties, pharmacokinetics, and clinical application. This natural resource holds broad spectrum therapeutic potential, including but not limited to anticancer, antidiabetic, hepatoprotective, along with many others. Li et al. focuses on the Tibetan medicine, Bang Jian, and comprehensively covered its ethnomedicinal values, phytochemistry, and therapeutic properties. Traditionally, dried flowers of this plant have been used for various lung diseases. Scientific studies validated it to have much wider pharmacological spectrum. Wu et al. discussed the extracellular vesicles obtained from traditional medicinal plants like *Zingiber officinale*, *Citrus paradisi*, *Citrus limon*, *Ginseng quinquefolium*, along with many others. It is a comprehensive review discussing various properties of such plant-derived extracellular vesicles. Susilawati et al. focused on *Erythrina* genus plants of *Fabaceae* family and discussed their pharmacological and clinical potential. This review focused on the Goal 3: Good Health and Wellbeing of the sustainable goals. Not least, Fan et al. prepared a comprehensive review which examines computational advances (network analysis, deep learning) in TCM pharmacology, analysing formula-herb-component-target-phenotype-ZHENG databases, addressing homogeneity bottlenecks, and envisioning next-gen models for multi-target TCM research.

These 30 articles have definitely enriched the literature in ethnopharmacology. In order to further understand the frequently used terminologies, we have performed cloud computing on the abstracts of these 30 articles (Figure 1). Some of the most prominent keywords are Chinese, Tibetan, sepsis, anticancer, anti-inflammatory, polysaccharides, along with many others.

We are highly thankful to all the authors who have significantly contributed their high-quality articles in our Research Topic and we strongly encourage them to further promote it on social media, including X (previously Twitter). They can use some hashtags like #INPST, #DHPSP, #NPMND, #SCINATMED, #AcademicTwitter, #SCICOMM, etc., which can further help to gain broader readership to these articles, and in this way, these articles can reach to wider audience (Kletecka-Pulker et al., 2021; Singla et al., 2023a).

Considering the tremendous success of this potential Research Topic, we have also released 2nd volume of this Research Topic, as "Reviews in Ethnopharmacology: 2025". We invite the authors, readers, and reviewers to contribute in this Research Topic and submit their review articles.

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Funding

The author(s) declare that financial support was received for the research and/or publication of this article. This work was supported by the Sichuan Science and Technology Program (Grant No. 2025YFHZ0213).

Acknowledgments

We extend our sincere gratitude to all the authors who contributed their valuable research to this topic. We are also deeply thankful to the reviewers for dedicating their time and expertise to the manuscript review process, despite their demanding schedules. Their insights and constructive feedback have been instrumental in advancing the quality of this scholarly work.

Conflict of interest

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The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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The author(s) declare that Generative AI was used in the creation of this manuscript. The author(s) declare that Generative AI like ChatGPT and Deepseek have been used partially, in order to improve small sections of the manuscript. But majority of the work is free from the involvement of Gen AI role.

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TYPE Review
PUBLISHED 14 November 2023
DOI 10.3389/fphar.2023.1289901

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OPEN ACCESS

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RECEIVED 06 September 2023
ACCEPTED 03 November 2023
PUBLISHED 14 November 2023

CITATION
Fan M, Jin C, Li D, Deng Y, Yao L, Chen Y, Ma Y-L and Wang T (2023), Multi-level advances in databases related to systems pharmacology in traditional Chinese medicine: a 60-year review.
Front. Pharmacol. 14:1289901.
doi: 10.3389/fphar.2023.1289901

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Multi-level advances in databases related to systems pharmacology in traditional Chinese medicine: a 60-year review

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The therapeutic effects of traditional Chinese medicine (TCM) involve intricate interactions among multiple components and targets. Currently, computational approaches play a pivotal role in simulating various pharmacological processes of TCM. The application of network analysis in TCM research has provided an effective means to explain the pharmacological mechanisms underlying the actions of herbs or formulas through the lens of biological network analysis. Along with the advances of network analysis, computational science has coalesced around the core chain of TCM research: formula-herb-component-target-phenotype-*ZHENG*, facilitating the accumulation and organization of the extensive TCM-related data and the establishment of relevant databases. Nonetheless, recent years have witnessed a tendency toward homogeneity in the development and application of these databases. Advancements in computational technologies, including deep learning and foundation model, have propelled the exploration and modeling of intricate systems into a new phase, potentially heralding a new era. This review aims to delves into the progress made in databases related to six key entities: formula, herb, component, target, phenotype, and *ZHENG*. Systematically discussions on the commonalities and disparities among various database types were presented. In addition, the review raised the issue of research bottleneck in TCM computational pharmacology and envisions the forthcoming directions of computational research within the realm of TCM.

KEYWORDS

TCM databases, systems pharmacology, formula-ZHENG relationship, complex biological system, network analysis

1 Introduction

Chinese herbal medicines have primarily originated from foods. Over long periods of practical living experience, the medicinal properties of many herbs were gradually established (Hou and Jiang, 2013; Gu and Pei, 2017). Subsequently, foods with therapeutic properties were progressively separated and designated for specialized use as medicines (Hou and Jiang, 2013; He et al., 2018; Long et al., 2022). Human foraging practices frequently entail the amalgamation of various food sources, a tendency that has played a

significant role in the creation of Traditional Chinese Medicine (TCM) formulas (Hou and Jiang, 2013). From its inception, TCM may have involved the application of herbal combinations. Some of these herbal combinations were stable and clearly effective and were therefore documented and passed down as formulas through generations. This resulted in the creation of over 300,000 known formulas (Li et al., 2008), laying the foundation for clinical TCM treatments. However, pharmacological research of TCM formulas faces the significant challenge of analyzing combinations of 100 or more chemical compounds (which are also named components) per formula (Zhao et al., 2010). Statistics on the total amount of targets corresponding to each compounds in PubChem Bioassays database is 3.7 in average (Jalencas and Mestres, 2013; Hu et al., 2014). According to the number mentioned above, a given TCM formula could potentially regulate over 370 targets. Thus, the "one drug-one target" pharmacological research methodology is insufficient to explain the therapeutic effects and mechanisms of action associated with TCM formulas (Ding et al., 2020).

Deciphering the intricate pharmacological mechanisms associated with herbs and formulas is a monumental task for researchers in the field of TCM (Wang et al., 2021b; Li et al., 2022b). Due to the "black box" nature of complex biological systems, studies of formula efficacy would do well to take a more macroscopic approach (Yao et al., 2013; Huang et al., 2023), i.e., research needs be designed using a "system-to-system" framework for clinical and pharmacological investigations of entire formula instead of disassembling formulas and studying the components (Liang et al., 2012). This approach involves observing the relationships between formulas (input) and effects in biological systems (output). The research philosophy behind chemical drug development is fundamentally guided by reductionism, with antagonism serving as a primary principle (Saks et al., 2009). The key paradigm of drug discovery revolves around the creation of inhibitors or activators that specifically target particular molecular entities (Jendza et al., 2019; Gong et al., 2023). Over time, this approach has proven imperfect due to the discovery of off-target responses, which may have toxicological impacts or cause other side effects. Given the extensive range of enzymatic systems, classes, and isoforms that have been identified in biological systems, the development of many target-specific agents has relied on trial-and-error methodologies (Méndez-Lucio et al., 2016; Paydas, 2019). However, regulation of targets by formulas does not always require an extremely high level of specificity, and exceptionally high activity levels may not be necessary (Méndez-Lucio et al., 2016). Formulas themselves constitute complex systems, wherein synergistic interactions between components can lead to optimal effects to maximize impacts on the human biological systems (Chen et al., 2018). Research of the pharmacology associated with specific formulas therefore necessitates unveiling (or partially) of the "black box" that is synergistic interactions between components and their interactions with the human biological system. This requires accurate simulation of the alterations that occur in various nodes within the biological system due to regulation by a specific formula (Tan et al., 2019). The goal is to establish correlations (and ideally quantitative relationships) between changes in a formula and changes in clinical phenotypes.

Advances in computational biochemical analyses have ushered in a new age of TCM research (Barabási et al., 2011; Wang et al., 2022a). Cooperative regulation of multiple targets by multi-component medicine is an effective strategy for altering the output of complex systems (Csermely et al., 2005; Zhang et al., 2014; Ramsay et al., 2018). Mathematical models that reflect complex systems are exceptionally potent tools in systems biology research (Kitano, 2002; Liu and Barabási, 2016; Zhao et al., 2019). The advent of artificial intelligence (AI), particularly deep learning, has allowed the accumulation of TCM data with unprecedented depth and complexity (Chen et al., 2019). Studying a substantial number of effective formulas (rather than individual formulas) using phenotypes or clinical manifestations as outputs can allow elucidation of the intricate relationships among formulas, herbs, components, targets, phenotypes, and *ZHENG*. Computer science is a powerful tool that facilitates TCM research by allowing both establishment of relationships and large-scale collection of relevant data (Zhang et al., 2019a). However, it is crucial to exercise caution in utilizing such tools to ensure that results are grounded in reality. The establishment of trustworthy, accurate TCM databases will thus be a pivotal step in unraveling the complexities of herbs or formulas (Saks et al., 2009).

Research into the pharmacology underlying TCM necessitates the accumulation of extensive data for multiple parts of the TCM system: formulas, herbs, components, targets, phenotypes, and *ZHENG* (Han et al., 2017). Clear delineations of various relationships (e.g., formula-component, component-target, and target-phenotype relationships) are vital (Xu et al., 2021; Zhao et al., 2023b; Gan et al., 2023). Since 1960, databases have been developed and are now available for use in computational TCM research (Figure 1). In this review, we conduct a retrospective examination of the establishment of these databases, with a particular emphasis on comparison based on the inclusion of formula, herb, component, target, biological function, phenotype, and *ZHENG* data. We aim to consolidate and analyze the relationships between various entities within these databases, including formula-component, component-target, target-phenotype, and phenotype-*ZHENG* relationships. This review summarizes the trends, identifies gaps in the existing research, and suggests directions for future development of the databases related to systems pharmacology in TCM.

2 TCM formula databases

Initially, TCM formulas included only a small number of herbs. The herbs were consistently combined and administered in fixed proportions, which were documented and transmitted over time (Zha et al., 2015). With the evolution of medical practices, current formulas encompass not only ancient formulas but also modern empirical formulas and commercially prepared Chinese patent medicines. These formulas comprise the fundamental data within TCM formula databases, and there is a total of 21 such databases. These databases primarily contain fundamental information such as formula compositions, therapeutic functions, indications, and methods of use. Of the 21 databases, 18 are academic and 3 are commercial (Table 1). TCM-ID was one of the earliest TCM formula databases which brings the concept of formula-herb-component-target

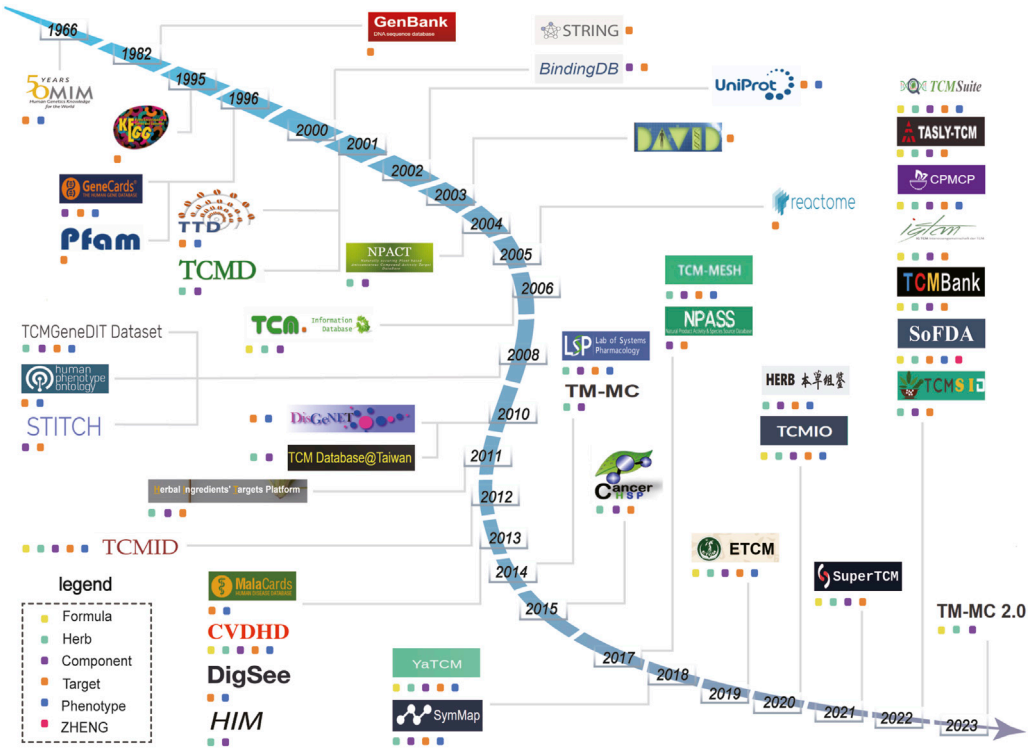

FIGURE 1
Timeline showing the establishment of databases related to traditional Chinese medicine (TCM) from 1966 to 2023. Different colored squares represent the main entity data contained in the database. The yellow squares represent "Formula". The green squares represent "Herbs". The purple squares represent "Components". The orange squares represent "Target". The blue squares represent "Phenotype". The red squares represent "*ZHENG*".

relationships in the form of databases (Chen et al., 2006). Chinese Medicine Think Tank (a big data analysis platform for TCM) houses the largest collection of TCM formula resources, including ~300,000 formulas (Chinese Medicine Think Tank-a big data analysis platform for TCM, 2017). Over the Counter TCM Database and the Database of Standardized TCM Chinese Patent Drugs focus on marketed TCM formulas (Database of Standardized for Chinese patent drugs, 2017; OTC Chinese Herbal Medicine Database, 2017). These databases provide more comprehensive records of Chinese patent Drugs. DRUGDATAEXPY and Pharnexcloud are the major databases used in the pharmaceutical industry (DRUGDATAEXPY, 2009; Pharnexcloud, 2021). In addition to providing extensive formula resources, they also enable queries related to research, clinical trials, marketing, production inspections, and sales of specific formulas.

In 2016, with the emergence of databases like BATMAN-TCM that allow for customized predictive analysis of the pharmacological mechanisms of TCM formulas, databases for TCM were no longer limited to the functions of browsing and searching (Liu et al., 2016). The development of database functions became more geared towards analysis and prediction, and it was only then that TCM formula databases began to be utilized in a truly meaningful way. The principles of herbal compatibility are crucial for the effectiveness of TCM formulas. Formula databases not only allow for direct retrieval of herbal composition of formulas but also enable algorithmic-based analysis of the patterns governing herbal combinations. Through analysis of existing TCM formulas, CPMCP has summarized frequently used herbal combinations in TCM clinical practice. This functionality has helped to uncover the habitual pairings and contraindications between various herbs, shedding light on principles of herbal compatibility (Sun et al., 2022). Huabing data, an intelligent TCM big data platform, enables screening of disease-related formulas based on input herbal combinations. It dynamically calculates and analyzes the top 20 herbs, symptoms, diseases, and functions related to the treatment of certain diseases using formulas present in the database (Huabing data platform, 2023). TCMIP allows the selection of target TCM formula groups based on criteria such as formula composition and primary diseases treated. It calculates the frequency of herb usage, herb properties, inter-herb associations, and the frequency of formula targets within a selected formula group (Wang et al., 2021a). This approach facilitates innovative research of TCM formulas. For example, researchers have constructed a scoring system for the post-effects of drug combinations based on formula-herb relationships. The scoring system is utilized to recommend the most effective herbal combinations for certain diseases (Niu et al., 2023). The use of analytical algorithms on data from these databases can accelerate explorations of the intricate networks underlying TCM formula efficacy (Wang et al., 2021b).

One effective approach to simplifying the study of the pharmacological mechanisms of formulas is to make use of databases such as, ETCM, TCMIP, LTM-TCM, TCM-ID, which enable direct prediction of the pharmacological mechanisms of formulas (Chen et al., 2006; Xu et al., 2019; Wang et al., 2021a; Li et al., 2022a; Zhang et al., 2023). However, it's important to note

TABLE 1 List of TCM formula databases.

Database name	Type	Number of formulas	Data source	Presentation modules directly related to TCM formula	Analysis modules directly related to TCM formula
TCM-ID Chen et al. (2006) (http://tcm.cz3.nus.edu.sg/group/tcm-id/tcmid.asp)	Academic database	7443 formulas	ChP-TCM, TCM formulas approved by China food and drug administration	Related herb, indication, reference	Targeted human proteins by the components of formula, targeted pathogenic microbes by the components of formula, GO associated to targeted human proteins, KEGG pathways associated to targeted human proteins, target expression in healthy human tissues
YaTCM Li et al. (2018a) (http://cadd.pharmacy.nankai.edu.cn/yatcm/home)	Academic database	1813 formulas	Literatures, ancient books, ChP-TCM (2015), TCMID	Related herb, reference, traditional explanation, traditional usage, traditional/modern application	Network and pathways analysis
TCMID 2.0 Huang et al. (2018) (http://www.megabionet.org/tcmid/)	Academic database	99582 formulas	Encyclopedia of TCM, literatures	Related herb, related formula component, use method, indication, reference	
TCMIO Liu et al. (2020) (http://tcmio.xielab.net)	Academic database	1493 formulas	ChP-TCM (2015)	Reference, efficacy, indication	Pathway enrichment analysis, network visualization
TCMIP v2.0 Wang et al. (2021a) (http://www.tcmip.cn/)	Academic database	3959 formulas	ETCM	Related herb, dosage form, administration, formula type, related *ZHENG*, indication, related target, related disease	GO analysis, reactome pathway, target prediction and functional analysis of formulas, analysis of medication rules in formulas, reverse searching for formulas based on enrichment analysis
LTM-TCM Li et al. (2022a) (http://cloud.tasly.com/#/tcm/home)	Academic database	48126 formulas	TCMID, TCM-ID, ChP-TCM, TCM typical formula database, NMPA protected traditional medicines, NMPA OTC database (TCM), NMPA national basic drug database (TCM), national service platform for academic experience of famous TCM doctor	Related herb, related symptom, reference, related component	The knowledge graph of prescript with related herbs and symptoms, statistics of components related to the formula, formula similarity analysis, formula gene target search and prediction, enriched pathways, formula target genes enrichment analysis
CPMCP Sun et al. (2022) (http://cpmcp.top)	Academic database	1469 Chinese patent drugs, 656 formulas	Compendium of national standards for Chinese patent medicines, Chinese medicine works	Related herb, related TCM symptom, related MM symptom, related component, related target, related disease, reference	Visualization of the following entities/associations: formula-TCM symptom-MM symptom-disease, formula-herb-component-target-disease
TCM-Suite Yang et al. (2022) (http://TCM-Suite.AImicrobiome.cn)	Academic database	6692 formulas	Database	Related herb, related component, related protein, related disease	Visualization of the following entities/associations: formula-herb-component
ETCM v2.0 Zhang et al. (2023) (http://www.tcmip.cn/ETCM2/front/#/)	Academic database	48442 formulas, 9872 Chinese patent drugs	Ancient books, ChP-TCM (2020)	Related herb, formula type, related symptom/sign, dosage form, related disease, efficacy, source, related component, related target, related enriched disease, related ZHENG, indication, quantitative information of marker component	Number distribution of components hitting the candidate targets, functional enrichment analysis of targets, feature distribution of components, expression heatmap of targets, similar formulas, similar Chinese patent drugs, network visualization
TM-MC 2.0 TM-MC 2.0, 2023 (https://tm-mc.kr/index.jsp)	Academic database	5075 formulas	Ancient books	Related herb, source, indication	

(Continued on following page)

TABLE 1 (*Continued*) List of TCM formula databases.

Database name	Type	Number of formulas	Data source	Presentation modules directly related to TCM formula	Analysis modules directly related to TCM formula
CNKI-TCM formula knowledge base CNKI-TCM formula knowledge base, (2023) (https://kb.tcm.cnki.net/TCM/TCM/Index?dbcode=ZYFF)	Academic database	Over 20000 formulas	Ancient books	Source, efficacy, use method, related herb, TCM song tip, indication, medical record	
Modern Application Database of Herbal Formulations Modern Application Database of Herbal Formulations, (2017) (http://cintmed.cintcm.com/cintmed/searchIndex/basic.html?dbtype=xdfj&pdh=15)	Academic database	9651 formulas	ChP-TCM, drug standards issued by the ministry of health-Chinese patent drugs	Source, efficacy, use method, related herb, dosage form, use method, related component, contraindication, adverse reaction, pharmacological action, clinical application, toxicological experiment, physicochemical property, manufacturer	
Database of Standardized for Chinese patent drugs Database of Standardized for Chinese patent drugs, (2017) (http://cintmed.cintcm.com/cintmed/searchIndex/basic.html?dbtype=zyfj&pdh=20)	Academic database	4052 Chinese patent drugs	Drug standards issued by the ministry of health - Chinese patent drugs (1989-1998)	Source, efficacy, use method, alias, related herb, dosage form, use method, contraindication, quality requirement, specification, storage, standard number	
Chinese Herbal Formulas Database Chinese Herbal Formulas Database, (2017) (http://cintmed.cintcm.com/cintmed/searchIndex/basic.html?dbtype=zyfj&pdh=14)	Academic database	85174 formulas	Literatures, Ancient books	Contraindication, pharmacological action, source, efficacy, use method, alias, related herb	
TCM Ancient Formulas Database TCM Ancient Formulas Database, (2023) (http://zyygx.cintcm.ac.cn:9698/zyygx/)	Academic database	Over 160000 formulas	Ancient books	Related syndrome, efficacy, use method	
OTC Chinese Herbal Medicine Database OTC Chinese Herbal Medicine Database, (2017) (https://cintmed.cintcm.com/cintmed/searchIndex/basic.html?dbtype=xdfj&pdh=21)	Academic database	2852 formulas	OTC Chinese herbal medicine released by the Chinese government (1999)	Source, efficacy, use method, alias, related herb, dosage form, type, contraindication, specification, adverse reactions, standard number, storage, quality requirement, pharmacological action	
Chinese Medicine Formulae Image Database Chinese Medicine Formulae Image Database, (2023) (https://library.hkbu.edu.hk/electronic/libdbs/cmfid/index.html)	Academic database	182 formulas	Commonly used Chinese formulas	Type, application, source, efficacy, related herb, use method	Introduction to formulas with illustrations
Chinese Medicine Think Tank- a big data analysis platform for TCM Chinese Medicine Think Tank- a big data analysis platform for TCM, (2017) (https://www.duguji.cn/login)	Commercial database	300000 formulas	Ancient books, the empirical formula of famous veteran teran doctors of TCM	Source, efficacy, related herb, use method	
DRUGDATAEXPY DRUGDATAEXPY, (2009) (https://db.yaozh.com/fangji)	Commercial database	34214 formulas	Drug standards issued by the ministry of health - Chinese patent drugs, ancient books	Dosage form, contraindication, indication, application, efficacy, related herb	
Pharnexcloud Pharnexcloud, (2021) (https://www.pharnexcloud.com/)	Commercial database	35000 formulas	ChP-TCM, ancient books, the empirical formula of famous veteran teran doctors of TCM	Indication, contraindication, source, efficacy, related herb, use method	
Huabing data platform Huabing data platform, (2023) (http://www.huabeing.com/pres/index)	Commercial database	200000 formulas		Source, efficacy, related herb, related disease, related symptom	Medication rules analysis in formulas compositions, the core function of auxiliary discovery in formulas, the auxiliary confirmation of the core components of formula

TCM, traditional Chinese medicine; GO, Gene ontology; OTC, over the counter; ChP-TCM, pharmacopoeia of the people's Republic of China; MM, modern medicine.

that the quality of data and the dimensions covered vary among these databases, which can impact the accuracy of predictive results. Further evaluation is necessary for the data in these databases. Besides of that dosage and usage have varied significantly between regions and dynasties (Zha et al., 2015). Therefore, the results of the verification of the usage and dosage of individual herbs within formulas, as well as the sources of this information, should also be indispensable data within the database. The dosage of an herb significantly determines the concentrations of its components within the human body fluid and then may impacts the activity of component's targets, which would be the key logic of both wet and dry experiments. However, most databases have not effectively cleaned dosage-related data during the inclusion of herbal formulas and data cleaning processes. Systematic research of ancient texts is essential to methodically organize and standardize ancient formulas. This highlights the needs of establishing uniform, rigorous standards, and quantitative dosage information for TCM data (Wang et al., 2021b).

3 Herb databases

Ancient Chinese people, through extensive medical practices over a long period of time, experimented with many medicinal substances derived from animals, plants, minerals, microorganisms, and other sources (Wang et al., 2017). These substances were documented, and their functions continuously verified over time. At present, TCM herbs encompass plant-based medicines, animal-derived medicines, and mineral-based medicines, with plant-based medicines being the predominant category. Herb databases are commonly used to compile fundamental information about herbs, such as properties, meridians, regions of usage, flavors, effects, and indications. This information came from various sources, including the Pharmacopoeia of the People's Republic of China (ChP-TCM), the fourth national survey on Chinese Materia Medica Resources, books, literatures, and dictionaries. A total of 24 databases related to herbs have been identified, 21 of which are academic and 3 of which are commercial (Table 2). The Pharmacloud database contains the largest number of herb resources at −18,000 (Pharnexcloud, 2021). TCMID holds the distinction of being the academic database with the most extensive collection of herb resources, encompassing a total of 10,846 (Xue et al., 2012). China's multi-ethnic composition means that various ethnic groups have discovered numerous herbs rooted in their own cultural practices. To enhance drug development, databases related to herbs from different ethnic groups have also been established, including databases for Tibetan, Mongolian, Uyghur, and Yao medicine.

The relationships between herbs and active components are currently key areas of focus in herb research (Fu et al., 2014; Zeng et al., 2022b). These linkages are included in herb databases. Active components are not only the primary materials that compose herbs but are also crucial for their therapeutic effects (Liu C. et al., 2018a). In 2018, following an update to the TCMID, there was a significant improvement in the coverage of herbs (Huang et al., 2018). Additionally, it introduced mass spectrometry (MS) data for these herbs, which served the purpose of distinguishing differences in the quality of herbs. Quantitative data for the characteristic components in each herb, as specified in the ChP-TCM, are available in the, ETCM and TCMIP databases (Wang et al., 2021a; Zhang et al., 2023). SymMap annotates components in four categories based on experimental MS data from ChP-TCM and from the literatures: quality control components, blood components, metabolite components, and other components (Wu et al., 2019). Utilizing herb-component relationship information from such a database, it is possible to construct more intricate features for herbs. This can be achieved, for example, by building heterogeneous herb-component-target networks. Such efforts enhance the accuracy of intelligent formula recommendation systems based on deep learning, such as FordNet (Zhou et al., 2021). Herb-component-target relationships in these databases also enable researchers to measure the effectiveness of specific herbs in treating diseases. This approach can then be used to identify herbs that are highly associated with specific diseases based on the importance of a particular target within a disease network (Wang et al., 2021d; Niu et al., 2023). For the identification of biological components in TCM, TCM-Suite gathered sequences and associated information for six marker genes: ITS2, matK, trnH-psbA, trnL, rpoC1, and ycf1 (Yang et al., 2022). Therapeutic efficacy of herbs is associated with the components and its content in the herb. A counterpart example in compound chemical drug is that there are fixed usage ratios for the synergistic effects of components (Ferrannini et al., 2022). For example, a fixed-ratio combination of insulin glargine and lixisenatide can better control the blood sugar levels in patients with diabetes (Aroda et al., 2016). Inappropriate ratios can lead to opposite effects (Létinier et al., 2023). In the context of components in herbs, the same principle holds true. Therefore, establishment of the herb-component relationships also requires the critical quantitative information-the content of components in herbs (Heinrich et al., 2022). Currently, there is a substantial accumulation of research on the identification and content measurements of components in herbs, including high-performance liquid chromatography, high-performance liquid chromatography-MS, and gas chromatography-MS, etc. (Arrizabalaga-Larrañaga et al., 2021; Papatheocharidou and Samanidou, 2023), but there is still a lack of databases for comprehensive aggregation and compilation of quantitative research data on components in herbs.

4 Component databases

Regardless of whether a so-called "herb" of interest is a plant, animal product, mineral-based medicine, the active components of which are chemical substances. Herbal component databases include information about the chemical components that have been extracted or isolated from single herbs or formulas. Such databases source their data from the literature, experimental data, and/or preexisting databases, encompassing essential details such as chemical structure, and CAS registry number of component. A total of 28 databases related to chemical components in TCM herbs have been identified (Table 3).TCM-Suite has the largest number of TCM chemical components at 704321 components, but it only 54,868 herb-component relationships (Yang et al., 2022). Some of these

TABLE 2 List of herb databases.

Database name	Type	Number of herbs	Data source	Presentation modules directly related to herb	Analysis modules directly related to herb
TCM Database@Taiwan Chen, (2011) (http://tcm.cmu.edu.tw/)	Academic database	453 herbs	Chinese medical texts and dictionaries	Type, related component	
TCMSP Ru et al. (2014) (http://sm.nwsuaf.edu.cn/lsp/tcmsp.php)	Academic database	499 herbs	ChP-TCM	Related component, related target, related disease	Network representation of component-target interactions, network representation of target-disease interactions
TM-MC 2.0 TM-MC 2.0, 2023 (https://tm-mc.kr/index.jsp)	Academic database	635 herbs	Literatures	Related component	
CancerHSP Tao et al. (2015) (http://lsp.nwsuaf.edu)	Academic database	2439 anticancer herbs	Literatures	Related component	
TCM-Mesh Zhang et al. (2017b) (http://mesh.tcm.microbioinformatics.org/)	Academic database	6235 herbs		Property, meridian, use part, efficacy, indication, related component, related disease, related research, related target, toxicity and side effect	
TCMID 2.0 Huang et al. (2018) (http://www.megabionet.org/tcmid/)	Academic database	10846 herbs	TCM-ID, books, literatures	Property, meridian, use part, efficacy, indication, related component	Herb-target-disease network
YaTCM Li et al. (2018a) (http://cadd.pharmacy.nankai.edu.cn/yatcm/home)	Academic database	6220 herbs	TCMID, TCMSP, TCM Database@Taiwan, books, literatures	Picture, efficacy, indication	Herb-herb network
TCMIO Liu et al. (2020) (http://tcmio.xielab.net)	Academic database	618 herbs	ChP-TCM (2015)	Species, family, use part, property, flavor, meridian, efficacy, indication	Formula-herb-component-target network, pathway enrichment analysis
TCMIP v2.0 Wang et al. (2021a) (http://www.tcmip.cn/)	Academic database	402 herbs	ETCM	Picture, type, description in Chinese/English, habitat, collection time, appearance, specification, property, flavor, meridian, indication, related disease, related formulas, chemical profiling and the corresponding candidate target gene list, quality control	GO analysis, reactome pathway, target prediction and functional analysis of herb, exploration and functional analysis of molecular mechanisms related to TCM medicinal properties, network mining in TCM
SuperTCM Chen et al. (2021) (http://tcm.charite.de/supertcm)	Academic database	6516 herbs	Books, databases	Use part, picture, related component, related target, related disease, related recipe	KEGG metabolic pathways
HERB Fang et al. (2021) (http://herb.ac.cn/)	Academic database	7263 herbs	SymMap, TCMID, TCMSP, TCM-ID	Use part, efficacy, related component, related target, related disease, related high-throughput experiment, ID mapping of TCMID database	
LTM-TCM Li et al. (2022a) (http://cloud.tasly.com/#/tcm/home)	Academic database	9122 herbs	TCMID, CancerHSP, TCMIP, ChP-TCM (2020)	property, herb toxicity, use part, herb smell, related formula	The knowledge graph of herb with related prescripts and components, summary of herb query results (number of TCM formulas containing this herb, number of components, number of herb's target, number of components supported by literature evidence, count of literature evidence), ADME of the herb component, herb-component target prediction, target gene set enrichment
HIT 2.0 Yan et al. (2022) (http://hit2.badd-cao.net)	Academic database	1250 herbs	Literatures	Efficacy, related target, related component, ID mapping of TCM-ID database	

(Continued on following page)

TABLE 2 (*Continued*) List of herb databases.

Database name	Type	Number of herbs	Data source	Presentation modules directly related to herb	Analysis modules directly related to herb
CPMCP Sun et al. (2022) (http://cpmcp.top)	Academic database	1560 herbs	ChP-TCM	Property, meridian, type, use part, related Chinese patent drug, related formula, related TCM symptom, related MM symptom, related component, related target, related disease, ID mapping of HERB, SymMap databases	Relationship network (herbs-Chinese patent drugs-formulas-TCM symptom-MM symptom-component-target-disease), the common compatibility of medicinal materials
TCMSID Zhang et al. (2022a) (https://tcm.scbdd.com)	Academic database	499 herbs	TCMSP, SymMap	Category, picture, related component	Automatically calculate and generate the network analysis diagram of herb-component-target-drug relationship
IGTCM Ye et al. (2023) (http://yeyn.group:96/)	Academic database	83 herbs		Related component, efficacy, indication	
SymMap v2 Wu et al. (2019) (http://www.symmap.org/)	Academic database	698 herbs	ChP-TCM (2020)	Property, meridian, class, use part, efficacy, ID mapping of TCMID, TCM-ID, TCMSP databases	Overview of the herb-component network, overview of the herb-*ZHENG*-TCM symptom network, functional enrichments of genes
TCM-Suite Yang et al. (2022) (http://tcm-suite.aimicrobiome.cn/)	Academic database	7322 herbs	Database	Property, meridian, efficacy, indication, use part, related formula, related component, related protein, related disease	Visualization of the following entities/associations: formula-herb-component-protein
ETCM v2.0 Zhang et al. (2023) (http://www.tcmip.cn/ETCM2/front/#/)	Academic database	2079 herbs	The fourth national survey on Chinese materia medica resources, ChP-TCM (2020), authoritative Chinese medical books and dictionaries	Family, habitat, use part, herb classification based on efficacy, property, flavor, meridian, indication, related Chinese patent drug, related formula, related target, related enriched disease, related component	Number distribution of components hitting the candidate targets, functional enrichment analysis of targets, feature distribution of components, expression heatmap of targets, similar herbs (based on components, targets), herb-component network
TCMBank Lv et al. (2023) (https://TCMBank.cn/)	Academic database	9192 herbs	TCM Database@Taiwan	Related components, property, efficacy, related disease, related gene, ID mapping of HERB, Timebank, TCM-ID databases	Relationship map (herb-components-targets-diseases), AI-assisted drug-drug interactions prediction model to produce the prediction results of the mutual exclusion of Chinese-Western medicine
The Chinese traditional medicine database The Chinese traditional medicine database, (2017) (https://cintmed.cintcm.com/cintmed/searchIndex/basic.html?dbtype=xdfj&pdh=5)	Academic database	8173 herbs	ChP-TCM, authoritative Chinese medical books and dictionaries	Property, toxicity and side effect, quality control, efficacy, processing method, use part, indication, family, habitat	
Huabing data platform Huabing data platform, (2023) (http://www.huabeing.com/pres/index)	Commercial database		Ancient books	Related component, related symptom, related disease, related herb, efficacy	
Pharnexcloud Pharnexcloud, (2021) (https://www.pharnexcloud.com/)	Commercial database	18000 herbs		Type, related formula, related component, efficacy, habitat	
DRUGDATAEXPY DRUGDATAEXPY, (2009) (https://db.yaozh.com/fangji)	Commercial database	2000 herbs		Habitat, efficacy	

TCM, traditional Chinese medicine; AI, artificial intelligence; ChP-TCM, pharmacopoeia of the people's Republic of China, GO, gene ontology; MM, modern medicine.

databases are more specialized: TCMIO, CancerHSP, and NPACT primarily focus on collecting information about active components related to tumors, whereas CVDHD contains data about active components associated with cardiovascular diseases (Mangal et al., 2013; Tao et al., 2015; 2015; Liu et al., 2020). Databases for herb components specifically offer a wealth of resources for modern drug development (Fu et al., 2016; Zhang et al., 2023).

TABLE 3 List of component databases.

Database name	Number of components	Data source	Presentation modules directly related to component	Analysis modules directly related to component
TCMD He et al. (2001) (http://repharma.pku.edu.cn/tcmd.html)	23033 components	Book: TCM: molecular structures, natural sources, and applications	Chemical structure, physicochemical property	
TCM-ID Chen et al. (2006) (http://tcm.cz3.nus.edu.sg/group/tcm-id/tcmid.asp)	5669 components	Books, literatures	Chemical structure, CAS	
TCM Database@Taiwan Chen, (2011) (http://tcm.cmu.edu.tw/)	20000 components	Chinese medical texts and dictionaries, Medline, ISI Web of knowledge	Chemical structure, molecular volume, molecular properties (ALogP, molecular polar surface area, number of hydrogen bond acceptors, number of hydrogen bond donors, number of rotatable bonds)	
CVDHD Gu et al. (2013) (http://pkuxxj.pku.edu.cn/CVDHD)	35230 compounds	CHDD, UNPD	Chemical structure, CAS, molecular properties (AlogP, number of hydrogen bond acceptors, number of hydrogen bond donors, number of rotatable bonds), docking results, cardiovascular-related disease, pathway and clinical biomarker	
NPACT Mangal et al. (2013) (http://crdd.osdd.net/raghava/npact/)	1574 bio- compounds	PubMed, literatures	Chemical structure, properties (physical, elemental, and topological), cancer type, cell lines, inhibitory values (IC50, ED50, EC50, GI50), related target, commercial supplier, *in-vitro* anticancer activity, *in-vivo* anticancer activity, CAS, ID mapping of PubChem database	Drug-likeliness filters, similarity search
TCMID 2.0 Huang et al. (2018) (http://www.megabionet.org/tcmid/)	43413 total components, 1045 formula components	TCM-ID, HIT, TCM Database@Taiwan, books, literatures	Chemical structure, related target, related herb, ID mapping of PubChem database	Component-targets network, component-targets-drug-disease network
HIM Kang et al. (2013) (http://www.bioinformatics.org.cn/)	361 components		*In-vivo* metabolism information, bioactivity, organ/tissue distribution, toxicity, ADME, clinical research profile	
TCMSP Ru et al. (2014) (http://sm.nwsuaf.edu.cn/lsp/tcmsp.php)	29384 components	PubChem, ChEMBL, ChemSpider	Related target, ADME, Chemical structure, CAS, related disease, related target, related herb, ID mapping of PubChem database	
CancerHSP Tao et al. (2015) (http://lsp.nwsuaf.edu)	3575 anticancer compounds	Books, literatures, PubMed, Google scholar, PubChem, ChemSpider	Pharmacological property, molecular property, anticancer activity, related target, related herb, reference, ADME	
BindingDB Gilson et al. (2016) (https://www.bindingdb.org/rwd/bind/index.jsp)	490000 compounds	ChEMBL, PubChem, UniChem, ZINC	Chemical structure, ligand, substrate, IC50, citation, inhibitor, related target	Protein-small molecule affinity
STITCH Szklarczyk et al. (2016) (http://stitch.embl.de)	430000 compounds		Chemical structure, related target	Protein-chemical interaction networks
TCMAnalyzer Liu et al. (2018b) (http://www.rcdd.org.cn/tcmanalyzer)	16437 components	TCMSP, TCMID	Chemical structure	Substructure search, scaffold search, 2D similarity search, 3D similarity search
ETCM v2.0 Zhang et al. (2023) (http://www.tcmip.cn/ETCM2/front/#/)	38298 components	ChP-TCM (2020), literatures	Chemical structure, molecular weight, physicochemical property, pharmacokinetic property, toxicology, related formula, related herb, related target	

(Continued on following page)

TABLE 3 (*Continued*) List of component databases.

Database name	Number of components	Data source	Presentation modules directly related to component	Analysis modules directly related to component
TCM-Suite Yang et al. (2022) (http://TCM-Suite.AImicrobiome.cn)	704321 components	Database	Chemical structure, related herb, related formula, related protein, related disease	Visualization of the following entities/associations: formula-herb-component-protein-disease
LTM-TCM Li et al. (2022a) (http://cloud.tasly.com/#/tcm/home)	34967 components	TCM-ID, TCMID, TCM-Mesh, TCMIP, TM-MC	Basic physical and chemical information, ADME, chemical structure, related herb, related formula, related target	Enriched signal pathways, component target genes enrichment analysis
TCMIO Liu et al. (2020) (http://tcmio.xielab.net)	16437 components	TCMAnalyzer, TCMSP, TCMID	Chemical structure, synonyms, related target	Herb-component-target network
TM-MC 2.0 TM-MC 2.0, 2023 (https://tm-mc.kr/index.jsp)	34081 components	Literatures	Chemical structure, related herb, related target, number of hydrogen bond acceptors, number of hydrogen bond donors, number of rotatable bonds	
HIT 2.0 Yan et al. (2022) (http://hit2.badd-cao.net)	1237 components	TCM-ID, PubChem, ChEMBL	Chemical structure, CAS, related target, related herb, ID mapping of ChEMBL, PubChem, TCM-ID databases	Automatic target-mining and my-target curation from daily released PubMed literatures
SymMap Wu et al. (2019) (http://www.symmap.org/)	19595 components	TCMID, TCMSP, TCM-ID	OB score, type, CAS, related herb, related *ZHENG*, related TCM symptom, related MM symptom, related target, related disease, ID mapping of PubChem, TCMID, TCM-ID, TCMSP databases	
SuperTCM Chen et al. (2021) (http://tcm.charite.de/supertcm)	55772 components	Databases, books	Chemical structure, related target, related KEGG pathway, related disease, related herb, related recipe, ID mapping of PubChem, ChEMBL databases	Herb-component relationships, component-target relationships, component-target-pathway relationships, component-target-disease relationships
HERB Fang et al. (2021) (http://herb.ac.cn/)	49258 components	SymMap, TCMID, TCMSP, TCM-ID	Chemical structure, related herb, ID mapping of TCMID database	Paper mined target genes, paper mined diseases, differentially expressed genes were calculated from high-throughput experiments deposited in the GEO database, GO enrichment analysis were performed based on the differentially expressed genes, KEGG enrichment analysis were performed based on the differentially expressed genes, Connectivity analysis was performed by mapping the differentially expressed genes of herb/component to CMap touchstone datasets, using the CMap website
TCMBank Lv et al. (2023) (https://TCMBank.cn/)	61966 components	TCM Database@Taiwan	Related herb, related target, related disease, OB score, molecular polar surface Area, number of hydrogen bond acceptors, number of hydrogen bond donors, number of rotatable bonds, C count, O count, CAS, ID mapping of PubChem, TCMID, TCM-ID, TCMSP, SymMap databases, chemical structure	Relationship map (herb-component-target-disease)
CPMCP Sun et al. (2022) (http://cpmcp.top)	26341 components	TCMID, TCMSP, TCM-ID	OB score, CAS, related formula, related herb, related TCM symptom, related MM symptom, related target, related disease, ID mapping of PubChem, SymMap databases	Component-herb-TCM symptom-MM symptom-target-disease relationship network

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TABLE 3 (*Continued*) List of component databases.

Database name	Number of components	Data source	Presentation modules directly related to component	Analysis modules directly related to component
TCMSID Zhang et al. (2022a) (https://tcm.scbdd.com)	20015 components	Literatures, TCMSP, SymMap	CAS, chemical structure, synonyms, ADME, related target, ID mapping of PubChem database	Herb-component-target-drug multilevel interaction network, component structural reliability evaluation, component structural classification
NPASS Zhao et al. (2023a) (http://bidd.group/NPASS)	43285 compounds	PubMed, PubChem, ZINC, ChEMBL, BindingDB, TCM-ID, TCM Database@Taiwan, TCMID, TCMSP, TM-MC, StreptomeDB, HerDing, TTD, ChEMBL, DrugBank, IUPHAR/BPS	Chemical classification, related herb, chemical structure, natural product quantity composition/ concentration, ADMET, chemically structural similarity, biological similarity, related target, synonym, synthetic gene cluster, physical and chemical property, ID mapping of PubChem, ChEMBL database	
TCMIP v2.0 Wang et al. (2021a) (http://www.tcmip.cn/)	7284 components		Chemical structure, ALogP, LogD, molecular solubility, molecular volume, molecular surface area, molecular polar surface area, number of hydrogen bond acceptors, number of hydrogen bond donors, number of rotatable bonds, ADMET, drug likeness weight, drug likeness grading, related target, related disease, CAS, reference, related herb, related formula, ID mapping of PubChem, ChEMBL databases	Herb-component-formula network
IGTCM Ye et al. (2023) (http://yeyn.group:96/)	1033 components		Chemical structure, OB score, external link (KEGG), reference	
Chinese Traditional Medicine Chemical Components Database Chinese Traditional Medicine Chemical Ingredients Database, (2017) (https://cintmed.cintcm.com/cintmed/searchIndex/basic.html?dbtype=xdfj&pdh=7)	27593 components	Books, literatures	Chemical structure, physicochemical property, pharmacokinetic property, clinical application	

TCM, traditional Chinese medicine; ChP-TCM, pharmacopoeia of the people's republic of China; GEO, gene expression omnibus; GO, gene ontology; MM, modern medicine; OB, oral bioavailability.

The relationships between components and targets represent a key link connecting two intricate systems: herbs and human biological systems (Stitziel and Kathiresan, 2017). Data mining and computational chemistry approaches are currently being used to collect and organize known component-target relationships and to predict and validate previously unknown component-target relationships (Chen et al., 2016). Several component databases TCM provide both information about known component-target relationships and functionalities for predicting such relationships. The HIT and HERB databases contain information about component-target relationships obtained through text mining of the literature (Fang et al., 2021; Yan et al., 2022). HIT categorizes component-target relationships component into three types: "Directly inhibit/activate," "Indirectly inhibit/activate," and "Enzyme substrate". Users can refer to the associated literature to learn more about specific component-target relationships. More importantly, HIT facilitates automatic target mining and curation of "My-target" information from newly released PubMed literature (Yan et al., 2022).

For components lacking reported relationships with a target, several computational chemistry approaches have been significantly developed. These approaches include ligand-based methods, target-based methods, and target-ligand methods, all of which aim to predict relationships between components and proteins (Sadybekov and Katritch, 2023). The SwissTargetPrediction is a widely used web tool, available online since 2014, designed to predict the most probable protein targets of small molecules. Predictions are made using the similarity principle through reverse screening. In the latest updated version, the models have been recalculated, achieving a success rate of at least one correct human target in the top 15 predictions for more than 70% of external compounds (Daina et al., 2019). BindingDB is a database that focuses on relationships between small molecules and their corresponding targets. The BindingDB website provides specialized tools that leverage its extensive data collection, allowing researchers to generate hypotheses for protein targets of a given bioactive component or to predict components that are bound by a particular protein. Additionally, the website offers virtual component screening using methods like maximal chemical similarity, binary kernel

discrimination, and support vector machines(Gilson et al., 2016). To meet the demand for predicting targets of components, component databases have also started incorporating target prediction functionality. SysDT is a model that was designed to predict potential targets of components within the TCMSP database (Ru et al., 2014). SysDT has demonstrated remarkable predictive performance for drug-target relationships (Yu et al., 2012). ETCM v2.0 uses a target identification method that is based on a two-dimensional ligand similarity search module within the D3CARP platform and utilizes data from Binding DB (Zhang et al., 2023). To enhance the accuracy of target prediction, TCMSID employs multiple target prediction methods, including similarity ensemble approach, SwissTargetPrediction, HitpickV2, PPB, PPB2, and CHEMBL (Zhang al., 2022a). LTM-TCM integrates component-target information from various sources, including the BATMAN-TCM, ChEMBL, and STITCH databases. LTM-TCM retains target scores from different sources to enable personalized target screening based on user-defined thresholds (Li et al., 2022a).

The systematic collection and organization of herb components in databases forms the foundation of target prediction to decipher the multiple pharmacological actions of a given compound. Target prediction methods have the potential to significantly shorten drug development timelines, but the accuracy of computational studies remains relatively low. In practice, even the most successful virtual screening campaigns typically result in only 10%–40% of candidate hits being confirmed through experimental validation (Sadybekov and Katritch, 2023). A multitude of virtual screening efforts produced predominantly discouraging outcomes. For instance, the antimalarial drug ebselen, which had been identified through an early virtual screening process, ultimately proved unsuccessful in clinical trials (Sadybekov and Katritch, 2023). Therefore, it is essential to conduct more comprehensive *in vitro* and *in vivo* studies and develop improved methods for evaluating the above results. These results recorded in online databases should also have clear indications of their sources, to aid researchers in assessing the reliability of the data.

5 Target and target-related biofunction databases

Targets are the smallest functional units within an organism, serving as the internal nodes of complex systems (Turkarslan et al., 2014). They carry out various functions in numerous pathways and phenotypic responses, acting as bridges between medicines and the human biological system (Pfister and Ashworth, 2017; Santos et al., 2017). Drug mechanisms of action involve interactions between components and their targets. The initial paradigm in this area posited that a single component would act on a single target (Koeberle and Werz, 2014). However, further research revealed that nearly all natural and human-synthesized components interact with multiple targets (Plazas et al., 2022). Target databases primarily encompass genetic and protein-related information. Existing types of target databases include drug target databases, disease target databases, and specific target databases. These databases typically include basic information such as the target type, function, and origin, which are often sourced from the literature. UniProt, NCBI, and GeneCards are examples of target databases that provide comprehensive genetic and protein sequence information along with functional details (Table 4) (Safran et al., 2010; Brown et al., 2015; The UniProt Consortium, 2023). ETCM, TCMID, YaTCM, HIT, HERB, DisGeNET, and other databases also include information about targets, but these primarily focus on the relationships between targets and components or diseases (Piñero et al., 2017; Li et al., 2018a; Huang et al., 2018; Huang et al., 2018; Xu et al., 2019; Fang et al., 2021; Yan et al., 2022; Zhang et al., 2023). They often therefore have a decreased emphasis on the functional details of targets. Researchers have used target databases for purposes such as analysis of target-phenotype relationships (e.g., SymMap) (Wu et al., 2019; Lv et al., 2023). Target relationships in the TCMSP, TCMID, and TCM-ID databases have been used to map symptom-related genes and herb-related targets to human protein interaction networks (Chen et al., 2006; Xue et al., 2012; Ru et al., 2014; Huang et al., 2018). Through analysis of their topological relationships within a network, the distances between gene nodes can be calculated to infer distances between symptom modules, providing information about symptom co-occurrence and similarity. This approach has been employed to evaluate herb effectiveness for specific symptoms. It is a robust method for deciphering the mechanisms of herb and for predicting early-stage drug efficacy for diseases of interest (Gan et al., 2023).

Biological pathways can be considered as subsystems within complex systems. They serve as a framework for conducting pharmacological TCM research. These pathways can provide explanations for the complex mechanisms that link herbs to physiological changes. They often play significant roles in elucidation of interactions between drugs and biological functions (Wang et al., 2022b). Many target function databases integrate information about genes and genomes with higher-level functional annotations (Zeeshan et al., 2020). These data can then be used to systematically analyze gene functions based on known biological processes in an organism. Such databases are thus commonly utilized in conducting gene functional enrichment analyses, pathway-related analyses, and protein-protein interaction analyses. KEGG is a reference database for biological interpretation of genome sequences and other high-throughput data. The primary functionalities for biological process analyses are biochemical pathway mapping, metabolic network construction, genome comparison and merging, and enzyme database construction for target molecules (Kanehisa et al., 2017). BioCyc compiles and references genomes and metabolic pathways from thousands of sequenced organisms (Karp, 2005). Reactome systematically generates ordered molecular transformation networks, resulting in formation of classical metabolic maps. This database also associates human proteins with their molecular functions, offering a resource that serves as both a record of biological processes and a tool for discovering new functional relationships from data such as gene expression levels or mutations in tumor cells. Additionally, it can predict target biological processes of ion channels (Jassal et al., 2019). DAVID database consists of six tools: the functional annotation clustering, the functional annotation chart, the functional annotation table, gene functional classification, gene ID conversion, and gene name batch viewer (Sherman et al., 2022). The STRING database is used

TABLE 4 List of target databases.

Database name	Number of targets	Data source	Presentation modules directly related to target	Analysis modules directly related to target
TTD Li et al. (2018b); Zhou et al. (2022) (https://db.idrblab.net/ttd/)	3578 targets	Literatures	Target type, related disease, biochemical class, related drug, related regulator, related target profile in patient, related target affiliated biological pathway, related model, related study, ID mapping uniport database	All known drugs of a target are clustered based on multiple or single drug-like properties, which is displayed using the hierarchical clustering map, heatmap and bar plot, sequence similarity searching
NPASS Zhao et al. (2023a) (http://xin.cz3.nus.edu.sg/group/ttd/ttd.asp)	7753 targets		Target type, organism of target, biological activities of natural product against the target, related component, related specie	
Uniprot The UniProt Consortium, (2023) (https://www.uniprot.org/)	Over 227 million protein sequences, 451000 proteomes	Completely sequenced viral, bacterial, archaeal, and eukaryotic genomes	Function, subcellular location, related disease/variant, showing features for signal, chain, disulfide bond, glycosylation, expression (tissue specificity, gene expression database, organism-specific database), interaction (binary interaction, protein-protein interaction database, chemistry) structure, family/domain, sequence/isoform, similar protein	Find a protein sequence to run BLAST sequence similarity search by uniport ID, the align tool enables users to make multiple sequence alignments which can now be viewed in two ways-the wrapped view allows for a quick scan of the alignment, and the overview allows researchers to zoom in/out and move through the sequences in a user-defined manner, find uniport entries through parts of their peptide sequences, retrieve/ID mapping
Genecards Rebhan, (1997); Safran et al. (2010) (https://www.genecards.org/)	418808 entries, 43626 HGNC approved, 21702 protein coding, 292000 RNA genes	Databases	Genomic, protein, family/domain, function, localization, pathway/interaction, related drug, related component, transcript, expression, ortholog, paralog, variant, related disease, related product, reference	
Genbank Benson et al. (2018) (https://www.ncbi.nlm.nih.gov/genbank/)			Sources, feature, origin, reference	
Pfam Mistry et al. (2021) (http://pfam.xfam.org/)	19632 entries, 655 clans		Description, reference, protein, taxonomy, proteome, alphafold, pathway, genome 3D	

for analysis of protein-protein interactions. Individual protein queries generate a network composed of all proteins that interact with the queried protein (von Mering et al., 2003). This is particularly valuable for exploring interactions among input proteins; for example, it can be used to analyze the connections among differentially expressed proteins identified from proteomic data (Szklarczyk et al., 2021).

6 Phenotype databases

From the perspective of a complex system, the state of an organism corresponding to any abnormal phenotype is an abnormal steady state (Tyler et al., 2016). Such an abnormal steady state entails multiple nodes balance within the system. Likewise, interventions should target several nodes simultaneously to effectively restore the system to its normal steady state. Phenotype databases primarily focus on collecting data related to diseases, symptoms, and other phenotype-related entities. These databases provide robust datasets for those researching the mechanisms underlying TCM efficacy, primarily sourced from the literature and from other databases. Currently, a total of 13 databases have been compiled that provide detailed descriptions of diseases and symptoms (Table 5). TCMBank is the most comprehensive repository of disease-related resources, encompassing 32,529 data points (Lv et al., 2023).

The relationship between a target and the corresponding phenotype serves as a crucial bridge connecting a biological mechanism to the pathological manifestation in the human body. This connection was first established through the discovery of mutation-phenotype relationships. OMIM is a comprehensive repository that focuses on genetic and phenotypic data and interrelationships between the two. This database plays a pivotal role in naming and categorizing genetic phenotypes, thereby exerting a significant influence on the field of genetics (Funk et al., 2022). With the advent of the post-genomic era, the goal of deciphering the biological functions of target has evolved into the larger goal of delineating the intricate relationships between multiple genes and phenotypes. HPO and DisGeNET are comprehensive databases for analyzing and interpreting human gene-disease

TABLE 5 List of phenotype databases.

Database name	Number of phenotypes	Data source	Presentation modules directly related to phenotype	Analysis modules directly related to phenotype
OMIM Amberger et al. (2015) (http://omim.org)	7894 phenotypes	Biomedical literatures	Gene-phenotype relationship, clinical synopsis, phenotypic series, description, clinical feature, cytogenetics, molecular genetics, reference; external links: protein, animal models, clinical resources	Graphical representation of phenotype/gene relationship
LTM-TCM Li et al. (2022a) (http://cloud.tasly.com/#/tcm/home)	1928 TCM symptoms		Related formula	Intelligent TCM formula based on existing formulas for the symptom, frequency of components in TCM formulas for the symptom, frequency of symptoms co-occurred with the given, the combination frequency of herb in formulas
HPO Köhler et al. (2021) (https://hpo.jax.org)	over 156000 annotations to hereditary diseases		Hierarchy, synonyms, reference, related gene, related disease	Profile search (allow you compare your profile across species with a specific set of genes or diseases)
DisGeNET Piñero et al. (2017) (http://www.disgenet.org)	30170 diseases	MeSH, OMIM, DO, ICD-9, ClinVar, GWAS Catalog, UniProt, GAD, BeFree, Wikipathways, ChEMBL, Gene Expression Atlas	Semantic type, phenotypic abnormality, disease ontology, summary of gene-disease association, evidence for gene-disease association, summary of variant-disease association, evidence for gene-disease association, summary of disease-disease association, disease mapping, ID mapping of UMLS, MeSH, OMIM databases	
MalaCards Rappaport et al. (2017) (http://www.malacards.org/)	20000 diseases	OMIM, GT, GR, GHR, Orphanet, NIH RD, Wiki, DISEASES, DO, NiNDS	Aliases/classification, related disease, related symptoms/phenotype, related drugs/therapeutics, genetic test, anatomical context, reference, related gene, variation, disease gene expression data, pathways, GO term	
SymMap Wu et al. (2019) (http://www.symmap.org/)	2518 ZHENGs/symptoms, 1148 MM symptoms, 14086 diseases	OMIM, MeSH, Orphanet, UMLS, MalaCards	*ZHENG* type, symptom locus, symptom property, symptom type, related MM symptom, related disease	
ETCM v2.0 Zhang et al. (2023) (http://www.tcmip.cn/ETCM2/front/#/)	8045 diseases	HPO, OMIM, DisGeNET, ORPHANET	Global category, anatomical category, related target	
TCMID 2.0 Huang et al. (2018) (http://www.megabionet.org/tcmid/)	4633 diseases, 2679 TCM diseases	OMIM	Description, clinical feature, related target	
TCMGeneDIT Fang et al. (2008) (http://tcm.lifescience.ntu.edu.tw/)	3360 diseases	PharmGKB	Related target	
SuperTCM Chen et al. (2021) (http://tcm.charite.de/supertcm)	8634 diseases	ICD-10-CM	ICD-10-CM detail, related component, related target, related herbs, related recipe	
HERB Fang et al. (2021) (http://herb.ac.cn/)	28212 diseases	Literatures, DisGeNET	Disease type, related component, related target, related herb	
TCMBank Lv et al. (2023) (https://TCMBank.cn/)	32529 diseases	TCM Database@Taiwan	Disease type, related target, external links: DisGeNET, MeSH, DO, HPO, UMLS	Relationship map (herb-component-target-disease)

(Continued on following page)

TABLE 5 (*Continued*) List of phenotype databases.

Database name	Number of phenotypes	Data source	Presentation modules directly related to phenotype	Analysis modules directly related to phenotype
CPMCP Sun et al. (2022) (http://cpmcp.top)	14434 diseases	MeSH, SIDER, UMLS, ChP-TCM, research on the standardization of TCM terminology, and pathology terminology standardization	Related component, related herb, related formula, related target, related disease/MM symptom/ TCM symptom	Click on the uncomfortable body part to filter for symptoms that may match
	1148 MM symptoms			
	2285 TCM symptoms			

MM, modern medicine; DO, disease-ontology; UMLS, unified medical language system; ChP-TCM, pharmacopoeia of the people's republic of China; TCM, traditional Chinese medicine.

networks (Piñero et al., 2017; Köhler et al., 2021, 1). CPMCP and SymMap also include both TCM symptoms and modern medicine (MM) symptoms in an attempt to bridge TCM and modern medicine-based research through symptom associations (Wu et al., 2019; Sun et al., 2022).

7 ZHENG database

TCM involves a unique, intuitive understanding of physiological states. *ZHENG* differentiation and treatment (辩证论治) is the fundamental approach guiding clinical practice in TCM. Diagnoses and treatments are made by taking into account the individual differences between patients (Zhou et al., 2014; Wang and Zhang, 2017). *ZHENG* is a summary of the pathological and physiological discrepancies at each stage of a disease. It is also determined by factors such as the disease site and the nature of the disease (Wang et al., 2022a). TCM practitioners prescribe different formulas based on the *ZHENG* to achieve therapeutic efficacy. Explorations of *ZHENG*-formula and *ZHENG*-phenotype relationships represent a challenging area of research in both clinical practice and foundational TCM studies. A *ZHENG* database, SoFDA, has been constructed to record and collect *ZHENG* data (Zhang et al., 2022b). It includes both macroscopic data, such as ZHENG, phenotypes, and TCM formulas, and microscopic data (molecular mechanisms). Such databases promote a deeper understanding of ancient systematic medicine, TCM, and modern medicine. SoFDA implements two common association measures (Jaccard and Cosine similarity) to quantify relationships between clinical entities (e.g., *ZHENG*, phenotypes, and formulas). This allows users to compute the degree of indirect associations between the three entities in terms of six shared features: symptoms, genes, enriched gene ontology (GO) terms, enriched pathways, network modules, and network density. However, databases specifically focused on *ZHENG* are currently limited in number, and there are few comprehensive phenotypes analyses related to *ZHENG*.

8 Discussion

8.1 The emergence of network analysis triggered a surge in data generation and database construction

Database evolution is closely linked with current research trends and challenges over time (Sorokina and Steinbeck, 2020). Beyond serving as robust repositories for vast amounts of data, databases related to TCM systems pharmacology also represent pivotal milestones in summarizing the alternations of states in the TCM research. In this review, we retrospectively trace databases pertinent to computational analyses in TCM. Our primary focus is the detailed exploration and comparison of data structures within databases containing formula, herb, component, target, phenotype, and *ZHENG* data (Figure 1). Additionally, we delve into the intricate relationships between these entities within relevant databases. Systems biology is the cornerstone in the establishment of databases related to TCM systems pharmacology. It was until the emergence of component-target databases such as BindingDB, which summarize a large number of component-target relationships based on experimental data, allow mathematical simulation of component-target relationships, effectively addressing the challenge of identifying targets for numerous components (Gilson et al., 2016; Mendez et al., 2019). The emergence of component-target relationships as an area of study has bridged the gap between TCM and biological systems. In 2007, Yildirim et al. applied the principles of network biology by integrating and analyzing drug-gene and drug-protein interaction data. Their work revealed that the majority of drugs exert their effects through indirect modulation rather than direct targeting of disease-associated proteins (Csermely et al., 2005). Building upon this foundation, Hopkins proposed the research methodology of network analysis in pharmacology. He posited that drugs act on multiple targets and demonstrated enhanced efficacy and reduced toxicity through interactions among these multiple targets (Hopkins, 2008). The field of network analysis, which answers research questions from an inherently integrated standpoint, coincides remarkably well with the fundamental principles of TCM (Li and Zhang, 2013; Wang et al., 2021b). Over the course of Chinese history, thousands of herbs and over 300,000 formulas have been applied as medicines (Li et al., 2008). Often, the certain single herb appears in multiple formulas, each of which yields a distinct effect (Wang et al., 2021d). Compared to commercially available synthetic drugs, herbs exhibit a larger quantity of components with higher complexity. Consequently, there is a greater need to collect and organize information to uncover the patterns associated with herbal combinations and their therapeutic effects. Possibly driven by this rationale, the TCMID database was launched in 2012, including relationships between and among formulas, herbs, components, targets, and phenotypes. The inception of this database marked the emergence of the core chain of pharmacological research using herb (Xue et al., 2012; Huang et al., 2018). Subsequent databases related to TCM systems pharmacology have largely promoted establishment of relationships

between and among the same entities. However, these newer databases have also offered enhanced capabilities for computational analyses.

In TCM, the stable coexistence of various clinical manifestations is defined as *ZHENG*, which is also the integrative description on the current status of complex biological system (Tang et al., 2008). Empirical explorations in TCM focus on establishing direct relationships between formulas and *ZHENG*. For patients sharing common pathological characteristics, TCM practice calls for the use of similar but not entirely identical formulas (Wang et al., 202a). Diagnosing and treating patients based on *ZHENG* differentiation can enhance the clinical effectiveness of the treatments. The integration of *ZHENG* and modern personalized medicine approaches could serve as a breakthrough for addressing current challenges in medical practice (He et al., 2008; Su et al., 2012; Chen et al., 2013). A significant amount of omics research is employed to uncover the physiological mechanisms of patients with different *ZHENG* (Wu et al., 2021; Akhoundova and Rubin, 2022). Experimental studies have revealed that patients with different *ZHENG*, but the same disease exhibit distinct biomarkers (Shang et al., 2022). The accumulation of this data can provide more accurate features for computational analysis of *ZHENG*. The rapid development of AI has enabled the training with and analysis of large datasets and led to advancements in personalized medicine. AI has been utilized to learn from tongue images and clinical diagnostic information, aiding in clinical diagnosis (Kanawong et al., 2012; Tang et al., 2021; Chen and He, 2022). Several computational studies have described the use of information about relationships between targets, phenotypes, and symptoms to recommend appropriate clinical formulas (Li et al., 2007; Kanawong et al., 2012; Zhou et al., 2014). It has become possible to reveal the essence of *ZHENG* based on a wealth of information, including phenotypes, and AI model. It thus appears that the process through which herbs exert their therapeutic effects follows the core formula-herb-component-target-phenotype-*ZHENG* chain (Figure 2). In fact, this core relationship chain built with extensive data may aid us in exploring from one entity to another, e.g., starting from a drug entity to explore its clinical applications, it offers an approach to uncover new clinical uses of existing drugs, thereby expanding our understanding and utilization of pharmaceutical resources. Similarly, application in an opposite direction is recommending personalized medications based on clinical phenotype entity. This interconnectivity, grounded in large-scale data, provides a robust framework for enhanced drug discovery and personalized medicine. It enables the identification of tailored therapeutic solutions catering to individual patient's unique clinical presentations and needs (Zhang et al., 2019b). Indeed, there have been studies that utilize such relationship chains for recommendation of personalized medication. Researchers have created gene expression profiles for 189 diseases, then analyzed the perturbation characteristics of herbs based on the herb-component-target relationships within the database. Finally, they predicted the optimal combinations of herbs for treating diseases based on the mapping relationship between herbs and diseases (Chen et al., 2018). However, these studies are still in their early stages of research, and a substantial number of experiments are needed before they can be applied in clinical research.

Pharmacological research is concerned with the process of regulating biological systems through medications; TCM employs complex chemical systems to regulate biological systems (Liang et al., 2012). TCM research is based on the accumulation of extensive clinical experience, through which numerous associations between specific *ZHENG* and corresponding formula are established (Yang et al., 2020). Patients are primarily differentiated into subtypes to enable selection of suitable formulas (Wang et al., 2021c). Another aspect of TCM research involves discovering the efficacy of specific herbs for particular phenotypes. This allows for the incorporation of "specific herbs" into formulas, which are modified accordingly to address a specific patient's disease state beyond the corresponding *ZHENG*. However, there is a lack of necessary research into the material bases and action mechanisms of formulas and herbs metioned above (Xu et al., 2019). This limitation has confined the development of new medical knowledge to the accumulation and extension of clinical experience. In contrast, modern medicine, which is based on chemistry and molecular biology, has be used to elucidate chemicalstructures, functions, and targets, providing modern pharmacology with an extremely precise perspective at the micro scale (Penrod et al., 2011; Zeeshan et al., 2020). However, the rate of new chemical drug production is slowing (Sadybekov and Katritch, 2023). During the development of modern pharmacology, a plethora of component-target relationships have been established (Santos et al., 2017). The establishment of these relationships has provided TCM research with numerous paradigms and methods. This, in turn, has endowed TCM pharmacology with the ability to unveil the formula systems regulating human biological systems, opening the "black box". The core chain (formula-herb-component-target-phenotype-*ZHENG*) bridges the gap between macroscopic and microscopic levels; to some extent, it also explains the interactions between formula systems and the biological system at the molecular level (Figure 2). This framework has made it possible to conduct TCM systems pharmacology research.

8.2 Data accumulation encounters a research bottleneck in TCM computational pharmacology

To date, there has been a significant accumulation of data at various levels within the formula-herb-component-target-phenotype-*ZHENG* chain. The relationships between members of each level have been effectively organized and summarized in various databases. The research in TCM has leveraged the concept of networks, thereby advancing towards the approach with more characteristic's systems science (Huang et al., 2017). However, in complex system, both formulas and modified herbal prescriptions are administered at specific quantities in practical applications, meaning that the herbal composition of formulas is quantitative, and the components within the herbs are quantitative (Luan et al., 2020). In the process of pharmacological research, the effective dosage of a drug is crucial and therefore carefully examined (Spencer and Jarvis, 1999). However, in the context of TCM databases and computational studies of TCM formulas, there are few quantitative calculations and little dosage information. Indeed, not only in TCM but also for active components in general, there

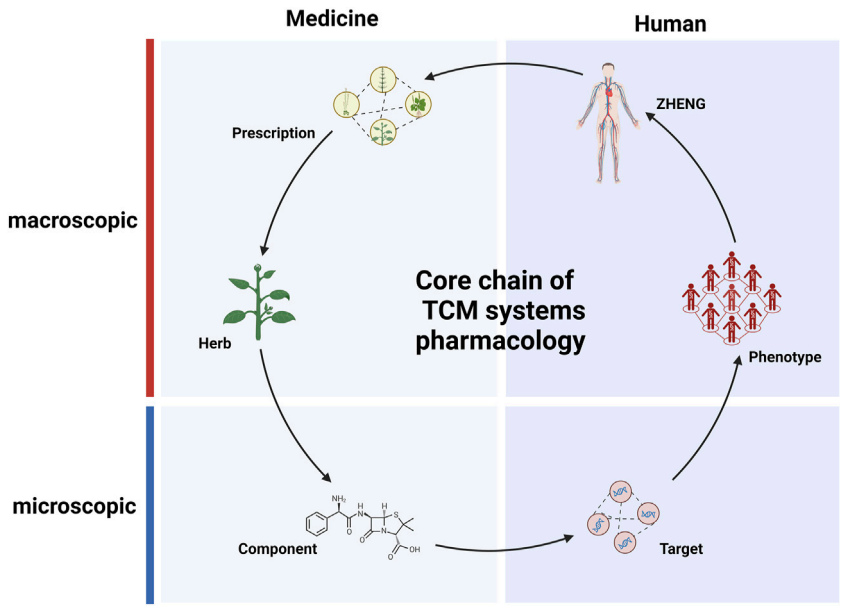

FIGURE 2
The central chain of pharmacological research in traditional Chinese medicine (TCM). Solid arrows represent primary relationships between entities within various hierarchical databases related to TCM.

have been few studies that provide absolute quantitative or relative quantitative (i.e., proportional) information. This approach raises doubts about the accuracy of computational predictions of the composition, efficacy, and mechanism of action associated with formulas. For instance, polyphenols could interact with multiple targets due to their unique nature of multiple hydrogen donor if not considering the effective concentration (Luca et al., 2020), but not all drug-target relationships identified through these methods necessarily translate into therapeutic effect, which presents one of the major limitations in *in silico* research. However, predictive research should apply "quantitative algorithm" to calculate the inhibition rate to the very target but not component-target relationship only; second, components must accumulate to a sufficient concentration around the target in cellular and animal experiments after passing through the cell membrane or even gastrointestinal tract and liver (Manukyan et al., 2019; Luca et al., 2020; Khojah et al., 2021).

Pharmacological research in TCM necessitates both qualitative and quantitative investigations of relationships between parameters in the "formula-herb-component-target-phenotype-*ZHENG*" chain. The relationships among these entities are highly intricate, constituting not one-to-one but rather many-to-many relationships. This complexity is reminiscent of neural networks, which are characterized by extensive intricate connections (Ma et al., 2014). Quantitative studies can be likened to parameters such as weights and biases in a neural network (Lu et al., 2022). In a previous study, the introduction of a novel coefficient aimed to replicate the proportional quantities of components relative to the weight of an herb of interest within a specific formula (Chu et al., 2020). This coefficient also serves to evaluate the pharmacological impact of antiarrhythmic herbal medicine Xin Su Ning capsule across various pertinent biological pathways (Wang et al., 2019b). However, the complex network of quantitative information requires systematic collection in relevant databases to facilitate systems pharmacology research of herbs.

8.3 Upcoming paradigm shifts in TCM pharmacological research

TCM databases offer a wealth of foundational data for pharmacological analyses of complex systems (e.g., formulas). They play pivotal roles in accelerating TCM-based computational science and pharmaceutical research. Moreover, these databases are essential for deciphering the intricate relationships among entities in the formula-herb-component-target-phenotype-*ZHENG* core chain. At present, such databases are primarily used for data retrieval rather than aiding in the discovery of new drugs/formulas or novel pharmacological mechanisms. However, many researchers have begun harnessing the extensive relationships described in databases such as those discussed here to simulate complex formulas. This approach aids in exploration of herb combination patterns (Niu et al., 2023), development of innovative drugs (Li et al., 2010), identification of mechanisms of disease intervention through herb (Gan et al., 2023), and enhancement of clinical research (Zhao et al., 2015; Wang et al., 2021c).

The essence of formulas and medicinal plants is a mixture of compounds. Referring to a single compound, the functionalities of chemical components are determined by their structures (Xiong et al., 2022). Chemical drugs exhibit limited structural diversity and target just over 700 different proteins. The constrained coverage of this chemical space is insufficient to address all modulable or pathological physiological mechanisms that occur in human

disease states (Lipinski and Hopkins, 2004; Reymond, 2015; Stocker et al., 2020). Natural products, which are often referred to as single components in TCM, are numerous and display a wide range of chemical structures (Lachance et al., 2012). This diversity enables them to target a broader spectrum of receptors (Lipinski, 2016). These component therefore represent a valuable repository of potential therapeutic agents (Li et al., 2008). To date, a substantial body of research on TCM formulas has identified the key active components and core mechanisms of action (Zhang et al., 2017a; Wang et al., 2019a; Xu et al., 2020). This information continues to be instrumental in aiding the development of combination drugs composed of multiple components. Once the relationships between a significant number of components combinations and their therapeutic effects are understood, it becomes possible to create new formulas consist of those components based on specific requirements (Keith et al., 2005). This approach can minimize issues related to drug quality control and reduce the costs associated with drug development.

Advances in deep learning and foundation model (Hamet and Tremblay, 2017; Du et al., 2021) indicate that it is increasingly feasible to simulate the complex network encompassing the core chain. The emergence of foundation models is expected to provide tools with precise computational capabilities and entirely new perspectives on pharmacological calculations (Du et al., 2021; Zeng et al., 2022a). Additionally, foundation model-based generative AI has shown immense disruptive potential across various industries, including healthcare and medicine (Singhal et al., 2023b; Xiong et al., 2023). Currently, generative foundation models and medical models fine-tuned based on them have demonstrated strong general capabilities in many medical tasks (Singhal et al., 2023a). They have shown preliminary potential to simulate the corresponding relationships between the entities within the core chain. In the medical field, foundation models can be leveraged to perform various types of tasks, such as extracting key information from electronic health records and analyzing patient symptoms to make disease diagnoses (Xiong et al., 2023). These models can assist in automating data extraction and standardization procedures, leading to a substantial reduction in the time required to establish comprehensive medical databases (Singhal et al., 2023a). The integration of databases related to TCM with foundation models hold significant potential for establishing a knowledge graph in the field of TCM system pharmacology. This integration can enable the generation of knowledge graphs that encompass the relationships between various entities of TCM, formulas, herbs, *ZHENG*, and their pharmacological effects. It can facilitate the development of a question-and-answer system that provides relevant analytical solutions. Furthermore, the expansion of relevant data relationship dimensions in the systems pharmacology database may enhance the depth of computation in foundation models and improve the accuracy of computation. Using foundation model, a dataset comprising a substantial number of effective formulas and the corresponding phenotypes could be used to elucidate the intricate relationships among the entities of the core chain.

In summary, research on databases has made significant and substantial progress in recent times. A vast amount of data related to formula, herb, *ZHENG*, and diseases has been accumulated. A core chain of interrelated relationships has been established, linking the research entities. Furthermore, computational methods are now being employed to simulate and analyze the relationships between entities within this core chain. Currently, while there isn't a single database that can provide computational services to model the complex relationships among all entities in the core chain mentioned above, it's anticipated that with the advancement of technology, this stage is not too far off in the future. However, regarding the existing entities and relationships within the core chain, there are still numerous significant issues that cannot be overlooked. The presence of these issues poses a potential risk of failure in future computational pharmacological research (Sadybekov and Katritch, 2023). Most databases are interconnected resources, and even new databases are often updates or extensions of existing ones, with limited substantive changes to older data. An illustrative example is that some of these databases operate under the principle that if a specific chemical structure demonstrates activity, it's likely to have a similar effect on structurally similar sites. Therefore, we need more and better experiments for evaluation, and literature studies also contain many false positives/false negatives, so it's crucial to maintain clear data sources when incorporating them into the database. For the entire core chain, there is indeed the potential for quantitative calculations, which could enhance the rigor and accuracy of computational research in TCM. It's worth noting that there currently might not be corresponding databases or reports available to support this quantitative approach. It is hoped that in the future, databases will address this issue and foster greater collaboration between different domains, ultimately advancing the modernization and scientific exploration of TCM.

Author contributions

MF: Writing–original draft, Writing–review and editing. CJ: Writing–review and editing, Writing–original draft. DL: Writing–original draft. YD: Writing–original draft. LY: Writing–review and editing. YC: Writing–review and editing. Y-LM: Writing–review and editing. TW: Writing–review and editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was supported by the National Natural Science Foundation of China (82374075 to TW), Taishan Scholar Youth Project of Shandong Province (TW). This work was also supported, in part, by National Natural Science Foundation of China (82274128 to LY), Joint Fund of Shandong Provincial Natural Science Foundation (ZR2021LZY020 to LY).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 16 November 2023
DOI 10.3389/fphar.2023.1281150

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OPEN ACCESS

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RECEIVED 22 August 2023
ACCEPTED 31 October 2023
PUBLISHED 16 November 2023

CITATION
Susilawati E, Levita J, Susilawati Y and Sumiwi SA (2023), Pharmacology activity, toxicity, and clinical trials of Erythrina genus plants (Fabaceae): an evidence-based review.
Front. Pharmacol. 14:1281150.
doi: 10.3389/fphar.2023.1281150

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Pharmacology activity, toxicity, and clinical trials of Erythrina genus plants (Fabaceae): an evidence-based review

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The concept of using plants to alleviate diseases is always challenging. In West Java, Indonesia, a local plant, named *dadap serep* has been traditionally used to reduce blood glucose, fever, and edema, by pounding the leaves and applying them on the inflamed skin, or boiled and consumed as herbal tea. This plant belongs to the Erythrina genus, which covers approximately 120 species. The scope of this review (1943–2023) is related to the Global Development Goals, in particular Goal 3: Good Health and Wellbeing, by focusing on the pharmacology activity, toxicity, and clinical trials of Erythrina genus plants and their metabolites, e.g., pterocarpans, alkaloids, and flavonoids. Articles were searched on PubMed and ScienceDirect databases, using "Erythrina" AND "pharmacology activity" keywords, and only original articles written in English and open access were included. *In vitro* and *in vivo* studies reveal promising results, particularly for antibacterial and anticancer activities. The toxicity and clinical studies of Erythrina genus plants are limitedly reported. Considering that extensive caution should be taken when prescribing botanical drugs for patients parallelly taking a narrow therapeutic window drug, it is confirmed that no interactions of the Erythrina genus were recorded, indicating the safety of the studied plants. We, therefore, concluded that Erythrina genus plants are promising to be further explored for their effects in various signaling pathways as future plant-based drug candidates.

KEYWORDS

anti-inflammatory, Erythrina genus, flavonoids, pterocarpans, toxicity

1 Introduction

Discovering herbal-based drugs is always challenging. Plants' metabolites, e.g., flavonoids and polyphenols, have been reported for their role in representing pharmacological activities (González et al., 2011; Rosdianto et al., 2020; Stromsnes et al., 2021).

In West Java, Indonesia, a local plant, named *dadap serep*, has been traditionally used to reduce blood glucose, fever, and edema. The leaves are usually pounded and applied to the inflamed skin, or boiled and consumed as herbal tea. In order to understand the reason this plant exhibits the ability to cure diseases, we search for scientific information related to the plant. *Dadap serep*, botanical name *Erythrina subumbrans* (Hassk.) Merr.) belongs to the Erythrina genus of the family Fabaceae. The Erythrina genus covers approximately

120 species worldwide. *Erythrina subumbrans* (Hassk.) Merr.) (Fabaceae) trees are large, up to 22 m tall. The leaves are 3-pinnated, with leaf blades almost round to rhombic shape, rounded base, larger tip, with a flat edge. These plants are widely grown in the Southeast Asia region (http://ipbiotics.apps.cs.ipb.ac.id/index.php/tumbuhanObat/297). The native range of this species is from China (Yunnan) to Tropical Asia and Southwest Pacific (https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:494601-1#source-KBD).

This article aims to review the pharmacology activities, *in silico* studies, toxicity studies in animal models, and clinical trials of the Erythrina genus plants, to ensure the safety of their administration to humans.

2 Radical scavenging activity of the phytochemicals

The Erythrina genus of the family Fabaceae includes trees, shrubs, and herbaceous plants with orange to bright red flowers, rich in pterocarpans and alkaloids (Rukachaisirikul et al., 2007a; Phukhatmuen et al., 2021), flavonoids (Nyandoro et al., 2017; Deviani et al., 2022; Simão et al., 2022), triterpenes, steroids, alkyl trans-ferulates, proteins, saponins, and lecithin (Kumar et al., 2010), gallic and caffeic acids (Muthukrishnan et al., 2016). Pterocarpans comprise a large group of isoflavonoids and function as phytoalexins (plant antimicrobial mechanism) and are mostly found in the Leguminosae (Fabaceae) family (Jiménez-González et al., 2011). Flavonoids are low-molecular-weight polyphenolic metabolites in plants that are further classified into subgroups, e.g., flavones, flavonols, isoflavones, chalcones, and anthocyanins (Panche et al., 2016). Pterocarpans, flavonoids, and alkaloids are considered responsible for Erythrina's pharmacology activities.

The radical scavenging activity of Erythrina genus plants has been verified by employing various methods, such as ferrous reducing antioxidant capacity (FRAC) (Afzal et al., 2022), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical (Dzoyem et al., 2014; Deviani et al., 2022; Fofana et al., 2022), and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) diamonium salt (ABTS) (Dzoyem et al., 2014).

The ethyl acetate fraction and lupinifolin, a flavanone isolated from *E. crista-galli* L. twigs collected in Cihideung, West Java, Indonesia, have confirmed its capability to scavenge 50% of the initial DPPH radicals with a value of 64.41 and 128.64 μg/mL, respectively, better than the other flavanones, e.g., citflavanone and lonchocarpol A. It was explained that the prenyl and the pyran in lupinifolin contribute to this capability (Deviani et al., 2022). Silver nanoparticles (Ag-NPs) synthesized from the leaf and bark extracts of *E. suberosa* Roxb. exhibited significant reducing activity towards ferric chloride solution (Afzal et al., 2022). The radical scavenging activity of *E. caffra* Thunb. (leaves) (Dzoyem et al., 2014) and *E. senegalensis* DC. (stem bark) (Fofana et al., 2022) were also delineated. Moreover, a previous *in silico* approach disclosed that the radical scavenging activity of Erythrina's flavanone, namely, mildbone and mildbenone, is predominantly associated with the second bond dissociation enthalpy of a second hydrogen atom transfer (Anouar, 2016). Mildbone and mildbenone, polar metabolites in Erythrina, were suggested to contribute to the various biological activities of Erythrina plants (Jiménez-Cabrera et al., 2020).

3 Pharmacology activities

Most of the studies described Africa and Brazil as the origin locations of Erythrina plants (Fabaceae). The scope of articles was those published from 1943 to 2023 (depicted in Figure 1).

Of these articles, we found 16 species with confirmed pharmacology activities of which are:

- *E. abyssinica* Lam. ex DC. (synonym = *Carallodendron suberifera* (Welw. ex Baker) Kuntze or *Chirocalyx abyssinicus* (Lam. ex DC.) Hochst. or *Chirocalyx tomentosus* Hochst. or *E. bequaertii* De Wild. or *E. comosa* Hua or *E. eggelingii* Baker f. or *E. huillensis* Welw. ex Baker or *E. kassneri* Baker f. or *E. mossambicensis* Sim or *E. pelligera* Fenzl or *E. platyphylla* Baker f. or *Erythrina suberifera* Welw. ex Baker or *E. tomentosa* R. Br. ex A. Rich. or *Erythrina warneckei* Baker f.)
- *E. addisoniae* Hutch. & Dalziel.
- *Erythrina americana* Mill. (synonym = *Corallodendron americanum* (Mill.) Kuntze or *Corallodendron coralloides* (Moc. & Sessé ex DC.) Kuntze or *Corallodendron roseum* (A.Dietr.) Kuntze or *E. carnea* Aiton or *E. coralloides* Moc. & Sessé ex DC. or *E. enneandra* DC. or *E. fulgens* Loisel. or *E. laeta* Dehnh. or *E. rosea* A.Dietr.)
- *E. burttii* Baker f.
- *E. caffra* Thunb. (synonym = *E. constantiana* Micheli or *E. fissa* C.Presl or *E. insignis* Tod. or *Erythrina viarum* Tod.
- *E. crista-galli* L. (synonym = *Corallodendron crista-galli* (L.) Kuntze or *E. fasciculata* Benth. or *E. laurifolia* Jacq. or *E. pulcherrima* Tod. or *E. speciosa* Tod. or *Micropteryx crista-galli* (L.) Walp. or *M. fasciculata* Walp. or *M. laurifolia* Walp.)
- *E. falcata* Benth. (synonym = *Corallodendron falcatum* (Benth.) Kuntze or *E. crista-galli inermis* Speg. or *E. martii* Colla)
- *E. lysistemon* Hutch., Kew Bull. (synonym = *E. caffra mossambicensis* Baker f.)
- *E. mildbraedii* Harms (synonym = *E. altissima* A.Chev. or *E. klainei* Pierre ex Harms or *E. problematica* P.A.Duvign. & Rochez)
- *E. senegalensis* DC. (synonym = *E. guineensis* G.Don or *E. latifolia* Schumach.)
- *E. sigmoidea* Hua. (synonym = *E. dybowskii* Hua or *E. eriotricha* Harms or *E. lanata* Taub. ex Gilg or *E. sudanica* Baker f.)
- *E. suberosa* Roxb. (synonym = *Corallodendron suberosum* (Roxb.) Kuntze or *E. alba* Wight & Arn. or *E. glabrescent* (Prain) R.Parker or *E. maxima* Wight & Arn. or *E. stricta suberosa* (Roxb.) Niyomdham or *E. sublobata* Roxb. or *M. suberosa* (Roxb.) Walp. or *M. sublobata* (Roxb.) Walp.)
- *E. subumbrans* (Hassk.) Merr. (synonym = *Corallodendron lithospermum* (Blume ex Miq.) Kuntze or *E. holoserica* Kurz or *E. lithosperma* Miq. or *E. secundiflora* Hassk. or *E. sumatrana* Miq.)

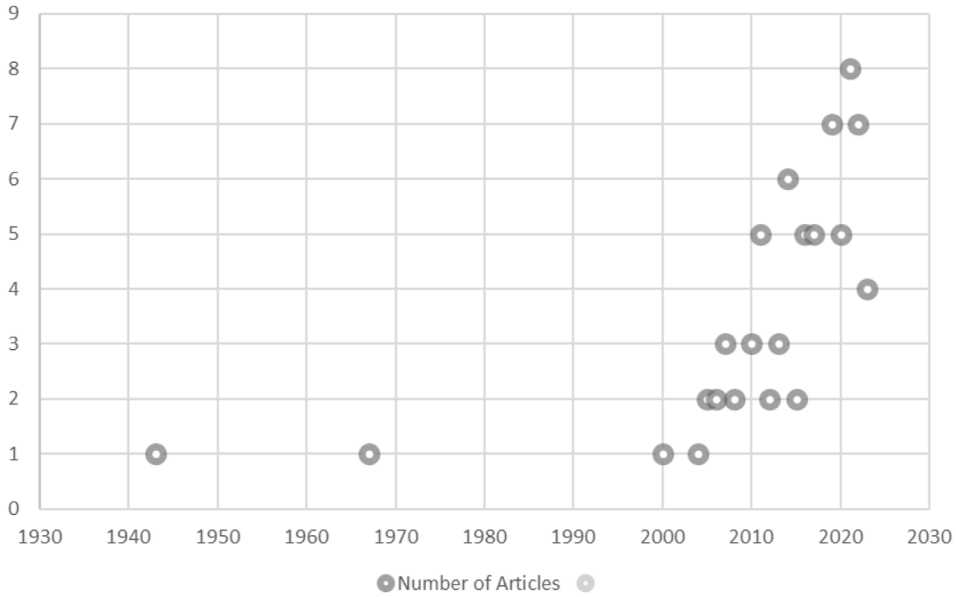

FIGURE 1
Scattered diagram of the number of reviewed articles plotted against the year of publication.

- *E. variegata* Linn. (synonym = *E. indica* Lam., or *E. orientalis* (L.) Merrill, or *E. corallodendrum* var. *orientalis* L.)
- *E. velutina* Willd. (synonym = *Chirocalyx velutinus* Walp. or *Corallodendron velutinum* (Willd.) Kuntze or *E. aculeatissima* Desf. or *E. aurantiaca* Ridl. or *E. splendida* Diels)
- *E. verna* Vell. (synonym = *Corallodendron mulungu* (Mart. ex Benth.) Kuntze or *E. mulungu* Mart. ex Benth. or *E. flammea* Herzog)

The most studied activities were antibacterial and anticancer, although a few works reported anti-inflammatory, antidiabetic, estrogenic, antifungal, antidepressant, and other activities. The stem bark was the most explored and considered an active part of the Erythrina genus plants, followed by the leaves, twigs, root bark, and seeds. The pharmacology activities of the Erythrina genus plants are summarized in Supplementary Table S1.

3.1 Antibacterial activity

Antibacterial activity was confirmed to belong to *E. subumbrans* (Hassk.) Merr. (twigs and roots) (Phukhatmuen et al., 2021), *E. verna* Vell. (stem bark) (Simão et al., 2022), *E. suberosa* Roxb. (leaves and bark) (Afzal et al., 2022), *E. lysistemon* Hutch., Kew Bull. (stem bark) (Sadgrove et al., 2020), *E. poeppigiana* (Walpers) O.F. Cook (leaves) (Dzoyem et al., 2014). A notable antibacterial activity of plant extracts is an auspicious result since extracts contain a mixture of metabolites (Dzoyem et al., 2014). It was described previously that the prenylation of flavonoids increases the ability to penetrate the membrane cell of bacteria by building a strong lipophilic arm to the molecule. An OH group attached to the same aromatic ring as the prenyl strengthens the antibacterial activity (Sadgrove et al., 2020). Alkaloids, a group of nitrogen-containing organic compounds, were reported for their cell membrane-damaging mechanism. Alkaloids could interfere with the energy metabolisms thus inhibiting bacterial growth (Djeussi et al., 2015; Sadgrove et al., 2020; Alencar de Barros et al., 2021).

Antibacterial mechanisms of plant metabolites may include, but are not restricted to, the inhibition of bacterial cell wall synthesis, alteration of the permeability of the cell membrane permeability, and/or blocking of the bacterial synthesis of protein. Implying this, Erythrina plants, that contain flavonoids and alkaloids, may have the potential to kill Gram-positive and Gram-negative bacteria (Dzoyem et al., 2014; Djeussi et al., 2015; Sadgrove et al., 2020; Alencar de Barros et al., 2021; Phukhatmuen et al., 2021; Afzal et al., 2022; Simão et al., 2022; Yenesew et al., 2005).

3.2 Anticancer activity

The anticancer activity of the Erythrina genus plants has also been validated and reported in 7 articles against different cancer cell lines. The ethyl acetate subfraction of *E. senegalensis* DC. stem bark exhibited anticancer activity against U373, MCF-7, A549, SKMEL-28, and B16F10 cells, despite its lower phenolic and flavonoid content (Fofana et al., 2022). Flavanones isolated from the leaves of *E. crista galli* L. were evaluated using the *Arabidopsis thaliana* pER8: GUS reporter assay while their molecular interactions with the residues Arg394, Glu353, and His524 in the active site of human estrogen receptor R (ERR) were studied, thus revealing their anticancer activity (Ashmawy et al., 2016). The essential oils contained in the leaves of *E. variegata* Linn. blocked the proliferation of breast cancer (MDA-MB-231 and MCF-7) cells and non-cancerous mammary epithelial cells (HMLE) (Xing et al., 2019). Another *in vitro* study of *E. suberosa* Roxb. Stem bark described that two isolated metabolites, namely, 4′-methoxy

licoflavanone, and alpinumisoflavone, inhibited HL-60 cell proliferation and induced apoptosis (Kumar et al., 2013). Alpinumisoflavone isolated from the stem barks of *E. lysistemon* reduced the viability and induced the apoptosis of human lung cancer cell lines H2108 and H1299 (Namkoong et al., 2011). Prenylated pterocarpans isolated from the stem bark of *E. addisoniae* Hutch. & Dalziel revealed weak to moderate toxicity towards H4IIE hepatoma cells and decreased activation of the ERK kinase (p44/p42) (Wätjen et al., 2007). Xanthoxyletin isolated from the aerial part of *E. variegata* L. induced apoptosis and cell cycle arrest in human gastric adenocarcinoma SGC-7901 cells (Rasul et al., 2011).

Taken together, apoptosis in various cancer cells may be considered the main mechanism of Erythrina metabolites. Apoptosis, identified by cell shrinkage and DNA fragmentation, is structured by a series of molecular reactions that could be stimulated extrinsically or intrinsically. Apoptosis is activated by caspases (cysteine-aspartic proteases) as effectors of the process (Hail et al., 2006). The intrinsic pathway of apoptosis is initiated when internal cellular stress occurs due to DNA damage or endoplasmic reticulum stress, which eventually activates the BCL-2 homology 3 protein. The extrinsic pathway is activated when death receptors (DR1-DR8) are induced by receptor binding or aggregation (Cavalcante et al., 2019).

3.3 Antidiabetic activity

Metabolites isolated from the twigs and roots of *E. subumbrans* (Hassk.) Merr. were confirmed to inhibit α-glucosidase activity and/or α-amylase activity (Phukhatmuen et al., 2021). Prenylated flavonoids isolated from the root bark of *E. mildbraedii* Harms inhibited PTP1B activity with IC_{50} values ranging from 5.3 to 42.6 mM as reported by Jang and co-workers in Korea. The assay was carried out using a human recombinant PTP1B kit. There is very little information regarding the pharmacology activity and mechanism, and no discussion has been provided since this work was presented as Notes (Jang et al., 2008). PTP1B, a tyrosine phosphatase protein, plays important contributions in intracellular signal transduction by regulating the cellular level of tyrosine phosphorylation. It downregulates insulin signaling by dephosphorylating the insulin receptor (IR) and insulin receptor substrate (IRS), thus dysfunction of this protein may lead to numerous ailments such as cancer, diabetes, and obesity (Sun et al., 2012).

An alteration in insulin signaling, predominantly in the IRS/phosphoinositide-3-kinase (PI-3K)/protein kinase B (PKB) axis, stimulates the development of insulin resistance. Insulin resistance is generally associated with obesity, which is a risk factor for type 2 diabetes mellitus (T2DM) (Wondmkun, 2020). Mature insulin is stored in granules until it is needed to be released when blood glucose levels are elevated, e.g., following a high carbohydrate intake. Several factors may also activate insulin release, e.g., amino acids, fatty acids, and hormones (Boland et al., 2017). The occurrence of insulin resistance in T2DM patients may also be caused by an abnormal synthesis of insulin, a mutation of insulin receptors, or the presence of an insulin antagonist (Al-Ishaq et al., 2019).

Interestingly, flavonoids are thought to play an important role in regulating carbohydrate hydrolysis, as well as insulin signaling and secretion (Al-Ishaq et al., 2019). It was announced that dietary intake of anthocyanins and anthocyanin-rich fruit (blueberries) was correlated with a lower risk of T2DM in both males and females (Wedick et al., 2012). A reduction in IL-6 and TNF-α was observed following the intake of different berries, suggesting an important role of these fruits in modulating the lipid profile and inflammatory cytokines in subjects with metabolic diseases (Venturi et al., 2023).

3.4 Anti-inflammatory activity

The anti-inflammatory activity of Erythrina plants was also reported, although limited. We found 4 articles that described the anti-inflammatory activity by various mechanisms as follows:

The fractions obtained from the dichloromethane stem bark extract of *E. verna* Vell have confirmed their inhibitory activity on nitric oxide (NO) production and blocked TNF-α activity. This *in vitro* study was performed on a murine RAW 264.7 macrophage cell line and the quantification of NO and TNF-α was done using a commercially enzyme-linked immunosorbent assay kit. In their study, Simão and others employed a bioassay-guided procedure, which resulted in two active flavonoid metabolites, namely, alpinumisoflavone and erythratidinone (Simão et al., 2022).

Previously, Machado and his co-workers studied the purification, biochemical characterization, and anti-inflammatory evaluation of a novel Kunitz trypsin inhibitor from *E. velutina* Willd. Seeds. The metabolites were purified by $NH_4(SO_4)_2$, fractionated, and eventually underwent trypsin-sepharose affinity chromatography and an RP-HPLC. It was confirmed that the metabolites isolated from the seeds of *E. velutina* Willd. Strongly inhibited trypsin by a non-competitive mechanism, reduced leukocyte migration, decreased TNF-α release, and stimulated IFN-α and IL-12 production, thus indicating a promising anti-inflammatory activity (Machado et al., 2013).

The leaves and bark extract of *E. abyssinica* Lam. ex DC. ointments exhibited low anti-inflammatory and wound-healing properties. It was reported by Marume and the research team that wounds of animals treated with the extract ointments revealed almost similar recovery, although not significantly different, to those of animals treated with 3% oxytetracycline ointment (Marume et al., 2017).

The ethanol extract of *E. senegalensis* DC. leaves were assayed *in vivo* using egg albumin-induced paw edema in the rat model and resulted in a strong activity in stabilizing the erythrocyte membrane, and significantly decreased albumin denaturation, platelet aggregation, phospholipase A2 and protease activity (Enechi et al., 2021). Alkaloids isolated from the bark of *Erythrina crista-galli* L., purchased in September 2004 in São Paulo, Brazil, inhibit the production of nitric oxide in RAW264.7 cells (Ozawa et al., 2010). There are numerous complex mechanisms and signaling pathways related to inflammation, thus these reports may not depict the whole picture of how the botanical drugs suppress the inflammatory process. Moreover, it should be noted that different assay methods may notably affect the results.

3.5 Other pharmacology activities

Erythrina plants have also been explored for other activities, e.g., estrogenic activity by increasing uterus and vaginal index and keratinizing vaginal cells, inducing uterine and vaginal hypertrophy associated with endometrial proliferation (Li et al., 2019), antiviral against respiratory syncytial virus (RSV) and herpes simplex virus (HSV-2) (Mollel et al., 2022), strong antifungal activities against *Candida albicans* and *C. glabrata* strains and clinical isolates (Harley et al., 2022), Leishmanicidal activity against the promastigote forms of *L. amazonensis* (Callejon et al., 2014), anxiolytic and anti-depressant (Chu et al., 2019), bone protective effect in the tibia of ovariectomized rats (Xiao et al., 2021), antihepatotoxicity by reducing the levels of transaminases, ALP, bilirubin, and LDH to normal (Mujahid et al., 2017), retention of fear memory but did not prevent the extinction of fear memory (de Oliveira et al., 2014), Aurora kinase inhibitor (Hoang et al., 2016), antiplasmodial (Rukachaisirikul et al., 2007b; Waiganjo et al., 2020), inhibitor of neuronal nicotinic receptors (Setti-Perdigão et al., 2013).

To obtain the total active metabolites that contribute to the pharmacology activities of Erythrina genus plants, we further tabulated these metabolites and their studies on different pharmacological assays in Supplementary Table S2.

4 *In silico* study

Regrettably, phenolic metabolites of the Erythrina genus plants have been very limitedly studied for their interaction with various proteins. However, the limited studies are described below.

Phenolic metabolites of *Erythrina* × *neillii* (a cross-hybrid between *E. herbacea* L. and *E. humeana* Spreng), were reported for their interaction with Arg136, Met34, and Gly139 of heme oxygenase-1 (HO-1), a macromolecule that plays a role in cellular protection and oxidative catabolism of heme. Stimulation of HO-1 may reduce cardiovascular disease (Gabr et al., 2019). Phaseollin of *E. variegata* L. built interaction with significant fitness score and hydrogen bonds with Tyr354 and Ser499 residues in both COX-1 and COX-2, which is considered a potent analgesic candidate (Uddin et al., 2014). Tyr354 is one of the key residues constituting the active site of human COX-1, along with Arg119, Tyr384, Ile433, His512, Phe517, and Ile522 (Dhanjal et al., 2015). Polyphenolic metabolites isolated from *E. crista-galli* L. could interact with Arg394, Glu353, and His524 residues of the human estrogen receptor R (ERR), thus revealing a promising phytoestrogenic activity (Ashmawy et al., 2016). Moreover, diprenylgenistein and phaseollin isolated from the twigs of *E. crista-galli* L. showed the highest binding affinity towards CDK2 protein, revealing its anticancer activity (Imanuddin et al., 2023). Cyclin-dependent kinases (CDKs) are proteins that regulate the transition of cell cycle phases. Cyclin E binds and activates CDK2 to promote S-phase entry and progression. Cyclin E/CDK2 complex eventually phosphorylates various substrates to control essential cellular processes (Fagundes and Teixeira, 2021).

There is no report on the *in silico* study of pterocarpans of the Erythrina genus plants, thus unlatching the chance to be further investigated. However, a few articles reported *in silico* studies of pterocarpans from other plants.

One article reported that pterocarpans isolated from *Desmodium gangeticum* could interact with tyrosine phosphate kinase similarly to the protein's inhibitors (Meena et al., 2022). Protein kinases are responsible for the phosphorylation process to activate the function of other proteins. Inhibitors of kinases interact in different modes depending on the specific binding sites and conformational aspects of the enzyme, which are determined by two conditions, e.g., (1) the Asp-Phe-Gly (DFG) motif, as part of the activation loop, and (2) the αC-helix. Most kinase inhibitors interact in the active form (conformational state: DFG-in, αC-helix-in), and are classified as type I inhibitors. Type II inhibitors attach to the inactive state (conformational state: DFG-out, αC-helix-out) (Abdelbaky et al., 2021). It is interesting to explore the binding modes of pterocarpans to kinases and whether these compounds are categorized as type I or type II inhibitors.

Pterocarpans isolated from *Indigofera aspalathoides* were reported could interact with Ser530 and Tyr385 in COX-1 and COX-2 binding pockets. The interaction with Ser530 is believed to be important in inhibiting the catalytic process of these enzymes (Selvam et al., 2004). In the biosynthesis of prostaglandin H2 (PGH2), the substrate of COX, namely, arachidonic acid, has to enter the cyclooxygenase narrow channel with its carboxylate group attaches (by building hydrogen bonds) to Arg120 and Tyr355, two residues located at the channel opening, and its ω-end occupies in a hydrophobic cavity at upwards position of Ser530 (Malkowski et al., 2000; Vecchio et al., 2010). It has been confirmed that acetylation of Ser530 by acetylsalicylic acid could inhibit the synthesis of prostaglandins (Giménez-Bastida et al., 2019).

Another *in silico* study confirmed that pterocarpans contained in *Sophora flavescens* interact with neuraminidase by occupying a binding pocket adjacent to the active site. The methanol extract of this plant at 30 ppm reduced neuraminidase activity by 90% (Ryu et al., 2008).

5 Toxicity studies

In fact, there are only very limited toxicity studies on Erythrina genus plants.

An acute toxicity study of *E. senegalensis* DC. stem extract in mice (*Mus musculus*) with doses of 1,250 to 12,500 mg/kg body weight (BW) did not induce mortality or significant behavioral changes. In a sub-chronic toxicity study in rats (*Rattus norvegicus*), daily oral administration of *E. senegalensis* DC. stem extract at the dose of 600 mg/kg BW resulted in a significant elevation in the relative BW but no significant alterations in the hematological parameters, suggesting its safety (Atsamo et al., 2011).

Chronic oral toxicity studies of *E. mulungu* Mart. ex Benth. Mart. ex Benth. (synonym = *E. verna* Vell. or *Corallodendron mulungu* (Mart. ex Benth.) Kuntze or *E. flammea* Herzog) stem bark extract in mice (*Mus musculus*) at a dose of 1,000 mg/kg BW resulted in alterations of biochemical parameters of the liver and cardiovascular functions. Hematological analysis indicated significant decreases in the red blood cells. Oral daily doses of

TABLE 1 Toxicity studies of the Erythrina genus plants.

Plant extract	Toxicity study	Animal model	Results and doses	Reference
E. senegalensis DC. aqueous stem extract was prepared from the stem bark (collected in Dschang, West region of Cameroon, Africa, in February 2007)	Acute	Mice (*Mus musculus*)	• No mortality	Atsamo et al. (2011)
			• No behavioral changes at doses of 1,250–12,500 mg/kg BW which indicated the safety of the plant extract	
	Sub-chronic	Rats (*Rattus norvegicus*)	• A significant elevation in the relative BW	
			• No significant alterations in the hematological parameters	
			• Doses of ≥600 mg/kg BW did not cause mortality which indicated the safety of the plant extract	
E. mulungu Mart. ex Benth. Stem bark extract	Chronic	Mice (*Mus musculus*)	• Alterations of biochemical parameters of the liver and cardiovascular functions at a dose of 1,000 mg/kg BW	Adeneye (2014)
			• Alterations of hematological analysis (significant decreases in the red blood cells)	
			• Increases the relative weights of the liver and heart at doses of 500 mg/kg BW and 1,000 mg/kg BW	
			• Increases the serum MDA levels and lipid peroxidation marker at doses of 500 mg/kg BW and 1,000 mg/kg BW	
			• Significantly changes the histopathological of myocardial and degeneration of hepatocytes	
			• A dose of 1,000 mg/kg BW did not cause mortality which indicated the safety of the plant extract	
E. variegata Linn. methanolic leaves extract was prepared from the leaves (collected in Sumedang, West Java, Indonesia)	Sub-chronic	Male Wistar rats (*Rattus norvegicus*)	• Slight changes in hematological parameters but were within the normal ranges	Herlina et al. (2017)
			• Increase of BUN, SGOT, and SGPT	
			• Higher doses increased damage to the liver and kidney cells	
			• Doses of 250, 500, and 1,000 mg/kg BW did not cause toxicity in animal models	
			• A dose of 1,000 mg/kg BW did not cause mortality which indicated the safety of the plant extract	

500 and 1,000 mg BW of *E. mulungu* Mart. ex Benth. Stem bark extract increased the relative weights of the liver and heart and serum malondialdehyde levels. Significant histopathological changes in myocardial and degeneration of hepatocytes also occurred (Adeneye, 2014).

A sub-chronic toxicity study of the methanol extract of *E. variegata* Linn. leaves on male Wistar rats (*Rattus norvegicus*) revealed slight changes in hematological parameters but were within the normal range, except for BUN and SGPT. Histopathological examination indicated increased damage to the liver and kidney cells, however, doses of 250, 500, and 1,000 mg/kg BW did not cause toxicity in animal models (Herlina et al., 2017). Table 1 tabulates the toxicity studies of the Erythrina plants.

The toxicity of the metabolites contained in the Erythrina genus plants was not commonly reported. However, an article written by Unna and Greslin explained that erythroidine, an alkaloid isolated from *Erythrina americana* Mill., and other alkaloids, namely, 9-erythroidine, erythramine, erythraline, and erythratine revealed a curarizing effect similarly to that of the crude seed extracts (Unna and Greslin, 1943). It was pointed out that the curarizing effect of a drug changes the electrical activity of the cerebral cortex chiefly due to hypoxia and hypotension (Skorobogatov, 1967). The other major metabolites such as flavonoids and pterocarpans have been proven as safe.

6 Clinical study

There are very limited studies on the efficacy and safety of the Erythrina genus plants in humans. We found only 2 articles, both using *E. mulungu* Mart. ex Benth. (synonym = *E. verna* Vell. or *Corallodendron mulungu* (Mart. ex Benth.) Kuntze or *E. flammea* Herzog) as an antianxiety in patients who underwent dental surgery.

Study 1. A randomized, triple-blind, clinical control trial was performed on 200 patients who underwent oral surgery of their

third mandibular molars (consort number NCT02065843). The patients were treated with either *Passiflora incarnata* (500 mg), *E. mulungu* Mart. ex Benth. (500 mg), or midazolam (15 mg) orally 60 min prior to the surgery. The anxiety level of the patients was analyzed using questionnaires and measurements of heart rate, blood pressure, and oxygen saturation (SpO2). It was reported that *P. incarnata* revealed a similar effect to midazolam but differed from placebo and *E. mulungu* Mart. ex Benth., indicating that *E. mulungu* could not be used to reduce the anxiety of the patients (da Cunha et al., 2021).

Study 2. A similar randomized, double-blind, crossover study was performed on 30 healthy adult patients who underwent bilateral surgery of asymptomatic, impacted mandibular third molars (consort number NCT01948622). The patients were treated with either *E. mulungu* Mart. ex Benth. (500 mg) or a placebo (500 mg) orally 60 min before the surgery. To avoid pain and swelling after surgery, the patients were given a single dose of intra-muscular dexamethasone and an antiseptic mouthwash containing chlorhexidine gluconate 30 min before surgery. The level of anxiety was analyzed using questionnaires and measurements of blood pressure, heart rate, and oxygen saturation. It was reported that *E. mulungu* Mart. ex Benth. had shown an anxiolytic effect without significant changes in physiological parameters (Silveira-Souto et al., 2014).

7 Botanical drug-drug interaction

Extensive caution should be considered when prescribing botanical drugs for patients parallelly taking a narrow therapeutic window drug. The botanical drugs may dangerously alter the main drug or cause ineffectiveness. Taking this into consideration, we studied the Herb-Drug Interaction Chart (Mills and Bone, 2005; ESCOP, 2023; Standard Process, 2023) and found that no interactions of the Erythrina genus were recorded, indicating the safety of the studied plants.

8 Conclusion

There are 16 of the 120 species of the Erythrina genus plants with confirmed pharmacological activities. Antibacterial and anticancer were the most studied activities, although a few works reported anti-inflammatory, antidiabetic, estrogenic, antifungal, antidepressant, and other activities. The extracts of the Erythrina genus have revealed anticancer activity against human glioblastoma astrocytoma (U-373), human breast cancer cell line with estrogen, progesterone, and glucocorticoid receptors (MCF-7), human epithelial breast cancer (MDA-MB-231), human melanoma (SKMEL-28), murine melanoma (B16F10), human promyelocytic leukemia (HL-60), lung cancer (H2108, H1299, H411E), human adenocarcinoma alveolar basal epithelial (A549), and human gastric cancer (SGC-7901) cells. Studies of the effect of these plant extracts on non-cancerous cells are limited, nevertheless, a study reported that the Erythrina plant extract did not show cytotoxicity on HMLE (immortalized human mammary epithelial), a non-cancerous cell.

The stem bark was the most explored and considered the main active part of the Erythrina genus plants. Sixty-three metabolites have been isolated from various parts of the plants, comprised of pterocarpans, flavonoids, alkaloids, triterpenoids, glycosides, steroids, chromenes, coumarins, and carboxylic acids. The most abundant classes are pterocarpans and flavonoids.

There are very limited toxicity studies on Erythrina genus plants, among those are *E. senegalensis* DC., *E. mulungu* Mart. ex Benth. (synonym = *E. verna* Vell. or *Corallodendron mulungu* (Mart. ex Benth.) Kuntze or *E. flammea* Herzog), and *E. variegata* Linn. All these studies indicated the safety of the higher doses of the plant extracts in animal models. The toxicity of the metabolites contained in the Erythrina genus plants was not commonly reported. However, alkaloids isolated from *E. americana* Mill. namely, erythroidine, 9-erythroidine, erythramine, erythraline, and erythratine were reported for their curare-like effect on frogs.

There are very limited human studies on Erythrina genus plants. Only one species, *E. mulungu* Mart. ex Benth. has been reported as a weak anxiolytic in dental surgery in randomized, controlled clinical trials. It should be noted that the selected articles have employed different experimental designs.

Most of the pharmacological studies were carried out on plant extracts, not as nutraceuticals or supplements, or pure metabolites, and almost all studies reported significant statistical analysis results. Not all articles described their procedures in detail, thus affecting the reliability of the studies and limiting our interpretations. Nonetheless, we feel optimistic that the Erythrina genus plants are promising to be further explored for their effects in various signaling pathways as future plant-based drug candidates. We are certain this review has unraveled comprehensive perspectives of Erythrina genus plants thus providing beneficial insights into plant-based drug discovery.

Author contributions

ES: Formal Analysis, Writing–original draft. JL: Conceptualization, Methodology, Supervision, Writing–review and editing. YS: Formal Analysis, Supervision, Writing–original draft. SS: Conceptualization, Supervision, Writing–original draft.

Funding

Financial support for this study is facilitated by Universitas Padjadjaran via the Directorate of Research and Community Engagement.

Acknowledgments

The authors thank the Rector of Universitas Padjadjaran, West Java, Indonesia for facilitating the APC of this article. The present study was conducted in the framework of the dissertation of the first author at the Faculty of Pharmacy, Universitas Padjadjaran, West Java, Indonesia.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2023.1281150/full#supplementary-material

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frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 01 December 2023
DOI 10.3389/fphar.2023.1272241

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OPEN ACCESS

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RECEIVED 03 August 2023
ACCEPTED 20 November 2023
PUBLISHED 01 December 2023

CITATION
Wu P, Wu W, Zhang S, Han J, Liu C, Yu H, Chen X and Chen X (2023), Therapeutic potential and pharmacological significance of extracellular vesicles derived from traditional medicinal plants. *Front. Pharmacol.* 14:1272241. doi: 10.3389/fphar.2023.1272241

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Therapeutic potential and pharmacological significance of extracellular vesicles derived from traditional medicinal plants

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Medicinal plants are the primary sources for the discovery of novel medicines and the basis of ethnopharmacological research. While existing studies mainly focus on the chemical compounds, there is little research about the functions of other contents in medicinal plants. Extracellular vesicles (EVs) are functionally active, nanoscale, membrane-bound vesicles secreted by almost all eukaryotic cells. Intriguingly, plant-derived extracellular vesicles (PDEVs) also have been implicated to play an important role in therapeutic application. PDEVs were reported to have physical and chemical properties similar to mammalian EVs, which are rich in lipids, proteins, nucleic acids, and pharmacologically active compounds. Besides these properties, PDEVs also exhibit unique advantages, especially intrinsic bioactivity, high stability, and easy absorption. PDEVs were found to be transferred into recipient cells and significantly affect their biological process involved in many diseases, such as inflammation and tumors. PDEVs also could offer unique morphological and compositional characteristics as natural nanocarriers by innately shuttling bioactive lipids, RNA, proteins, and other pharmacologically active substances. In addition, PDEVs could effectively encapsulate hydrophobic and hydrophilic chemicals, remain stable, and cross stringent biological barriers. Thus, this study focuses on the pharmacological action and mechanisms of PDEVs in therapeutic applications. We also systemically deal with facets of PDEVs, ranging from their isolation to composition, biological functions, and biotherapeutic roles. Efforts are also made to elucidate recent advances in re-engineering PDEVs applied as stable, effective, and non-immunogenic therapeutic applications to meet the ever-stringent demands. Considering its unique advantages, these studies not only provide relevant scientific evidence on therapeutic applications but could also replenish and inherit precious cultural heritage.

KEYWORDS

traditional medicinal plants, extracellular vesicles, lipids, pharmacology, therapy, tumor, inflammation

1 Introduction

Extracellular vesicles (EVs), which are biological membrane-bound vesicles of 40–150 nm, are secreted by all mammalian cells (Colombo et al., 2014; Peng et al., 2020). Despite being initially considered as cellular debris and therefore underestimated, EVs are now recognized as significant factors in controlling numerous physiological and

pathological mechanisms in multicellular organisms. Further investigation has revealed that these EVs are surrounded by a double layer of fats and proteins, containing various functional nucleic acids, lipids, proteins, RNAs, and other elements. Although most EVs share analogical structures, the uptake and signal transduction capacity primarily rely on the heterogeneity of their source and components. Due to these distinctive features, EVs play a vital role in facilitating intercellular communication and the transfer of information and exchange of materials in both prokaryotes and higher eukaryotes. Since their first discovery in the 1980s, mammalian extracellular vesicles (MEVs) have been extensively researched, and their biogenesis mechanisms and functions of mediating intercellular transmission have been gradually elucidated (Colombo et al., 2014; Abels and Breakefield, 2016; Isaac et al., 2021). For example, the arrangement of lipids in a bilayer enables MEVs to trap both water-loving and water-fearing molecular substances. Moreover, MEVs have also been reported to have remarkable specific targeting capabilities (Shao et al., 2020; Wu et al., 2022; Zhang et al., 2022). Intriguingly, EVs possess physiological activity and have the ability to cross bio-membrane barriers, evade phagocytosis by the reticuloendothelial system, and extend the lifespan of drugs (Duan et al., 2021; Nguyen and Fuhrmann, 2022; Xie et al., 2023). Although they exist in the nanodomain, EVs are considered low toxicity and capable of decomposing. As a result, extensive research has been conducted on EVs in diverse scientific fields, such as drug delivery systems, nutrition, disease treatment, and clinical diagnosis. Furthermore, EVs have proven effective in treating cardiovascular diseases, cancer, and neurodegenerative diseases and facilitating tissue repair (Cheng and Hill, 2022; Zhou et al., 2022). For instance, Qiao et al. developed a composite EVs based on HT1080 cells, which enclosed doxorubicin, leading to a substantial increase in the concentration of doxorubicin in the tumor tissue and augmenting its effectiveness against tumor cells (Qiao et al., 2020). In addition, MEVs are also applied to deliver vulnerable reagents, such as nucleic acid and lipids. According to recent research, it has been proved that the EVs obtained from dendritic cells contained siRNA, effectively reducing the expression of beta-site amyloid precursor protein cleaving enzyme-1 (Alvarez-Erviti et al., 2011). Moreover, EVs originating from brain endothelial cells have also been demonstrated to cross blood-brain bio-membrane barriers and transport the anti-tumor medications doxorubicin and paclitaxel to the brains of mice (Khan et al., 2021; Fang and Liu, 2022b). Based on these characteristics, the MEVs have been regarded as promising drug delivery strategies in previous studies.

Despite the therapeutic potential of MEVs for drug delivery, their clinical application is still plagued by several challenges, including quick blood clearance, potential cancer stimulation, low production, and high expenses (Imai et al., 2015; Yamashita et al., 2016; Charoenviriyakul et al., 2017; Baldini et al., 2018). Accordingly, exploring safe, high-yield, and readily available sources of EVs has become a talking point for all researchers. According to the history of plant-derived extracellular vesicles (PDEVs), the first observed PDEVs were in 1967 and first isolated in 2009 (Figure 1). Since then, the research on PDEVs has been deepening and bringing the field into a period of diversified development. Interestingly, extracellular vesicles derived from plants (PDEVs) have been discovered in different plant species and possess exceptional qualities, making them a subject of increased interest in recent times. For example, PDEVs do not contain human or zoonotic pathogens and pose reduced immune risks *in vivo*. Hence, the PDEVs bestow non-immunogenic characteristics absent in MEVs and other artificial drug delivery methods (Zhuang et al., 2015; Baldini et al., 2018; Cao et al., 2019). PDEVs containing proteins, DNA, RNA, lipids, and other substances can acquire specific bioactive properties from the original plants, such as antioxidant, anti-inflammatory, and anti-cancer effects (Ju et al., 2013; Zhuang et al., 2015; Liu B. et al., 2020; Han et al., 2021). Furthermore, PDEVs, which have biological capabilities, can be utilized as carriers for molecules to enhance the therapeutic effects of drugs through synergistic effects. The transfer of these PDEV-loaded bioactive compounds can significantly impact the biological behaviors of recipient cells, particularly those related to various diseases like tumors and inflammation. Recent research has demonstrated that multiple PDEVs possess the ability to withstand challenging conditions in the gastrointestinal tract and overcome biological obstacles (Liu et al., 2023). It suggests that PDEVs may have great promise as platforms for delivering drugs. Various separate teams have utilized biochemical technologies to show the significant functions of PDEVs as potential intermediaries in intercellular communication and the exchange of bio-information among different species (Feng et al., 2023). For instance, it has been demonstrated that *Helianthus annuu* derived PDEVs containing lectins play a role in inflammation, cancer, and autoimmunity disease (Regente et al., 2009). Phosphoric acid can be transported by PDEVs derived from *Zingiber officinale Roscoe* to enhance chronic periodontitis while regulating pro-inflammatory cytokines can help alleviate colitis symptoms and preventing microglia activation can moderate encephalitis (Sundaram et al., 2019; Liu and Liu, 2022; Sundaram et al., 2022). Furthermore, various PDEVs from *Aloe ferox*, *Acrotome angustifolia*, Beta vulgaris, *Anthemis arvensis*, and *Vaccinium myrsinites* are being developed for disease therapy (De Robertis et al., 2020; Zeng et al., 2021; Chen et al., 2022; Mahdipour, 2022). Although growing evidence has also revealed that PDEVs could be absorbed by animal cells, some differences still exist between PDEVs and MEVs. For example, an apparent difference between PDEVs and MEVs is that PDEVs are abundant in phospholipids, such as phosphatidylethanolamines (PE), phosphatidic acids (PA) and phosphatidylcholine (PC). In contrast, MEVs mainly comprise sphingomyelins and cholesterol (Zhang et al., 2016b). Here, we summarized the advantages and disadvantages of PDEVs and MEVs in Table 1.

Accordingly, the magnitude of PDEVs in the field of biomedicine has witnessed a growing appreciation. Although PDEVs were discovered approximately a decade before MEVs, there is still limited understanding of the research conducted on drug delivery systems associated with PDEVs (Duan et al., 2021; Xu et al., 2021; Nguyen and Fuhrmann, 2022; Zhou et al., 2022). Therefore, this review aims to offer a brief summary of the PDEVs investigation, including the generation, formulation, thorough analysis, and loading methods in detail. Specifically, the focus is on revealing the capacity of PDEVs as therapies for diseases and as effective systems for delivering drugs based on ongoing

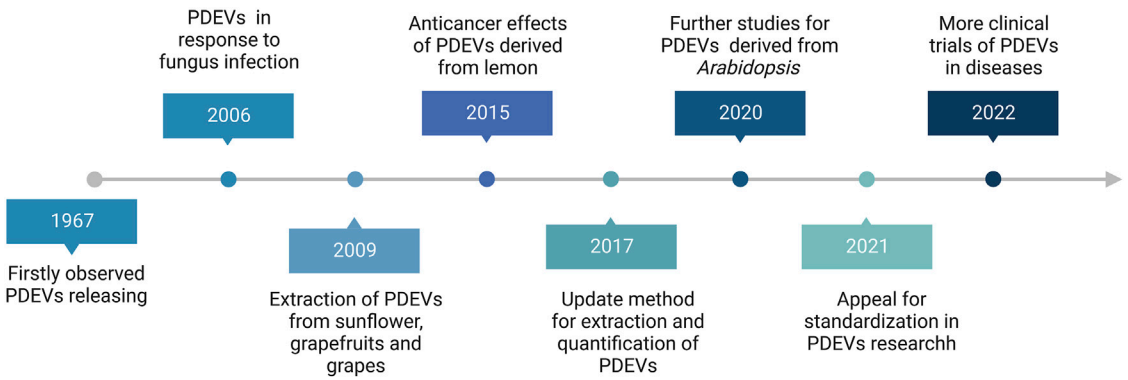

FIGURE 1
The brief research history of Plant-derived extracellular vesicles (PDEVs).

TABLE 1 The advantages and disadvantages of PDEVs and MEVs.

Strategies	Advantages	Disadvantages	References
PDEVs	Diverse types and abundant sources; Low toxicity; Relatively high extraction efficiency; Excellent drug carriers; High in phospholipids, easily absorbed; Excellent oxidation resistance; Broad adaptability to inflammatory diseases; Improve gut microorganisms	The database is incomplete, and the identification of specific functions and components is difficult; High dose dependence; There are few clinical trials, and the reliability of research results is low	Logozzi et al. (2022), Qin et al. (2022), Ratnadewi et al. (2022), Shinge et al. (2022), Wei et al. (2023)
MEVs	High stability; High stability in the digestive system; Less adverse reactions; Great potential for artificial transformation	High cost The database is incomplete, and the identification of specific functions and components is difficult; High dose dependence; There are few clinical trials, and the reliability of research results is low	Suharta et al. (2021), Cong et al. (2022), Liu et al. (2022), Wei et al. (2023)

research. We also tried to thoroughly evaluate the role of PDEVs as a lucrative mechanism for delivering drugs. In conclusion, we present our distinct perspectives on pharmacological significance, toxicity and immunogenicity, clinical utilization, possibilities, and obstacles of PDEVs.

2 Biogenesis of PDEVs

The presence of cell walls implies that plant cells possess more intricate communication methods than MEVs, making it difficult to investigate the forming process of PDEVs. According to recent research, the potential development of PDEVs may occur in various ways, such as through multivesicular bodies (MVBs), exocyst-positive-organelles (EXPOs), autophagosomes, and vacuolar pathways (Cui et al., 2020; Fang and Liu, 2022a). The primary types of PDEVs consist of the endosome (50–150 nm), microvesicles generated by the protrusion of the cell membrane (150–1,000 nm), and apoptotic bodies (1–1.5 μm), categorized by their origin within the cell, biological purpose, and physical characteristics (Fang and Liu, 2022a; Cheng and Hill, 2022).

The combination of MVBs and the plasma membrane (PM) results in the liberation of the intraluminal vesicle (ILV) and is controlled by the endosomal sorting complex required for transport (ESCRT), which is the predominant route for EVs creation in mammals. MVB pathway in plant cells bears a resemblance to that of mammalian cells (van Niel et al., 2022). The first observation of the MVBs occurred in the 1960s through a transmission electron microscope (TEM) (Ly et al., 2023). Numerous studies on different subjects, including unconventional protein secretion (UPS) and interactions between plants and pathogens, have validated the presence of PDEVs and their biogenesis mechanisms (An et al., 2006; Regente et al., 2012). For example, MVBs could discharge vesicles by merging with the PM or releasing them to particular infection locations while the fungus invades plant tissue (An et al., 2006). Multiple studies have additionally indicated that these MVBs, originating from the leaf tissues of *Arabidopsis thaliana* at the site of fungal infection, could be marked with Rab5-like GTPase ARA-6 and TETRASPANIN(TET) 8-GFP (Cai et al., 2018; Cai et al., 2019). Specifically, the ARA-6 is situated at the site of infection and persists on the PM for subsequent transportation. Simultaneously, the Tetraspanin-8 is located on the released vesicles, resembling the CD63 marker of MEVs, and is anticipated to function as a marker for plant EVs (Cai et al., 2018). In addition, studies also proved that the fusion between MVBs and PM was also promoted by plant-microbe symbiosis, and these EVs were released into the periarbuscular space between plants and fungi.

There is also supporting evidence for alternative routes, including secretion through EXPO, autophagosomes, and fusion between vacuoles and the plasma membrane (Ly et al., 2023). Autophagosomes and EXPO are spherical double-membrane organelles. In spite of their comparable structure, EXPOs do not

coexist with autophagosomes, nor do they experience any impact from nutrient deprivation. As a result, these two cellular components are regarded as separate entities. EXPO, possessing a dual-layered composition, has the capability to merge with the plasma membrane and discharge its internal vesicles into the extracellular space (Wang et al., 2010). However, EXPO exhibits morphological differences compared to MVBs and operates separately from endosomes and autophagosomes. Following fusion with the PM, EXPO secrete vesicles into the extracellular environment, which are larger by 200–500 nm (Cai et al., 2021). The recent discovery that glycosyltransferases and EXPO markers are found together in tobacco indicates that PDEVs derived from EXPO might play a role in releasing arabinogalactan proteins in plants. In addition to the MVBs and EXPO pathways discussed earlier, vacuoles may also play a role in forming PDEVs. Vacuoles containing hydrolytic enzymes are crucial organelles in plant defense (Pérez-Bermúdez et al., 2017; Wang et al., 2017). To combat pathogen infection, plants regulate the merging of vacuoles and plasma membranes, releasing substances like hydrolases and defense proteins into the external environment (Hatsugai et al., 2009). According to recent research, it has been discovered that central vacuoles in Arabidopsis originated from the transition of MVBs to small vacuoles (SVs) and the subsequent fusion of SVs (Cui et al., 2019). The fusion of autophagosomes and MVBs in yeast cells results in the formation of secondary vesicles, indicating a potential co-secretion of PDEVs through these secretory pathways (Manjithaya and Subramani, 2010).

Consequently, PDEVs have been discovered to have a crucial role in protecting the immune system and facilitating communication between cells. Nevertheless, the precise mechanisms of biogenesis for PDEVs remain uncertain, and PDEVs from various plant origins might be released in intricate ways. Hence, it is imperative to conduct additional research on the biogenesis process of PDEVs, as this could potentially aid in the systematic regulation of their secretion in the future.

3 Preparation and characterization of PDEVs

Utilizing PDEVs as naturally sustainable raw resources could be a promising approach in the medical field (Hernández-Díaz et al., 2021). MEVs have demonstrated significant promise in the biomedical domain, and PDEVs have also garnered considerable interest. In comparison to the MEVs, PDEVs offer a more comprehensive range of choices and enhanced safety (De Palma et al., 2020; Ly et al., 2023). However, achieving successful isolation of these PDEVs may be essential for practical applications. PDEVs are typically obtained directly from the extracellular surroundings, like apoplastic fluids or root exudate. These involve immersing plant raw samples in an infiltration buffer and isolating PDEVs by multiple methods, including ultracentrifugation, precipitation with polyethylene glycol, immunoaffinity, and size exclusion chromatography (Wang et al., 2021; Ali et al., 2022; Lai et al., 2022; Yin et al., 2022). Actually, the proper separation technique should be applied according to the final purpose of the PDEVs, among which basic research and clinical application are the bases for the selection of methods (Ströhle et al., 2022). However, there are currently limited separation methods for PDEVs, most of which refer to MEVs (Jackson et al., 2023). Due to the differences between animal cells and plant cells, such as starch and cellulose, the same isolation technique may be less effective in the extraction of PDEVs (Gardiner et al., 2016). As a result, we summarized the advantages and disadvantages of different isolation methods in Table 2 and provided a brief overview of the typical preparation and characterization of PDEV. It is predictable that an appropriate separation method could be more conducive to the acquisition of PDEVs with high purity, high yield, and high activity.

3.1 Preparation of PDEVs

The preparation of PDEVs follows similar methods established for MEVs. The extraction and purification technique used could be vital for the functioning of PDEVs since they are frequently present in a highly intricate environment. In existing research, numerous studies have aimed to enhance both extraction specificity and efficiency (Yang et al., 2021). Every method has its unique benefits and limitations. For example, a more precise strategy, like immunoaffinity, usually yields a pure sample but lacks efficiency and recovery. On the other hand, several methods, like membrane filtration, which have high efficiencies, may lack specificity and purity. However, it is important to mention that the functioning of PDEVs relies on the undamaged bilayer membrane (Ju et al., 2013; Wen et al., 2023). Therefore, it is crucial to exercise caution when extracting and storing it to avoid any deterioration. It can be achieved by employing protease inhibitors, keeping them at low temperatures, avoiding the freeze-thaw process, and maintaining a neutral pH (Li et al., 2017; Cheng et al., 2019). Extraction methods such as ultracentrifugation, ultrafiltration, immunoaffinity capture, precipitation, or microfluidics often rely on their distinct properties, such as their density, size, shape, and protein composition (Suharta et al., 2021). For instance, some studies suggested that blending is a superior choice for acquiring increased amounts of phytochemicals from grapefruits compared to juicing or manually squeezing (Uckoo et al., 2012). Wang and others determined that out of blending, high-speed centrifugal juicing, and low-speed juicing, the latter two preserved the most excellent phytochemical profiles and antioxidant activities of 19 vegetables, such as *Brassica oleracea*, *Raphanus raphanistrum*, and *Beta vulgaris* (Wang et al., 2021). This section summarized the typical techniques frequently employed for isolating PDEVs (Table 2).

3.1.1 Ultracentrifugation

Among the techniques mentioned above, ultracentrifugation is often utilized as the primary method for EVs isolation due to its straightforwardness, user-friendliness, cost-effectiveness over time, reasonable time requirement, and lack of complex sample preparation (Li et al., 2017). Accordingly, ultracentrifugation usually divides into a differential or a density gradient centrifugation (Figure 2A). Differential centrifugation relies on the separation of particles by their density, size, and shape through a sequence of high-speed centrifugation in a uniform medium (Li et al., 2017; Qiao et al., 2020). Actually, PDEVs are commonly extracted using the method of differential centrifugation (Gardiner et al., 2016). Various sizes of particles in the medium can separate different components with different rates of sedimentation. Generally, the intact cells could be eliminated at a low speed through

TABLE 2 Typical strategies and examples of applications to prepare PDEVs.

Strategies	Isolation condition	Advantage	Disadvantage	References
Ultracentrifugation	A range of centrifugation with different speeds and gradient ultracentrifugation. Considered as the gold standard in EVs extraction because of its capacity to extract EVs in a relatively high-purity fashion	Fast procedure; no limitations on sample volume; pure preparations; low cost	Vesicles trapping; loss of PDEVs; the possibility of clogging	Zhang et al. (2021)
Precipitation	Combination of centrifugation and clustering agents like PEG 6000 to trap EVs	The simplicity of the procedure, low cost, preservation of PDEVs integrity	Retention and contamination of the polymer	Jia et al. (2022)
Immunoaffinity	Using the interaction between the antigens on the surface of EVs and matching antibodies	Purity, high selectivity; convenient operation, low cost	High reagent cost; low yields; difficult to analyze complete vesicles	Sundaram et al. (2020)
Size-based isolation (SEC)	Separating similar-sized particles with ultrafiltration, size-exclusion chromatography, and flow field-flow fractionation, which typically resulted only in an EVs-enriched sample	Purity; reproducibility; preserves vesicle integrity; scalability; prevents PDEVs aggregation	Complexity; limitations on sample volume; specialized equipment; a small number of samples; high cost	Sidhom et al. (2020b)
Commercial isolation kit	EVs isolation kit	Suitable for small samples, simple steps, fast procedure	Impurity; low production; expensive reagents	Muraoka et al. (2020)

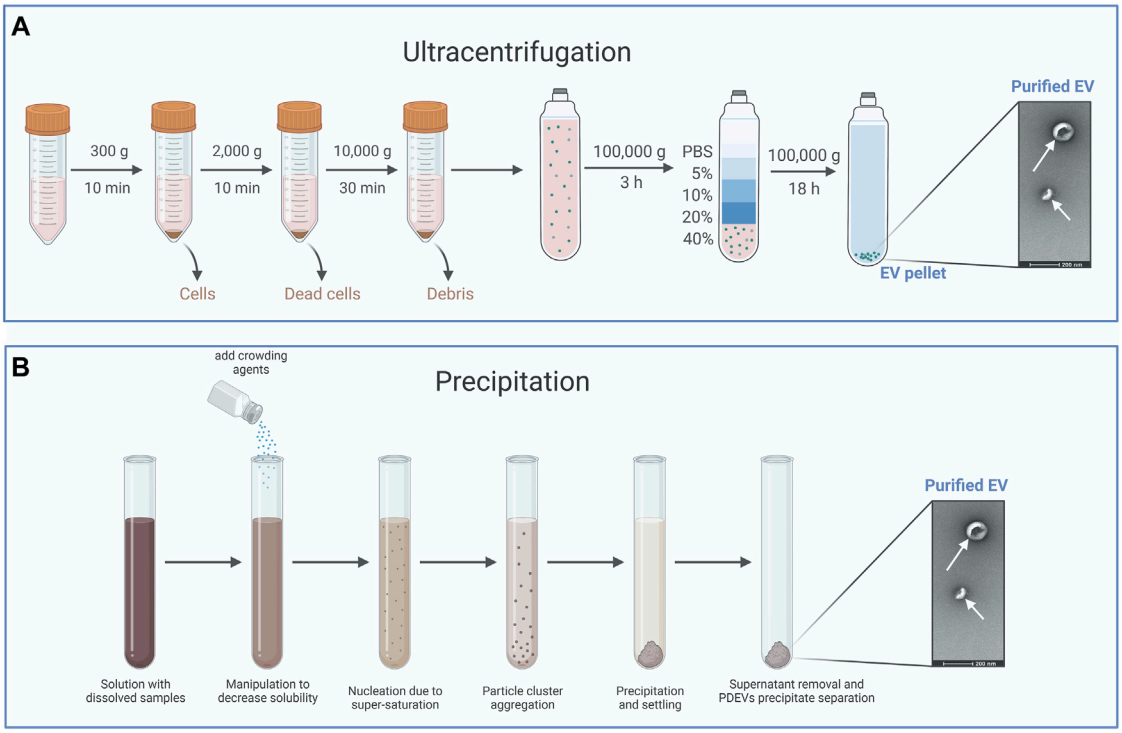

FIGURE 2
The typical method for the isolation of PDEVs. **(A)** Ultracentrifugation is often utilized as the primary method for extracellular vesicle isolation and usually divides into a differential or a density gradient centrifugation. **(B)** Precipitation isolates PDEVs by regulating the solubility and dispersion of PDEVs. For the separation of PDEVs, polyethylene glycol (PEG) is commonly employed as a coprecipitate.

centrifugation. At the same time, high-speed centrifugation can separate the sizable pieces and undamaged organelles. Ultimately, PDEVs are separated and enhanced through ultracentrifugation. It should be emphasized that prolonged and repeated ultracentrifugation can potentially disrupt the structure of the PDEVs. However, an additional cushion was incorporated at the base of the centrifuge tube, demonstrating its efficacy in enhancing the condition (Li et al., 2017; Yang D. et al., 2020; Lai et al., 2022). Differential centrifugation is the most common technology to extract PDEVs now. Despite the drawbacks of time-consuming processes and expensive costs, this technology offers the benefits of easy operation and a plentiful yield of PDEVs.

The density gradient centrifugation principle is based on the varying sedimentation coefficients of particles. Different particles settle in the area with varying density gradients under the influence of centrifugal force (Lai et al., 2022). Sucrose density gradient centrifugation is commonly used for purifying PDEVs that are acquired through differential centrifugation. The suspension of PDEVs was included in the solution with sucrose gradients. Following ultracentrifugation, the majority of PDEVs were typically concentrated within the density range of 1.13–1.19 g/mL. The primary origin of PDEVs is the sucrose gradient found in the sucrose layer, ranging from 30% to 45% (Stanly et al., 2019; Yang D. et al., 2020). Density gradient centrifugation yields PDEVs with excellent purity and a well-maintained structure, although the process is intricate and time-intensive. Accordingly, the most mature technique for preparing PDEVs is the combination of density gradient centrifugation and differential centrifugation.

3.1.2 Precipitation

By regulating the solubility and dispersion of PDEVs, it is possible to induce the precipitation of PDEVs from the solution (Figure 2B). For the separation of PDEVs, polyethylene glycol (PEG) is commonly employed as a coprecipitate (Fang and Liu, 2022b). Hence, a less expensive technique for extracting EVs was developed, involving a sequence of centrifugation steps and purification with polyethylene glycol-6000 (PEG6000). The presence of this overcrowding agent has the potential to form a mesh-like arrangement capable of ensnaring PDEVs prior to their precipitation (Ali et al., 2022). The utilization of this technique can be extended to cause the precipitation of tiny particles and even viruses (Adams, 1973; Lewis and Metcalf, 1988). By modifying the PEG-6000 concentration employed during purification, this technique successfully yielded diverse average nanoparticles, measuring 365 nm, 304 nm, 256 nm, and 252 nm at PEG-6000 concentrations of 8%, 10%, 12%, and 15%, respectively, in contrast to the 403 nm achieved through ultracentrifugation. Despite having a lower yield and reduced bioactive polyphenol compounds, the PDEVs acquired through this technique exhibited comparable efficiency in macrophage cell uptake and profiles of small RNAs, proteins, and lipids compared to those obtained through ultracentrifugation (Kalarikkal et al., 2020). In addition, the precipitation technique tends to co-precipitate additional impurities that are not EVs, such as different vesicles, aggregates, protein complexes, and proteins of significant size (Zarovni et al., 2015; Castillo et al., 2018). For instance, Sreeram and others designed a technique for purifying PDEVs extracted from ginger roots utilizing PEG6000. These PDEVs precipitated by PEG6000 possess identical dimensions, capabilities, and biochemical makeup to PDEVs obtained through ultracentrifugation (Kalarikkal et al., 2020). Due to the straightforward procedure and excellent productivity, these techniques have also been employed in extraction kits (Umezu et al., 2021). While coprecipitation methods to isolate PDEVs exclusively have been limited to a few researchers, resulting from the tendency of coprecipitation techniques to cause EVs aggregation and coprecipitation of contaminants like lipoproteins (Gardiner et al., 2016).

3.1.3 Immunoaffinity

The immunoaffinity technique is also frequently used to extract PDEVs (Figure 3A). The success of this method relies on the interactions between identifying particular antibodies and distinct proteins located on the surface of EVs. The protein target must be exclusively present or abundant within the EVs, bound within the membrane, and not present as a soluble component in the sample (Zarovni et al., 2015). The immunoaffinity technique can coat magnetic beads with specific antibodies that target proteins found in the membrane of PDEVs (Gardiner et al., 2016). This technique exhibits a high level of specificity and rapid separation rate. For instance, recent research successfully utilized the interaction between the phosphatidylserine on the surface of EVs and the T-cell immunoglobulin and mucin domain-containing protein 4 (Tim4) based on $Ca2^{+}$. Therefore, it allowed the liberation of the EVs from the beads through the application of $Ca2^{+}$ chelators (Yang M. et al., 2020). Furthermore, Field-flow fractionation (AF4) is an innovative method of isolating PDEVs based on their particle dimensions (Sitar et al., 2015). The Field-flow fractionation, in combination with light scattering detection, is appropriate for separating and characterizing a diverse range of analyses due to the vast array of mobile eluents available. For example, the AF4 method effectively isolated EVs from B16F10 cells (Zhang et al., 2018). Furthermore, PDEVs were effectively isolated from citrus and bitter gourd through dialysis and electrophoresis. The morphology and quantity of PDEVs separated using this technique were comparable to ultracentrifugation, as revealed by nanoparticle tracking analysis (NTA) or transmission electron microscopy (TEM) analysis (Yang M. et al., 2020; Yang et al., 2021). Additionally, aqueous biphasic systems were employed to extract PDEVs from garlic, with consistent particle size and a diverse range of labeled proteins (Özkan et al., 2021). However, the immunoaffinity technique has a limitation due to the heterogeneity of PDEVs. And only a fraction of EVs in a sample would contain the desired antigen. In contrast, the remaining subpopulation lacking this antigen would not be trapped by the antibody employed in the laboratory. Consequently, resulting from the absent study for the surface composition of PDEVs, the immunoaffinity method has not been studied extensively for extraction.

3.1.4 The size exclusion chromatography (SEC)

Another method for preparing PDEVs is based on size exclusion chromatography (SEC) (Figure 3B). By considering the correlation between the hydrodynamic radius of the particles present in the sample and the pore size of the resin beads within the column, this technique also effectively separates EVs (Hajj et al., 2013; Fang and Liu, 2022b). Due to the larger hydrodynamic radii, EVs were unable to pass through the pores, leading to their early elution. In contrast, components with smaller hydrodynamic radii, capable of entering the pores, experienced late elution. Some studies evaluated the efficacy of ultracentrifugation, precipitation with polyethylene glycol, and SEC for isolating PDEVs from cabbage. The SEC technique was employed to isolate PDEVs, which exhibited greater yields and more comprehensive functional properties compared to ultracentrifugation (Monguió-Tortajada et al., 2019; Martínez-Greene et al., 2021). In regards to purity, SEC produced outcomes that were over 20 times greater than the two techniques mentioned before (Nordin et al., 2015). These EVs also could preserve biological functionality and structure intact. Jae et al. successfully applied SEC to isolate PDEVs from carrots and cabbage, resulting from the SEC not exerting more changes in their physicochemical properties (You et al., 2021). The SEC

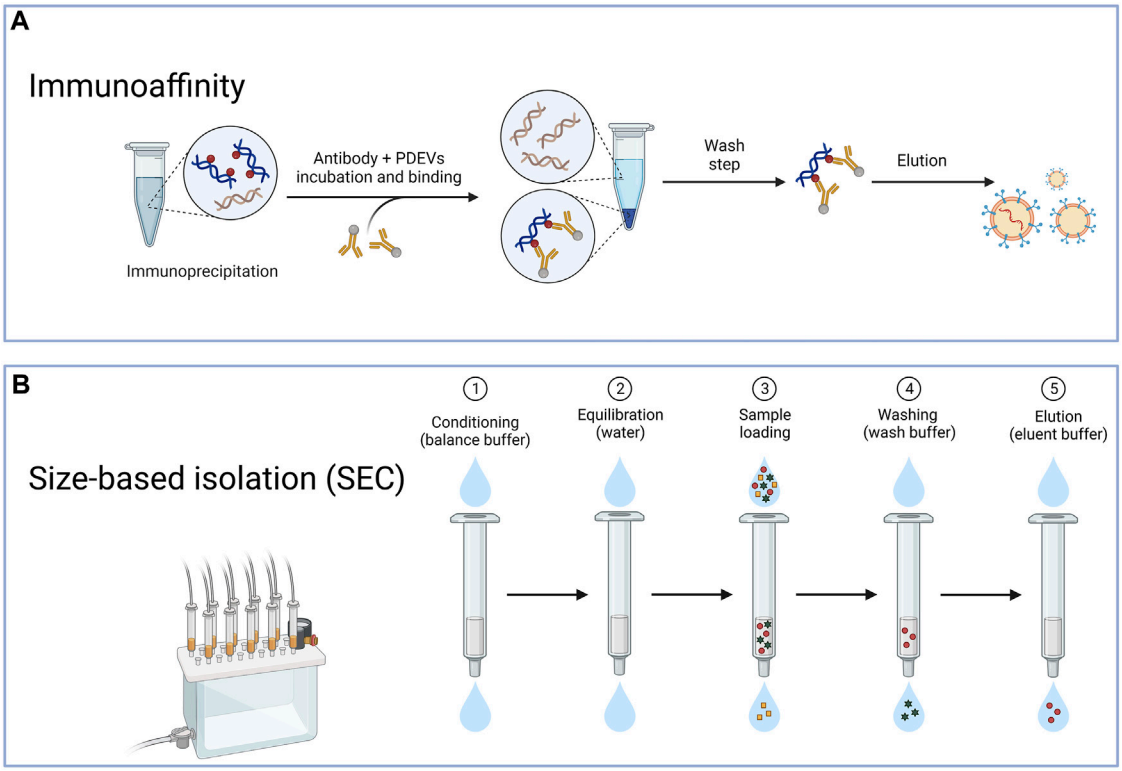

FIGURE 3
The typical method for the isolation of PDEVs. **(A)** The immunoaffinity technique is also frequently used to extract PDEV based on the interactions between identifying particular antibodies and distinct proteins located on the surface of extracellular vesicles; **(B)** Another method for preparing PDEVs is based on size exclusion chromatography (SEC) through the correlation between the hydrodynamic radius of the particles present in the sample and the pore size of the resin beads within the column.

method is recognized as the primary technique for extracting PDEVs due to its simplicity, scalability, affordability, and rapid separation rate. It has been applied to extract PDEVs from cucumbers, tomatoes, and peppers (Sidhom et al., 2020a; You et al., 2021). As SEC depends on gravity, it has the potential to maintain the original bioactivity and structures of PDEVs. Although obtaining PDEVs with higher purity can also be achieved by utilizing HPLC, challenges may arise in terms of scaling up and the availability of equipment (Yang D. et al., 2020).

3.2 Characterization and contents of PDEVs

As the most potential candidate for drug delivery platforms, biochemical and physical characterization of PDEVs may be crucial. For example, accurate analysis of their physicochemical properties is significant for drug delivery applications. The description of the characterization and structure of particles also can verify the effective extraction of PDEVs. PDEVs have been reported to transfer multiple cargoes to recipient cells and transform their phenotype, containing the surface morphology, lipids, proteins, RNA, and small molecular substances of PDEVs (Wang et al., 2022). For instance, PDEVs packaged with secondary metabolites could facilitate them to pass the membrane barrier (Woith et al., 2021). Accordingly, these bioavailable molecules encapsulated on PDEVs make them unique and powerful tools for health-beneficial purposes. However, an extensive database of the physicochemical properties related to PDEVs is still unavailable due to the diversity of plants. Therefore, we summarized the currently typical characterization of PDEVs to display their natural clinical and pharmaceutical benefits.

3.2.1 Morphological and physicochemical characterization

The assessment of morphology is essential in the study of PDEVs since it offers researchers direct insights into the characteristics. In most studies, the features, including shape, size, and surface charge, have been the primary focus of physical investigations of PDEVs. For instance, the majority of studies have adopted TEM and revealed that the morphology of PDEVs is typically round or saucer-shaped and characterized by lipid bilayers with lipid bilayers, which is regarded as the classic extracellular vesicle morphology. The size distributions of PDEVs separated through the disruption process range from 18–1,000 nm and exhibit negative surface charges. Recent studies suggested that the alteration of environmental pH or temperature may modify the size and charge of PDEVs. Up to now, PDEVs have been characterized by several approaches, including dynamic light scattering (DLS), nanoparticle tracking analysis (NTA), and electron microscopy (EM). Dynamic light scattering (DLS) has the capability to evaluate the hydrodynamic size of PDEVs through the fluctuation of light scattering. However, the size of

PDEVs may not be accurately analyzed by dynamic light scattering, resulting from their polydispersity and potentially low scattering contrast (Sokolova et al., 2011; Hadisurya et al., 2023). The size may differ depending on the testing technique, as each method operates based on distinct principles and preparations. Moreover, nanoparticle tracking analysis (NTA) is the most common method to assess the size distribution and provides information on the number of PDEVs (McIlvenna et al., 2023). Despite the absence of standardized configurations and the limited scattering index of PDEVs, NTA is still the prevailing method for characterizing PDEVs.

In addition, the stability and optimal preservation of PDEVs have not been extensively elucidated compared with MEVs or synthetic liposomes. However, it is crucial to clarify the capability of PDEVs for biological management and scalability through these necessary measures. Therefore, various experiments have been carried out to examine the durability of PDEVs in diverse environments by modifying variables such as pH ranges, temperature, freeze-thaw cycles, and external manipulation. For example, numerous studies implicated that PDEVs derived from *Z. officinale*, *Daucus carota*, and *Aframomum sulcatum* could remain stable across a broad pH range under physiological temperatures (Mu et al., 2014). Moreover, the pH level may affect the size and surface charge of PDEVs. For example, PDEVs derived from *Citrus paradisi* maintained their size and surface charge in neutral and alkaline pH conditions while increasing in acidic conditions (Wang et al., 2014). Similarly, the PDEVs of *Z. officinale* exhibited an increased size and a transition in surface charge from negative to positive in an acidic solution (Zhuang et al., 2015; Zhang et al., 2016a). PDEVs derived from *Citrus paradisi* and *Citrus limon* also showed significant resistance to the digestive environment in both simulated conditions and mouse models. In addition, several studies indicated that PDEVs might be unstable under boiling or sonication. The antiproliferative function of PDEVs extracted from citrus fruits was destroyed under sonication or boiling conditions (Alzahrani et al., 2023). Consistently, the PDEVs obtained from apples were susceptible after boiling and sonication (Fujita et al., 2018). PDEVs derived from *Ginseng quinquefolium* could promote the polarization of macrophages compared with un-sonicated samples (Cao et al., 2019). It is crucial for researchers to understand that sonication is a commonly employed technique for drug incorporation (Wang et al., 2013; Zhang et al., 2016c; Zhuang et al., 2016; Ly et al., 2023; Raguraman et al., 2023). Therefore, the morphology of PDEVs should be evaluated during drug loading. PDEVs have been extensively applied as drug carriers, resulting from their adjustable dimensions and excellent stability. For example, it is possible to improve the stability and bioavailability of the delivery platform by combining hydrophobic agents with *Z. officinale* isolated from PDEVs. Without affecting biological activities, *Citrus paradisi*-derived PDEVs could be modified with biomolecules such as zymosan A, folic acid, or curcumin. Moreover, these lipid particles exhibited more excellent stability compared to cationic liposomes when exposed to a 10% bovine serum solution at 37°C (Wang et al., 2013).

3.2.2 Proteins

The description of the distinctive protein composition and function in PDEVs is crucial for further classification of PDEVs. According to the International Society for Extracellular Vesicles (ISEV), it is recommended that researchers should document a minimum of three proteins in each EVs sample, such as transmembrane and cytosolic proteins (Lötvall et al., 2014). Interestingly, PDEVs usually comprise a low concentration of proteins, including cytosolic proteins like actin and proteases, and membrane proteins acting as channels and transporters, like aquaporins and chloride channels (Zhang et al., 2016a; Alzahrani et al., 2023; Ipinmoroti et al., 2023; Wang et al., 2023). Cao and others detected 3,129 proteins in PDEVs derived from *G. quinquefolium* by mass spectrometry. According to Gene Ontology, these proteins were categorized into three groups, which are biological processes, cellular compartments, and molecular functions (Cao et al., 2019; Ou et al., 2023). Other groups analyzed the protein composition of PDEVs derived from *Citrus limon* and identified 162 proteins related to transportation (Stanly et al., 2019). Surprisingly, 56.7% of these proteins matched with proteins extracted from MEVs, according to the ExoCarta protein database (Fang and Liu, 2022b). However, extracellular vesicles derived from mammals comprise over 1,000 proteins, whereas PDEVs, like those obtained from *G. quinquefolium*, only have 28 proteins (Ju et al., 2013; Zhang et al., 2016a). Interestingly, some studies reported that the surface protein of PDEVs derived from *Allium sativum* may control their absorption by HepG2 (Song et al., 2020). Subsequently, they found a significant decrease in the absorption of PDEVs after inhibiting CD98 receptors on HepG2. In detail, Song et al. indicated that the interaction of lectin-like protein from PDEVs with the glycoprotein CD98 on the cell surface facilitated the uptake of *A. sativum*-PDEVs. This discovery provided a deeper comprehension of how cells take up PDEVs. On the other hand, the PDEVs contain proteins that help the plant to be resistant to stress (Rutter and Innes, 2017). For example, Rutter et al. detected the proteomics of the apoplastic PDEVs from *Arabidopsis* leaves and revealed that they contained a high concentration of stress response-related proteins (De Vallée et al., 2023). In a recent study, researchers compared the proteomics between apoplastic and whole-leaf PDEVs from *A. thaliana*. As expected, PDEVs obtained through tissue rupture methods contained more composition of proteins than those obtained exclusively from extracellular fluid (Liu Y. et al., 2020). Furthermore, several proteins present in MEVs have also been identified in PDEVs, including GPI-anchored proteins, annexins, heat-shock protein 70 (HSP70), and aquaporin proteins (Ju et al., 2013). In summary, complete profiling of proteins inside PDEVs is quite tricky due to the varying protein databases needed for matching in different plants.

3.2.3 Lipids

Lipids have been considered the main composition of PDEVs, as the lipid bilayer composition influences their efficiency taken up by recipient cells and used for therapeutic purposes. Moreover, the proportion of lipids has a crucial role in the drug encapsulation and transportation capacity of PDEVs. The diversity in lipid composition determines the physical distribution and transport function of PDEVs. For instance, the primary lipid in PDEVs of *Citrus paradisi* and *A. sativum* was phosphatidylcholine (PC). In contrast, PDEVs derived from *Z. officinale* mainly contained phosphatidic acid (PA) as their main lipid component (Wang et al., 2014; Teng et al., 2018). However, PDEVs have been found to lack cholesterol and sphingomyelin, unlike MEVs and synthetic liposomes (Teng et al., 2018). In addition, this peculiarity in lipid

compositions impacted the distribution of PDEVs. For instance, the lipid composition and structure of PDEVs played a crucial role in their distinctive absorption by the cells in the intestine. PDEVs derived from *Vitis amurensis* were plentiful in PA (53.2%), primarily found in the intestinal tract, and rarely absorbed by intestinal stem cells and macrophages (Ju et al., 2013; Mu et al., 2014; Zhuang et al., 2015; Zhang et al., 2016a). By contrast, the enrichment of PC lipids by PDEVs determined the migration from the gut to the liver (Teng et al., 2018). The presence of phosphatidylcholine (PC) was observed to promote the movement of PDEVs into liver cells, as demonstrated by analyzing images of mouse intestinal cells and liver cells at 1–6 h after consuming fluorescence-labeled PDEVs (Teng et al., 2018). PDEVs derived from *Z. officinale* migrated from the gut to the liver via the bloodstream and were absorbed by hepatocytes and Kupffer cells, resulting in significant accumulation in the liver (Zhuang et al., 2015; Kilasoniya et al., 2023). In the same way, *Z. officinale*-PDEVs produced using the thin-layer dispersion technique were absorbed by RAW264.7 and colon-26 cells and persisted in the stomach, ileum, and colon, indicating a focus on the intestines (Zhang et al., 2017). Mouse intestinal stem cells rarely absorbed PDEVs derived from *V. amurensis*, whereas they could absorb recombinant liposomes, which then accumulated in the intestinal tract (Ju et al., 2013; Record, 2013). Therefore, it is crucial to lucubrate the lipid role of PDEVs to elucidate the mechanism of PDEVs and utilize them in drug delivery systems.

3.2.4 miRNAs

miRNAs, which are small RNA molecules approximately 22 nt in size, do not possess coding properties. Their primary roles include regulating the cleavage or translation of mRNAs and inducing the expression of specific target genes (Bartel, 2004; Olmi et al., 2023). Numerous studies have confirmed the existence of abundant miRNAs in PDEVs, typically thought to regulate gene expression in mammals (Chin et al., 2016). Besides, PDEVs are also proposed to protect miRNAs against degradation during transportation. Some studies also implicated that PDEVs have the potential to facilitate the interaction between the gut microbiota and the host immune systems by impacting the composition of gut microbes due to their possession of diverse miRNAs (Teng et al., 2018). Previous studies have detected 418 miRNAs in different species of PDEVs, suggesting that the over-expressed miRNAs might participate in inflammatory responses and signaling pathways associated with cancer (Xiao et al., 2018). Moreover, various methods, including gel electrophoresis and mass spectrometry, have been applied to validate miRNAs in PDEVs derived from *V. amurensis* (Ju et al., 2013). For instance, Zhang et al. identified 125 different miRNAs in *Z. officinale*-PDEVs, mediating immunomodulatory or metabolic regulation (Zhang et al., 2016a; Yin et al., 2022). Recent studies have demonstrated the ability of miRNA from PDEVs to regulate the composition of the gut microbiota. For example, miR167a from *Z. officinale*-PDEVs may activate the aryl hydrocarbon receptor (AHR) signaling pathway to alleviate ulcerative colitis by promoting IL-22 expression (Teng et al., 2018). Zhou and others demonstrated that miRNA-2911 derived from *Lonicera sempervirens* has the ability to suppress Influenza Virus A (IVA) (Zhou et al., 2015). Hence, the miRNA derived from PDEVs holds promise for therapeutic implications. Accordingly, due to the ability of miRNA to engage in intercellular communication, further study of PDEVs may be considered as one of the most potential therapeutic strategies in the future, especially as drug delivery systems.

4 PDEVs-based bioactive molecule delivery systems for therapeutic agents

PDEVs contained compounds inherit the bioactive properties of their source plants, and at the same time, their distinct structures also suggest the potential in the field of biomolecule transportation. For example, PDEVs, consisting of a double layer of lipids, enable the incorporation of hydrophobic medications, enhance the effectiveness of medicines, and efficiently accommodate hydrophilic chemicals to prevent adverse effects resulting from drug leakage (Nemati et al., 2022). Recent studies have implicated that the application of PDEVs has been shown to enhance the bioavailability, solubility, and stability of biomolecules (Kalani et al., 2016). For example, the PDEVs derived from *V. amurensis* were applied to encapsulate the methotrexate (GDN-MTX), preserving the anti-inflammatory properties of GDN-MTX and enhancing the uptake of GDN-MTX by intestinal macrophages (Wang et al., 2014). Moreover, the GDN-MTX effectively alleviated symptoms of ulcerative colitis, reduced the secretion of pro-inflammatory cytokines by intestinal macrophages, and mitigated the adverse effects of MTX while playing a more substantial anti-inflammatory impact in the mouse colitis model (Wang et al., 2014). Recent studies also suggest that PDEVs from *V. amurensis* coated with white cell membranes on their surface have the ability to improve target and accumulate at the site of inflammation in various models. Furthermore, these membrane fusion techniques also were proven to improve the targeting of PDEVs, prolong the drug circulation time, and enhance the accumulation of the medicine in the target tissue (Wang et al., 2015). In addition, several researchers also found that combining PDEVs with ligands, such as folic acid (FA), could enhance their targeting ability. For example, FA-modified PDEVs derived from *Z. officinale* could encapsulate the doxorubicin without size change, delay the doxorubicin release, produce sustained cytotoxicity, and improve the tumor targeting ability (Zhang et al., 2016c). Similarly, functional heparin (HR)-modified PDEVs from *Citrus limon* could load doxorubicin with a slight size variation, which effectively improved the internalization efficiency of HR-PDEVs (Xiao et al., 2022). At the same time, the diverse endocytosis capacity of HR-PDEVs efficiently dissipated intracellular energy, decreasing drug efflux (Xiao et al., 2022).

Accordingly, we summarized various PDEVs that have been extensively used to develop novel drug delivery systems for treating particular diseases or maintaining normal functions (Figure 4). Different edible and medical plants are applied to isolate therapeutic PDEVs with diverse functionalities. This review also lists the main types of PDEVs for their therapeutic applications (Table 3).

4.1 PDEVs as inherent therapeutic agents

Despite being a relative innovation, PDEVs have already been employed to alleviate various diseases resulting from their advantageous healing properties and precise targeting of specific tissues (Wang et al.,

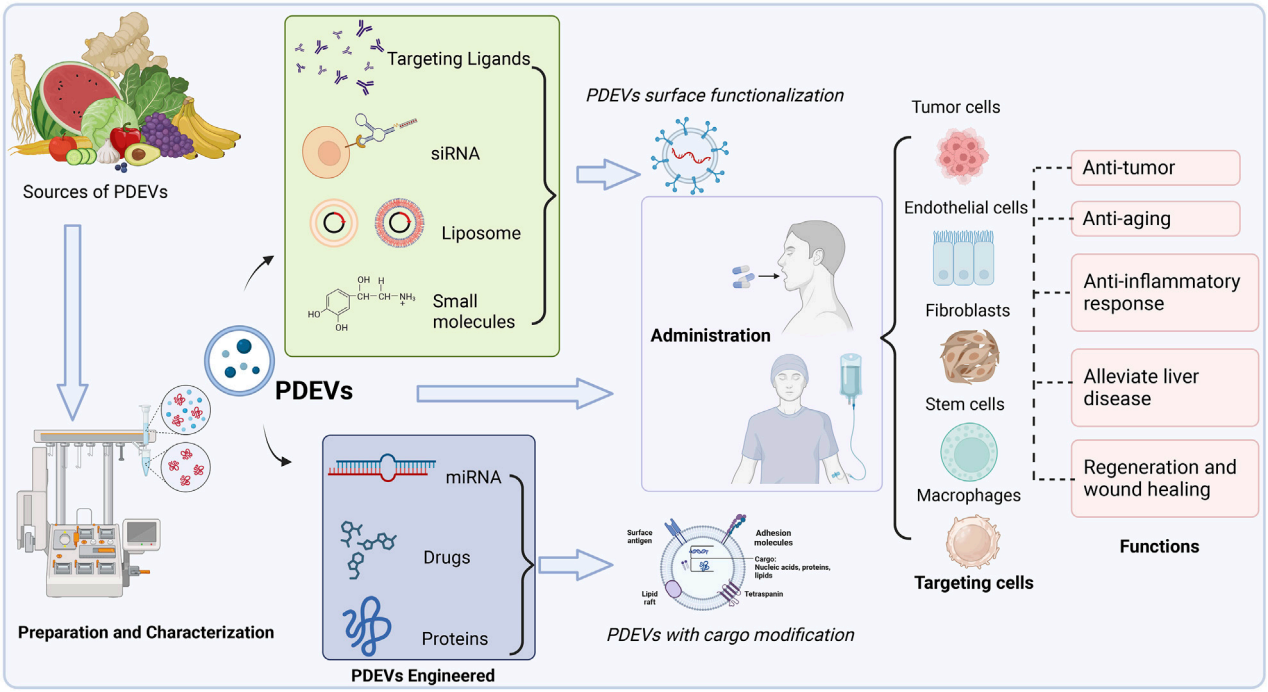

FIGURE 4
Multiple sources of PDEVs have been extensively used to develop novel drug delivery systems for treating particular diseases or maintaining normal functions. PDEVs and engineered PDEVs both are directly applied as therapeutic agents or delivery platforms, which could precisely target the different cells by administration to the body.

2014). Especially, PDEVs were identified to bind to receptors and serve as recognition elements for remote locations due to the biomolecules composed in PDEVs, including proteins, lipids, and miRNAs (Wang et al., 2014; Teng et al., 2018). Therefore, these PDEVs may be directly applied as therapeutic agents. For instance, PDEVs from *V. amurensis* have been proven to relieve dextran sulfate sodium (DSS)-induced colitis in mice (Ju et al., 2013). Interestingly, these PDEVs also enhanced the growth of intestinal stem cells, which could potentially generate enduring intestinal organoid structures *in vitro*, possibly being the sole source of such stem cells (Deng et al., 2017). Furthermore, these PDEVs dramatically improve the regeneration of mucosal epithelium and successfully revive the structural integrity of the entire intestinal tract (Deng et al., 2017). Similarly, some studies found that PDEVs derived from *B. oleracea* displayed significant preventative and curative properties in relation to three forms of colitis (Wang et al., 2014). In detail, these PDEVs exhibit strong preventive properties against both acute and chronic colitis *in vivo* experiments. These were supported by evidence of heightened levels of anti-inflammatory cytokines and increased histoscore, as indicated by a decrease in colon shortening, loss of weight, and mucosa inflammation (Wang et al., 2014). Additionally, these *B. oleracea*-PDEVs were found to preserve the immune environment of the intestines while causing minimal negative responses by activating the Adenosine monophosphate-activated protein kinase (AMPK) pathway (Wang et al., 2014). Furthermore, PDEVs also have been reported to alleviate some liver diseases (Zhuang et al., 2015). *Zingiber officinale* has been applied in traditional medicine for centuries, providing numerous health advantages due to its inherent chemical components like shogaol and gingerol (Brahmbhatt et al., 2013; Kandemir et al., 2019). The expression level of a set of detoxifying/antioxidant genes, including glutamate-cysteine ligase modifier subunit (GCLM), heme oxygenase-1 (HO-1), glutamate-cysteine ligase catalytic subunit (GCLC), and NADPH quinone oxidoreductase-1 (NQO1), was increased by *Z. officinale*-derived PDEVs, leading to alleviating liver damage. In addition, the analysis of tissue showed that mice administered *Z. officinale*-PDEVs experienced a significant decrease in fat deposits within the liver. Moreover, *Z. officinale*-PDEVs markedly reduced levels of triglycerides and the weight of the liver. The mice that were given *Z. officinale*-PDEVs also exhibited histopathological alterations in the liver, indicating that PDEVs safeguard the liver against the toxicity of alcohol.

Interestingly, PDEVs also have dramatic anti-tumor activity. Raimondo et al. have reported that PDEVs derived from *Citrus limon* significantly suppressed the growth of tumors and reduced the multiple angiogenesis-related cytokines secretion in chronic myeloid leukemia (CML) through the Tumor necrosis-related apoptosis-inducing ligand (TRAIL) pathway (Raimondo et al., 2015). At the same time, these PDEVs targeted the tumor site within 15 min, exhibiting enduring effects lasting over 24 h *in vivo*. Actually, these PDEVs participate in cellular interaction and contribute to eliminating cancer cells via activation of TRAIL signaling, displaying their potential as chemotherapy agents (Raimondo et al., 2015). Similarly, PDEVs derived from citrus fruits have demonstrated the ability to suppress the survival of cancer cells, such as LAMA84, SW480, and A549, dependent on both the dosage and duration, compared to normal cell lines (HS5, HUVEC, and PBMC). Additional analysis indicated that these PDEVs induced apoptotic cell death in tumor cells by activating the TRAIL pathway, which was further validated in the xenograft model

TABLE 3 Exploration, analysis, and potential medical applications of PDEVs.

Sources	Size (nm)	Drugs and contents	Target cells	Function	References
Grape	200–800 nm	proteins, lipids (PA, PE, PC), miRNA	Gastrointestinal tract (RAW-264.7)	Amelioration of DSS-induced colitis; Normalization of intestinal stem cell expression (Lgr5)	Ju et al. (2013)
Lemon	50–150 nm	Doxorubicin (DOX); miRNA, proteins, lipids	DOX-resistant ovarian tumor cells	Suppression of tumor cell growth and proliferation of chronic myeloid leukemia	Xiao et al. (2022)
Grapefruit	150–200 nm	Methotrexate (MTX), Doxorubicin (DOX), Curcumin (Cur)	Intestinal macrophages, Liver macrophages, Colon cancer	Enhances the chemotherapeutic inhibition of tumor growth through the delivery of therapeutic drugs	Wang et al. (2014)
	50–100 nm	miR17, miR-18a	GL26 brain tumor cells, Colon cancer	Amelioration in body weight loss and colon shortening in the mice model; Prolongation of survival rate	Zhuang et al. (2016)
	-	JSI-124, siRNA, paclitaxel (PTX)	Cancer cells	The enhanced therapeutical effect of IGNVs on inhibition of colitis; Prolongation of survival rate	Wang et al. (2013)
Broccoli	100 nm	AuNPs	Tumor cells	Amelioration of various types of colitis; Normalization of intestinal mucus layers	Deng et al. (2017), Singh et al. (2018)
Acerola	200–300 nm	miRNA	Bloodstream	Transports small RNA to the digestive system *in vivo*	Umezu et al. (2021)
Ginger	200–400 nm	Doxorubicin (Dox)	Colon cancer cells	Enhances the chemotherapeutic inhibition of tumor cell growth by delivering FA-mediated anti-cancer agents	Zhang et al. (2016c), Zhang et al. (2017)
			Colon-26 cells and HT-29 cells		
	100–200 nm	miRNA, proteins, lipids, siRNA-3WJ	Tumor cells (somatic cells, KB)	Inhibition of tumor cell growth with FA-3WJ siRNA ligand/GDENs	Li et al. (2018)
	50–100 nm	miRNA, proteins, lipids (PA, DGDG, MGDG, PC), metabolites (gingerol, shogaol)	Gastrointestinal tract (RAW 264.7)	Alleviation of the liver damage induced by alcohol	Zhuang et al. (2015)

(Raimondo et al., 2018). Specifically, it was shown that the proteomics analysis revealed a correlation between the suppression of acetyl-CoA carboxylase (ACC) and the anti-cancer impact of these PDEVs, specifically in the case of colorectal cancer (Raimondo et al., 2018).

Numerous research studies have focused on the development of drug-delivery systems that possess the ability to target particular cells, tissues, or organs specifically. PDEVs exhibited a promising potential due to their inherent capacity to target desired tissues specifically. *Zingiber officinale*-PDEV exhibited distinct transport characteristics in a study focused on devising strategies to protect liver cells against the toxicity of alcohol. *Zingiber officinale*-PDEVs labeled with tetramethylindotricarbocyanine iodide (DiR) were observed to accumulate primarily in the liver *in vivo* assay. Using confocal microscopy to image immunostaining provided evidence that *Z. officinale*-PDEVs could enter and shift the hepatic vascular system. Caco-2 cells were found to internalize PDEVs derived from apples following co-incubation (Fujita et al., 2018). Therefore, the inherent targeting capabilities of PDEVs are expected to enhance their application as natural drug delivery systems.

4.2 PDEVs engineering

PDEVs have exhibited some inherent advantages, containing stability, firmness, and an appropriate structure, enabling the inclusion of drugs in their lipid bilayer and precise targeting of specific tissues (Wang et al., 2013; Wang et al., 2015; Teng et al., 2018). Recent studies also suggested that PDEVs could transport biomolecules safely and maintain long-term circulation in the blood when administered systemically. Besides inherent biological activities, PDEVs could be engineered as desired drug delivery platforms for the precise delivery of poorly soluble agents or therapeutic compounds, such as surface functionalization and content modification. Recent studies have focused on modification techniques to improve the drug delivery precision and efficiency of PDEVs (Chen et al., 2023).

4.2.1 PDEVs membrane surface functionalization

Numerous studies have also indicated that PDEVs inherently possess the capability to target cells specifically. The surface of PDEVs, consisting of different lipids, exhibited distinct advantages like stability and precise targeting (Wang et al., 2013; Huang et al., 2023). The lack of studies compared with MEVs has resulted in limited knowledge about the fundamental mechanisms responsible for their natural targeting capabilities. However, recent studies implicated that the specificity of PDEVs is conferred by a specific alignment of proteins and lipids. According to research on the source plants, it has also been proposed that the selective absorption of PDEVs by particular cells is attributed to specific genes (that encode miRNAs and siRNAs) and small compounds, which act as external ligands for the targeting components on the cells (Wang et al., 2013; Teng et al., 2018). PDEVs exhibit excellent inherent targeting ability, minimal off-target effects, and rare toxicity compared to synthetic nanoparticles. It excludes the necessity for extra alterations to enhance biocompatibility, internal stability, and properties related to

pharmacokinetics, which would otherwise be necessary to increase their effectiveness as treatments when they are modified with particular ligands. Furthermore, PDEVs are regarded as optimal nanocarriers due to their inclusion of typical proteins involved in transfer and trafficking (Ju et al., 2013; Wang et al., 2014). Although they possess inherent targeting characteristics, numerous endeavors have been made to refine their targeting effects and enhance the efficacy of their intracellular delivery (Teng et al., 2018). PDEVs could be developed to precisely and consistently target organs or cells by modifications using targeting ligands, like chemicals and genetic drugs on their membrane, and merging with cell membranes (Wang et al., 2013). These biomolecular modifications on the surface of PDEVs have proved to improve the stability and uniformity of the nanostructures (Li et al., 2018).

Several modification techniques focus on the surface functionalization of PDEVs to incorporate cell membranes or ligands. For instance, some studies focused on improving the precision of PDEVs, and the lipid-thin film is formed by adding small molecules like folic acid (FA) to the extracted lipids from PDEVs, which are applied to fabricate nanovesicles. It also demonstrated the targeting effect of the ligands exhibited on the membrane surface via the assay of biodistribution *in vitro* and *in vivo* (Wang et al., 2013; Yáñez-Mó et al., 2015). Following intravenous administration, some studies demonstrated that FA-PDEVs remained detectable even after 48 h of circulation *in vivo*, thereby enhancing their potential to infiltrate tumors. Thus, FA-PDEVs might serve as a perfect carrier for delivering drugs against tumors without causing the adverse impact of freely circulating medications. Some studies developed a drug delivery system utilizing *Z. officinale*-PDEVs combined with siRNA as targeting ligands (Adams, 1973; Lewis and Metcalf, 1988). Different physical configurations and angles, referred to as arrow-tail and arrow-head, were achieved by manipulating the RNA three-way junction (3WJ) architecture. The analysis of their absorption by cancer cells revealed varying degrees of cellular internalization based on the orientation of the 3WJ nanostructure. In order to selectively focus on cancer cells that have an overexpression of folate receptors, arrow-tail siRNA was conjugated with FA, which served as a targeting ligand and attached to the outer surface of the ginger-PDEVs. The decreased levels of survivin mRNA levels confirmed the therapeutic potential of FA-3WJ-PDEVs. These vectors led to a significantly enhanced distribution of tumor tissues compared to unmodified PDEVs (Wang et al., 2013). Recent studies also designed an intelligent delivery platform by utilizing PDEVs covered with a plasma membrane derived from activated leukocytes, aiming to improve their therapeutic capabilities and targeting precision (Wang et al., 2015). Moreover, using the extrusion technique, PDEVs were ready to create a personalized transport mechanism by merging with the cell membrane derived from leukocytes. Zhuang et al. also designed biomimetic nanoplatforms that integrated electrodynamic Pd-Pt nanosheets and PDEVs from *Z. officinale* (Qiao et al., 2022). These engineered PDEVs endow great anti-infection with prolonged blood circulation and accumulation at infection sites. In this process, the engineered PDEVs could specifically target inflamed tissues and diminish the off-side impacts while transporting drugs (Wang et al., 2015).

4.2.2 PDEVs contents modification

The technology of encapsulation in the delivery system could transport biomolecules safely and maintain long-term circulation in the blood when administered systemically. As a result, PDEVs with content modification may be regarded as promising vectors for targeted delivery in tumorigenic disorders and chronic illnesses (Teng et al., 2018; Wang et al., 2019). Multiple techniques have been devised for loading diverse shipments into PDEVs. PDEVs can be loaded with desired cargo molecules using passive loading techniques, such as co-incubating with drugs (Vashisht et al., 2017). However, passive loading methods might not succeed in attaining a substantial encapsulation rate. Thus, subjecting PDEVs to sonication, freeze-thaw cycling, and similar mechanical treatments can destroy the integrity of their membranes and improve the efficiency of loading cargo. In order to prevent potential internal toxicity caused by impurities and achieve nanocarriers with consistent size, PDEVs can be designed using lipids and combined with cargo while preparing a lipid thin film (Li et al., 2018; Uddin et al., 2023). The method efficiently loads various shipments, including lipophilic drugs (DOX) and biomacromolecules like antibodies, siRNA, as well as DNA expression vectors (Wang et al., 2013; Zhang et al., 2016c). The encapsulation efficiency of doxorubicin through this method has resulted in high rates (95.9% ± 0.3%) (Zhang et al., 2016c). Moreover, conventional drug delivery systems loaded with some gene medicines, including siRNA and miRNA, have been lucubrated. However, the limited loading efficiencies of substances result in suboptimal therapeutic effects, and adverse consequences appear inevitable (Amreddy et al., 2017; Mainini and Eccles, 2020). In contrast, recent studies implicated that PDEVs could address these disadvantages (Zhu et al., 2022). For example, some studies suggested PDEVs from *Z. officinale* were used to carry specific siRNA targeting CD98 for the treatment of ulcerative colitis (Zhang et al., 2017). In comparison to a commercially accessible cholesterol and dioleoylphosphatidylethanolamine liposome product (DC-Chol/DOPE), it was identified that these PDEVs exhibited excellent biocompatibility, causing reduced toxicity and apoptosis in macrophage and eliminating colon-26 cells during *in vitro* assay (Zhang et al., 2017; Li et al., 2023). The PDEVs from *Z. officinale* were transfected with siRNA against CD98 using sonication to assess the mRNA expression of CD98. In detail, after being orally administered, the complexes of siRNA-CD98/PDEVs were successfully retained in the gastrointestinal tract and exhibited a notable decrease in CD98 expression in the gastrointestinal system compared to scrambled siRNA/PDEVs (Zhang et al., 2017). PDEVs derived from *Z. officinale*, which have low toxicity and high biocompatibility in contrast to synthetic liposomes, were discovered to be appropriate for transporting divalent metal-ion transporter-1 (Dmt1)-siRNA blunts to intestinal epithelial cells via reducing iron accumulation in hereditary hemochromatosis (Wang et al., 2019). Moreover, these PDEVs incorporating folic acid (FA) can enhance their capacity to specifically target the duodenum and seamlessly integrate into the intestinal tract through the proton-coupled folate transporter. In detail, they found that these PDEVs may decrease ferritin, transferrin saturation (TSAT), and non-heme Ferrum in multiple organs, such as the pancreas, heart, kidney, and liver, by reducing Dmt1-mRNA expression and iron loading (Wang et al., 2019).

4.3 Engineered PDEVs as the therapeutic system and bioactive molecule delivery system

Engineered PDEVs offer several unusual properties that render them highly appropriate as drug delivery systems for future medical

purposes, such as transporting hydrophobic medications, improving the target precision, evading enzymatic decomposition, and eluding immune response. For example, some content modification of PDEVs has ensured excellent stability and biocompatibility in diverse physiological environments, including the bloodstream and different pH. All of these stellar features, coupled with the discovery of their intrinsic therapeutic actions, make engineered PDEVs desired for their advent into the arena of bioactive molecule delivery applications. Here, we shed light on engineered PDEVs as the therapeutic system and drug delivery systems.

Several studies have proved that the surface functionalization of PDEVs can prevent the harmful effects of chemotherapy by transporting a higher amount of medication to tumors rather than normal organs. For instance, FA-modified PDEVs are predominantly gathered in tumor tissues, whereas unmodified PDEVs are primarily localized to the spleen and liver. Interestingly, Zhang et al. also found that FA- PDEVs loading doxorubicin (DOX) has a dramatic effect on colon cancer (Zhang et al., 2016c). FA-PDEVs-DOX or FA-PDEVs were fabricated using a conventional approach that relies on the hydration of lipid films, which is similar to the technique employed for producing liposomes. Compared with unmodified PDEVs, FA-PDEVs showed an enhanced capacity to target colorectal tumor cells via active FA and folate receptor (FR) interactions. While the FA-PDEVs exhibited dramatic reduction levels within the spleen and liver compared to PDEVs. It suggests that utilizing FA-PDEVs as the delivery platform may diminish the systemic toxicity of drugs toward healthy tissues. Following intravenous administration, some studies demonstrated that FA-PDEVs remained detectable even after 48 h of circulation *in vivo*, thereby enhancing their potential to infiltrate tumors. Thus, FA-PDEVs might serve as a perfect carrier for delivering drugs against tumors without causing the adverse impact of freely circulating medications. In addition, recent studies also designed an intelligent delivery platform by utilizing PDEVs covered with a plasma membrane derived from activated leukocytes, aiming to improve their therapeutic capabilities and targeting precision (Wang et al., 2015). By modifying the membrane-coated PDEVs, specialized inflammatory receptor-enriched plasma membrane-coated PDEVs (IPDEVs) were obtained, which possess distinct cellular targeting capabilities. To evaluate the inflammatory potential of IPDEVs or PDEVs, they were introduced to the upper layer of a transwell plate containing a cultured monolayer of human umbilical vein endothelial cells (HUVECs), which served as a blood-brain barrier model. The study yielded more significant quantities of IPDEVs migrating through HUVECs within 48 h compared to PDEVs, and the inclusion of chemokines further improved the efficiency of migration. The impact of IPDEVs on peripheral circulation was further validated in models of inflammation and chronic inflammatory cancer models. Compared to PDEVs, the inflammatory sites exhibited a notable rise in the accumulation of IPDEVs, and *in vivo*, IPDEVs and DOX were consistently administered for tumor treatment. Compared to free-DOX and PDEVs-DOX, IPDEVs demonstrated an improvement in tumor permeability and a decrease in tumor volume. As the result of inflammation is a prevalent occurrence in numerous affected tissues, the exceptional capacity of these engineered PDEVs to specifically target sites of inflammation renders them highly effective nanocarriers.

In addition, the technology of encapsulation in the delivery system could transport biomolecules safely and maintain long-term circulation in the blood when administered systemically. Multiple studies suggested that PDEVs with content modification may be regarded as promising vectors for targeted delivery in tumorigenic disorders and chronic illnesses. As a result, they have the capability to transport medications with accurate localization to specific tissues instead of causing widespread effects throughout the body, resulting in enhanced therapeutic results and reduced side effects. To evaluate the drug-carrying capability of PDEVs, multiple groups assess the effectiveness of ginger-PDEVs in loading and releasing doxorubicin through a pre-loading strategy (Zhang et al., 2016c). Furthermore, the drug release kinetics of PDEVs containing doxorubicin and DC-Chol/DOPE liposomes loaded with doxorubicin were analyzed at different pH levels (pH 5.5, 6.0, 6.5, 7.0, and 7.5). PDEVs from *Z. officinale* carrying doxorubicin could effectively secret DOX in an acidic tumor microenvironment, and these PDEVs diffused more rapidly compared to the liposomes (Zhang et al., 2016c). The capacity of PDEVs derived from *Citrus paradisi* to offer precise drug delivery to intestinal macrophages was also assessed (Wang et al., 2013). According to their findings, the primary immune cells of the intestine, known as intestinal macrophages, took up the majority of PDEVs from *Citrus paradisi* in the lamina propria of both the small and large intestines (Wang et al., 2013). However, liposomes that are readily accessible in the market exhibited significantly lower efficacy in directing their action toward intestinal macrophages. Moreover, PDEVs from *Citrus paradisi* were developed to conjugate with methotrexate (MTX), a chemical drug that suppresses the immune response and reduces inflammation (Zhuang et al., 2016; Niu et al., 2021). In a mouse model of ulcerative colitis induced by DSS, PDEVs from *Citrus paradisi* loading MTX could alleviate the loss of body weight and the shortening of the colon. These findings indicate alleviated colon tissue damage and infiltration of inflammatory cells in mice treated with grape-PDEVs-MTX (Niu et al., 2021). Accordingly, the PDEVs-MTX were effective in targeting intestinal macrophages for treating inflammatory-related diseases in the intestines (Nishad et al., 2021; Niu et al., 2021).

5 Conclusion and future perspectives

Ever since the identification of PDEVs, they have demonstrated remarkable characteristics in the fields of biotherapy, delivering medication, and traversing physiological obstacles. PDEVs, derived from plant sources, are believed to hold immense promise in the field of biomedicine, particularly as drug delivery systems. PDEVs present excellent benefits compared to artificial carriers and MEVs in terms of drug encapsulation, resistance to gastrointestinal fluid, cellular absorption, efficiency, and safety. However, the technological limitations currently constrain the utilization of PDEVs as drug delivery systems.

Firstly, one of the primary challenges encountered by drug delivery applications of PDEVs is the absence of a universal and standardized approach to separation techniques. In the laboratory, various techniques like ultracentrifugation, density gradient centrifugation, ultrafiltration, and size exclusion chromatography find extensive application for the isolation and analysis of PDEVs. In

comparison, the application of these techniques in the process of industrial transformation is hindered by the time-consuming and limited productivity. Researchers using ultracentrifugation may experience inconsistent results due to variations in centrifugation speed, force, and rotor type, as reported (Kameli et al., 2021b). Thus, it is imperative to investigate and develop a perfect isolation procedure to acquire consistent, steady, and productive PDEVs. Furthermore, it is also crucial to precisely analyze and regulate the quality and origin of raw materials owing to variations in plant types, growth phases, and geographical locations.

Further exploring the biological properties and transportation mechanisms of PDEVs remains one of the primary obstacles in utilizing them for drug delivery. PDEVs demonstrate exceptional tissue specificity and gastrointestinal resilience, yet this specific characteristic remains insufficiently elucidated. Further elucidating the tissue-specific targeting mechanism of PDEVs has the potential to overcome the challenge encountered in drug delivery applications. The ordered structure or lipid composition of PDEVs may enhance these capabilities of PDEVs. To understand these mechanisms of PDEVs, gaining a comprehensive comprehension of their physical characteristics might be the appropriate direction. The regulation of information in PDEVs appears to involve proteins, lipids, and miRNA, and the source of PDEVs may contribute to the variation in regulation. While articulating a comprehensive regulatory mechanism appears to be challenging, it may be more efficient to concentrate on regulating PDEVs in particular plants or species rather than across all plants. Simultaneously, the differentiation between regulatory mode and targeting mechanism facilitates the administration of diverse medications, enabling researchers to select from a range of PDEV origins based on their suitability for a particular ailment. Furthermore, the unpredictable element of the natural active component transported by PDEVs is not only capable of enhancing the effects of medications but also influencing their effectiveness (Nemati et al., 2022). Therefore, it is imperative to conduct comprehensive and profound research to elucidate the makeup of natural active constituents in PDEVs and the interaction mechanism between these constituents and medications to achieve efficient incorporation or collaborative therapeutic outcomes.

Insufficient targeting ability poses a challenge for the drug delivery application of PDEVs. On the other hand, proteins may have a critical role in receptor recognition. Several studies have indicated that PDEVs exhibit a lower protein count in comparison to MEVs, implying that PDEVs possess relatively limited selectivity (Pocsfalvi et al., 2018). The application of engineering was extensively utilized in the examination of MEVs, resulting in enhanced flexibility of MEVs (van der Koog et al., 2022). Thus, engineering PDEVs is expected to play a crucial role in the application of PDEVs as drug delivery platforms. PDEVs could also be modified using ligands like FA or peptides to enhance cargo targeting capability. The utilization of membrane fusion technology has the potential to combine PDEVs with synthesized liposomes, as well as mammalian EVs and bacterial EVs. The resulting hybrid PDEVs could potentially provide enhanced biological attributes. This strategy could improve the targeting precision and immune evasion capabilities of PDEVs.

In particular, several clinical studies on the therapeutic activities of PDEVs for clinical management have been registered (Alfieri et al., 2021). For example, the derivants of PDEVs as a delivery system to increase the bioavailability of oral curcumin are being tested on colorectal cancer patients (NCT01294072) (Wu et al., 2017). In a subsequent study, PDEVs derived from grapes and their derivates were proven to alleviate adverse reactions induced by chemoradiation in head and neck cancer patients, such as inflammatory and oral mucositis pain (NCT01668849) (Wu et al., 2017). Another clinical trial confirmed the prospects against inflammation of PDEVs from *Z. officinale* for effectively alleviating irritable bowel disease (NCT04879810) (Zhang et al., 2016a). Thus, considering these promising advantages, the researchers should carry out more preclinical and clinical trials to evaluate the bioactivities of PDEVs and engineered PDEVs in patients and define their minimum dosage. However, the application of PDEVs in clinical management is still limited by technology.

Firstly, one of the main problems of PDEVs translated from lab to clinical state is the lack of unified and standardized separation methods. These isolation methods have the problems of time-consuming and low yields in the industrial transformation (Kameli et al., 2021b). For example, ultracentrifugation may be a cost-effective and simple method, but it has been reported to disrupt PDEVs due to the centrifugation force and speed (Ramirez et al., 2018). Moreover, there have reportedly been inconsistencies in the separation of EVs by ultracentrifugation due to the rotor (Furi et al., 2017). Additionally, ultracentrifugation and other methods also have the risk of co-contamination with toxic proteins (Momen-Heravi et al., 2013). Further research of PDEVs to understand their biological role and transport mechanism in physiological or pathological processes may be one of the major challenges in the transformation from lab to clinical application (Cloet et al., 2021). For instance, components in PDEVs seem to be involved in the transport or regulation mechanism, and their different sources may be the primary reason (Kameli et al., 2021a). However, the current general regulation may be complicated to depict clearly. Therefore, it could be more effective to focus on the regulation of PDEVs in specific species rather than all plants.

Overall, drug delivery systems based on PDEVs demonstrated dramatic benefits in terms of biocompatibility, stability, distribution, and cellular absorption. Furthermore, employing PDEVs for the administration of biomolecules can decrease the adverse reactions of medications while also exhibiting the inherent capabilities of PDEVs. The unique attributes of PDEVs allow for the development of an innovative approach to drug delivery platforms. Since biomedical research on PDEVs is still in the infancy stages, there may be numerous challenges in utilizing PDEVs as drug carriers. Accordingly, further exploration of PDEVs in drug delivery is worthwhile, as these challenges can be overcome through additional research in the future.

Author contributions

PW: Conceptualization, Writing–original draft, Writing–review and editing. WW: Writing–original draft. SZ: Writing–original draft. JH: Writing–review and editing. CL: Visualization, Writing–review and editing. HY: Visualization, Writing–review and editing. XpC: Conceptualization, Supervision, Writing–original draft. XaC: Conceptualization, Writing–original draft.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was supported by grants from the Sichuan Science and Technology Program (Grant Nos 2022JDRC0131 and 2023NSFSC1759), the Sichuan Administration of Traditional Chinese Medicine (Gran No. 2023MS333), the China Postdoctoral Science Foundation (Grant Nos 2021M693784 and 2022T150078), the National Natural Science Foundation of China (No. 82004097), Potential Postdoctoral Program of Chengdu University of Traditional Chinese Medicine Grant No. BSH2019019.

Acknowledgments

We would like to acknowledge Yifan Bao (University of Vienna) for the construction of the figures, which are graphics created with BioRender.com. We also acknowledge the cited publications in this review, yet we were unable to mention them due to space limitations.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 14 December 2023
DOI 10.3389/fphar.2023.1295789

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OPEN ACCESS

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RECEIVED 17 September 2023
ACCEPTED 27 November 2023
PUBLISHED 14 December 2023

CITATION
Li Y, Zhang J, Fan J-y, Zhong S-h and Gu R (2023), Tibetan medicine Bang Jian: a comprehensive review on botanical characterization, traditional use, phytochemistry, and pharmacology. *Front. Pharmacol.* 14:1295789. doi: 10.3389/fphar.2023.1295789

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Tibetan medicine Bang Jian: a comprehensive review on botanical characterization, traditional use, phytochemistry, and pharmacology

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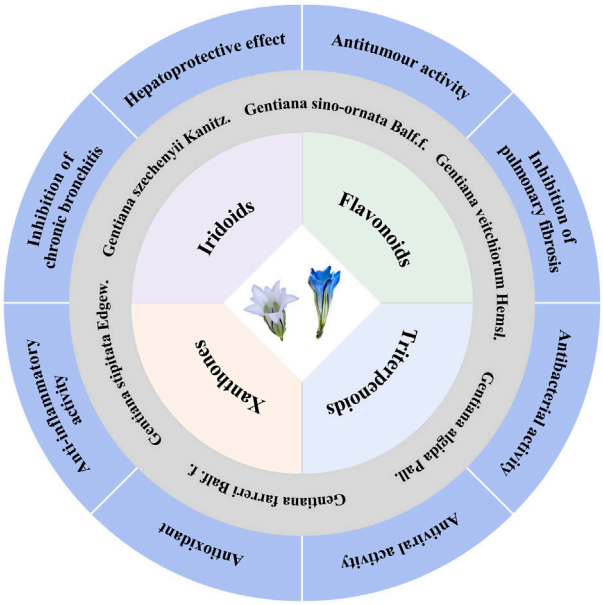
Tibetan medicine Bang Jian refers to a range of botanical drugs within the *Gentiana* genus. It serves as a prominent traditional Tibetan botanical drug primarily found in the ethnic minority regions of the Qinghai-Tibet Plateau in China. Traditionally, the dried flowers of Bang Jian, known as "Longdanhua" have been employed in Tibetan medicine to address detoxification, pharyngeal relief, acute and chronic bronchitis, bronchiectasis, lung infections, pulmonary fibrosis, and throat disorders. Surprisingly, there has been no comprehensive review published to date on Tibetan medicine Bang Jian. This passage systematically presents and critically assesses recent advancements in botanical characterization, traditional applications, phytochemistry, pharmacology, and clinical uses of Bang Jian, aiming to provide a scientific foundation for its reasonable use and further exploration. To date, researchers have isolated and identified 92 structurally diverse compounds, with a predominant presence of iridoids, flavonoids, xanthones, and triterpenoids. The crude extracts and metabolites derived from Bang Jian have been found to exhibit a wide range of pharmacological effects, encompassing anti-inflammatory, anti-tumor, anti-bacterial, antiviral, antioxidant, hepatoprotective properties, and protect the respiratory system. Nevertheless, detailed data on the biological effects, metabolic activities, and mechanistic research concerning active monomer metabolites remain insufficient. Consequently, there is a pressing need for comprehensive and in-depth research to guide rational clinical drug usage and evaluate the medicinal attributes of Bang Jian.

KEYWORDS

Bang Jian, botanical characterization, traditional use, phytochemistry, pharmacological activities

1 Introduction

Approximately 400 species fall within the genus *Gentiana*, widely dispersed across Southeast Asia and Europe (Zhang H. T. et al., 2009). Predominantly, they thrive at high altitudes in regions like Gansu, Sichuan, Yunnan, and other parts of China (Committee for the Flora of China, 2001). The roots and rhizomes of Gentian plants are frequently used in Chinese medicine to treat jaundice and eczema and are thought to have the effect of purging



GRAPHICAL ABSTRACT

liver and gallbladder fire. Interestingly, while Tibetan medicine often uses the flowers or whole grasses of Gentian plants to treat cough and remove heat from the lungs (Bai et al., 2016). It involves a variety of Gentian plants, which are collectively referred to as "Bang Jian" in Tibetan medicine literature.

Bang Jian, which translates to "beautification of the grassland," originates from the blossoms of various gentian plants within the *Gentiana* genus of the Gentianaceae family. Bang Jian primarily thrives in Tibet, Gansu, Qinghai, Sichuan, and Yunnan provinces (Committee for the Flora of China, 2001). Tibetan medicine literature often adopts the form of "classification." The categorization of Bang Jian often hinges on the flower's color, with the Jingzhu Materia Medica identifying three categories: white, blue, and black (Dimaer, 1986). However, the Bang Jian category in Tibetan botanical drugs comprises several species with intricate root origins. Prominent species include the white-flowered gentians *G. szechenyii* Kanitz. (*Gentiana szechenyii*), *G. algida* Pall. (*Gentiana algida*), and *G. stipitata* Edgew. (*Gentiana stipitata*), along with the blue-flowered gentians *G. veitchiorum* Hemsl. (*Gentiana veitchiorum*), *G. sino-ornata* Balf.f. (*Gentiana sino-ornata*), and *G. lawrencei* Burkill. (*Gentiana lawrencei*). A wide array of metabolites has been isolated from Bang Jian, encompassing iridoids, flavonoids, triterpenoids, xanthones, and organic acids. Notably, iridoids stand out as the most active metabolites, serving as markers to gauge Bang Jian's quality. Beyond its chemical complexity, Bang Jian has demonstrated a broad spectrum of biological functions. Modern pharmacological investigations have revealed its anti-inflammatory, anti-tumor, anti-bacterial, anti-viral, hepatoprotective, and respiratory protection. Clinically, it effectively addresses bronchitis, lung infections, bronchiectasis, pulmonary fibrosis and throat diseases (Liu et al., 2006; Zhang et al., 2014; Zhao et al., 2018).

These distinctive conditions contribute to its unique potency and efficacy. Typically found at high altitudes in alpine meadows or scrubland (Committee of State Administration of Traditional Chinese Medicine for Chinese Materia Medica, 2002), some Bang Jian species face resource depletion due to overharvesting, endangering their survival. *G. szechenyii*, for instance, is classified as a first-class endangered Tibetan medicine (Zhong et al., 2014). Therefore, it is crucial to raise awareness of the necessity for conserving this species by strengthening Bang Jian's protection measures and maximizing its utilization efficiency. With the ongoing progress and development in science, researchers should also actively conduct experiments to explore artificial cultivation methods suitable for Bang Jian, thus promoting its survival and sustainable use. Besides, Modern research has demonstrated the intricate chemical composition of traditional Chinese medicines, and there has been continuous advancement in analytical techniques to glean further insights into these medicinal substances. While existing literature has reported on the biological activity and chemical makeup of Bang Jian, recent advancements in technology have enabled the discovery of new compounds and facilitated more in-depth exploration of its pharmacological properties and clinical applications.

At present, some scholars have summarized the ethnic medicinal uses, phytochemistry and pharmacological activities of Gentianaceae in Tibetan Medicine. They mainly include Bang Jian, Ji Jie, Di Da, and Gang Ga Qiong. The inter-species differences are compared mainly for different species of Gentianaceae Tibetan medicines. However, comparisons of different origins of the same species are lacking. In addition, the pharmacological activities of Bang Jian are mostly concentrated on the prevention of upper respiratory tract infections (Chi et al., 2021). Other pharmacological activities of Bang Jian were not involved. In conclusion, the phytochemistry and other pharmacological activities of different origins plants in Bang Jian botanical drugs need to be further summarized.

Besides, as Bang Jian is the most species of Gentianaceae in Tibetan medicine, the market circulation is complicated. And there

are also mixed uses in hospitals. What's more, Bang Jian is a multi-original species. Its specific botanical characteristics, phytochemistry and pharmacological activity are different between each of the different species. This may affect the basis for its use in clinical practice. Therefore, it is necessary to carry out a more detailed and specific review of the Bang Jian of Tibetan botanical drugs. To the best of our knowledge, a comprehensive review of Tibetan medicine Bang Jian is currently absent. Consequently, this review seeks to consolidate more comprehensive knowledge from existing literature concerning Bang Jian's traditional usage, phytochemistry, pharmacological effects, and clinical research. Its aim is to establish a theoretical foundation for the reasoned advancement and utilization of Bang Jian, including the exploration of novel dosage forms.

2 Methods

The relevant information on Bang Jian was collected from PubMed, Web of Science, ScienceDirect, Google Scholar, Springer, Elsevier, CNKI, Baidu Academic, JSTOR and CBM database. Other literature sources include the Chinese Pharmacopoeia and Tibetan medicine classical books. Firstly, the keywords used included *Gentiana* (Tourn.) L, *G. szechenyii* Kanitz., *G. algida* Pall., *G. stipitata* Edgew., *G. veitchiorum* Hemsl., *G. sino-ornata* Balf.f., *G. lawrencei* Burkill., Longdanhua, Bang Jian (Longdanhua of Tibetan name), without any other restrictions, a total of 242 related documents were searched. Subsequently, on this basis, the search was repeated again with botanical characterization, traditional uses, phytochemistry, and pharmacological activity as secondary keywords, respectively. Finally, 91 representative relevant literatures were obtained. All of these are included in this review.

3 Botanical characterization

The Tibetan medicine Bang Jian is a collective name for part of the medicinal plants of the genus *Gentiana*. It is in the genus *Gentiana* mainly includes *Gentiana* Sect. *Frigida* (*G. algida*, *G. algida* var. *purdomii* (C.Marquand) T.N.Ho, *Gentiana atuntsiensis* W.W.Sm., etc.), Sect. Monopodiae Ser. Ornatae (*G. veitchiorum*, *G. sino-ornata*, *G. lawrencei*, etc.), Sect. Monopodiae Ser. Confertifoliae (*G. szechenyii*, *Gentiana stipitate*, *Gentiana confertifolia* C.Marquand, etc.) (Committee for the Flora of China, 2001). It has a long history of medicinal use in Tibetan medical treatment and is representative of the commonly used bulk of Tibetan medicinal botanical drugs, which are contained in many Tibetan medical classics and Materia medica.

Tibetan medicine literature often according to the "classification type" form to record the list: such as the "Jing Zhu Materia Medica" records, the list according to color is divided into three kinds of white, blue, and black flowers, called "Bang Jian Babao," "Bang Jian Wanbao" and "Bang Jian Nabao," and the "Four Medical Tantra Blue Sapphire" is divided into three types of white, blue, and miscellaneous flowers, miscellaneous flowers called Bang Jian Chawu (Disi, 2012). Although there are flower color classifications in the literature, Tibetan medicine in the actual recognition of the field and the use of medicinal botanical drugs often dilutes the blue flower and black flower distinction. In our group's preliminary investigation of the Bang Jian of three types of inter-list varieties of wild resources, Tibetan medicine market, and other investigations found that a total of 22 species, 4 varietas, including white-flowered gentian collated 8 species, 1 varietas, blue-flowered gentian collated 11 species, 2 varietas, black-flowered gentian 4 species, miscellaneous flowers gentian 5 species, 1 varietas (Zhong et al., 2014; Fu et al., 2018).

However, due to the complexity of the gentian plant, the three types of Tibetan medicine recorded in various Materia medica Bang Jian identification description are not clear, the source is complex, the classification is confusing, and there is a certain degree of subjectivity. The main species are *G. szechenyii*, *Gentiana purdomii*, *G. algida*, etc (Fu et al., 2018). In brief, several scholars on its varieties of evidence, the results of several scholars' examination of its species, as well as literature, specimen records, herbal market and local Tibetan hospital visits and investigations, found that *G. szechenyii*, *G. algida,* and *G. stipitate* are the mainstream varieties of white-flowered gentian, and that the mainstream varieties of blue-flowered gentian are *G. veitchiorum*, *G. sino-ornata*, and *G. lawrencei* (Figure 1) (Zhong et al., 2014; Tang et al., 2020). Some of the morphological characteristics are similar although Bang Jian is known as a taxonomically and phylogenetically challenging taxon.

According to "Du Mu Materia Medica" Born in a rocky meadow, with small leaves and numerous flowers (Xi, 2016). The "Four Medical Tantra Blue Sapphire" records that the leaves are small and green, elliptical in shape, and the stems are thin (Disi, 2012). Based on "Jing Zhu Materia Medica," white-flowered gentian grows on grassy slopes. The leaves are small, and the flowers are prosperous. The white-flowered gentian taste bitter which can cure febrile communicable disease, the blue-flowered gentian grows in very humid swampy grasslands in early autumn, with small leaves and light blue flowers, which are obvious, the black-flowered gentian grows in alpine meadows in mid-autumn, with a cyan surface on flowers, slightly larger than blue-flowered gentian. In addition, there is a mythological story about the flowering time of the three types of Tibetan medicine, white, blue, and black flowers of Bang Jian. It is believed that there are "three sisters of the fairy" in the autumn when all the drugs withered, the flowers withered, then turned into the list of three types of gentian, as a graphic description of the flowering time of the list of Tibetan medicine (Dimaer, 1986).

Based on recent morphological studies of medicinal botanical drugs in "Bang Jian," it has been found that, specifically, blue-flowered gentian is a perennial botanical drug with medium sized flowers and a few larger ones. The sepals are short, about one-third to one-half the length of the corolla, and the lobes are often shorter than the sepal tube, rarely as long as it. Most of the flower branches have flowers. Corolla funnel-shaped, sparsely inverted conical, 4–6 cm long. Their difference lies in the fact that the leaves in the middle and lower parts of the stem of the *G. veitchiorum* are wider, oval, or ovate lanceolate in shape, with a sharp apex but no small tip (Dimaer, 1986; Zhang, 2016). The stem leaves in the middle and lower parts of *G. lawrencei* and *G. sino-ornata* are narrow, linear-rectangular, or lanceolate, and the apex is acute. The corolla is light blue, with a yellowish white bottom and no spots. The difference between the two lies in the fact that there are no undeveloped twigs in the axils of the leaves of the *G. lawrencei*. And the leaves are often sparse, with finely linear in shape. There are very undeveloped branchlets in the leaf axils of *G. sino-ornata*. The leaves densely packed, linear lanceolate. *G. stipitate* and *G. szechenyii* have short stems. The leaves are densely packed in a lotus shape. The main root is thick, conical, or cylindrical. The lotus shaped leaf clusters of sterile stems are wider. Leaves and calyx lobes are broad with distinct cartilaginous edges (Wu and Wang, 2017). Most of

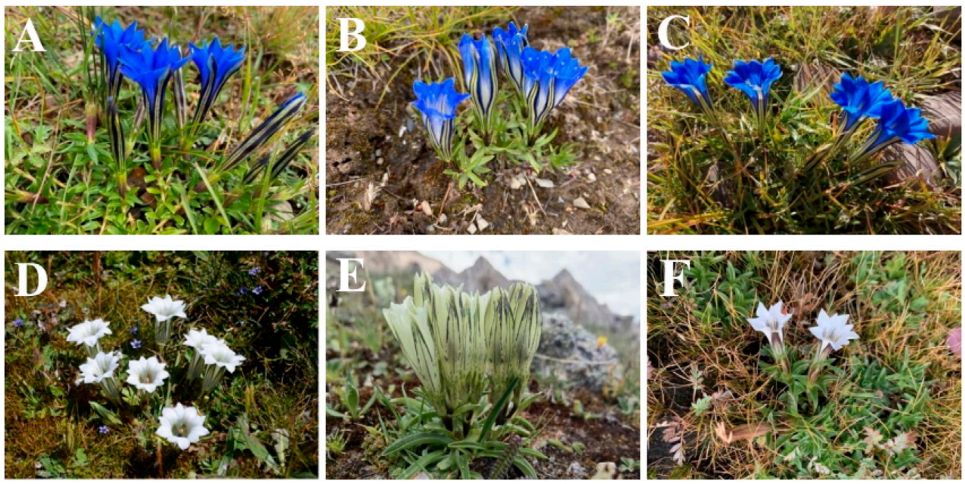


FIGURE 1
The plant pictures of mainstream varieties of Bang Jian. **(A)** *Gentiana szechenyii Kanitz.*, **(B)** *Gentiana algida* Pall., **(C)** *Gentiana stipitata* Edgew., **(D)** *Gentiana veitchiorum* Hemsl., **(E)** *Gentiana sino-ornata* Balf.f., **(F)** *Gentiana lawrencei* Burkill.

the flower branches are clustered and scattered, with a length of 7–10 cm; There are many pairs of stem leaves, with sparse lower parts and dense upper parts. The calyx lobes are oblanceolate, and the base shrinks. The corolla of *G. stipitate* is light blue gray. The branches are few and straight extending, 2–3 cm long. A few flower branches, straight extending, 2–3 cm long. The stem leaves are few and dense. The base of the calyx lobes is not narrowed. The corolla has deep blue gray wide stripes and spots, with white inside. And the calyx lobes of *G. szechenyii* are lanceolate (Ma et al., 2015). Unlike *G. szechenyii* and *G. stipitate*, *G. algida* have rhizomes or creeping stems, with well-developed rosette shaped leaf clusters (Ma et al., 2017). The shape of stem leaves is the same as the leaves of the lotus leaf cluster. The seeds are reticulately spongy and wingless. The calyx lobes are erect and neat with 1–3 (–5) flowers, and usually have sessile or shortly pedicels. The corolla has blue spots and no stripes. Summarized information on the characteristics of Bang Jian is given in Table 1 and Supplementary Material.

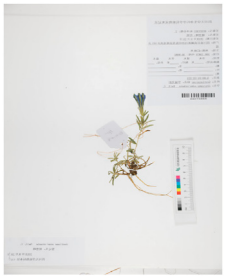
All species mainly grow on grassy slopes and forest margins at an altitude of 2,000–5,000 m in Gansu, Qinghai, Sichuan, Tibet, and Yunnan of China, while foreign distribution is mainly in India, northeastern Myanmar, the Soviet Union, Japan, and Nepal (Committee for the Flora of China, 2001).

As the Tibetan medicine Bang Jian is a multi-basic original plant, and there are more confusing products, the traditional identification method mainly based on morphological characteristics has its limitations. It is well known that DNA barcode technology has the advantages of not relying on the morphology of botanical drugs, excluding the subjective factors of the identifier, good reproducibility of the identification results, good versatility of the method, and easy to promote and standardize, etc (Kress, 2017; Fernandes et al., 2021; Yu et al., 2021). ITS sequence is one of the most commonly used molecular markers, which is widely used in the construction of phylogenetic tree and the analysis of population genetic structure. Our group constructed a phylogenetic tree by MEGA6.06 by combining the ITS sequences of the mainstream varieties of white-flowering gentian with the ITS2 sequences of the easily mixed varieties of white-flowering gentian retrieved from the GenBank database. The results showed that it was possible to separate the *G. szechenyii* from the *G. algida*, and that the ITS2 sequence could be used as a DNA barcode of *G. szechenyii* for the identification of *G. szechenyii* and its mixed forgeries (Zhang, 2016). However, it was also found that the ITS sequences of *G. veitchiorum* and *G. lawrencei* were highly heterozygous, and it was difficult to distinguish these two species by molecular fragment ITS, and further monoclonal sequencing was needed (Sun and Wang, 2019; Fu et al., 2020). It provides a reference for the barcode study and differentiation analysis of Tibetan medicine of Bang Jian.

4 Traditional uses

In China, as one of the traditional Tibetan medicines, Bang Jian has been recorded in many monographs on Tibetan medicines. According to textual research, as early as the 14th century AD, it was recorded in the Materia Medica Library: “It is believed that it is named according to its growing environment, such as Wawei and Yangou grass.” “Bang” refers to alpine meadow, and “Jian” means decoration. Ethnomedical applications of Bang Jian trace back to the middle of the eighth century B.C. Its medical values have been firstly recoded in the “Yue Wang Yao Zhen” that “Bang Jian” is used in the formula for lung diseases (Mao, 2012). For example, the medical book “Jing Zhu Materia Medica” records that the Bang Jian has a little astringent in taste, cool in nature, clears lung heat, treats poisonous diseases, cures all feverish diseases, detoxifies, and facilitates the throat. It has been recorded that blue flowers produce the same effect as white-flowered gentian but are cool in nature. The black one also has the effect of treating black scars and acne rashes, usually taken as a single flavor in water or powdered into a compound (Dimaer, 1986). In addition, Bang Jian was described detailed in the “Four Medical Tantra” reads: “Bang Jian ga bao (white flower) can cure laryngitis and detoxify heat” (Yutuo, 2005). It can be seen that “Bang Jian” has the exact effect in a very

TABLE 1 Summary of botanical characteristics and pictures of specimens of the dominant species of Bang Jian.

Classification	Species	Similarity	Differential	Herbarium pictures	Voucher no.
Blue flower	*G. veitchiorum*	① Flowers: medium, and a few larger ones	① The leaves in the middle and lower parts of the stem are wider, oval, or ovate lanceolate in shape, with a sharp apex but no small tip		KUN1479202
		② Sepals: short, about 1/3 to 1/2 the length of the corolla, and the lobes are often shorter than the sepal tube, rarely as long as it	② Corolla: dark blue		
		③ Most of the flower branches have flowers, corolla funnel-shaped, sparsely inverted conical, 4–6 cm long			
	G. lawrencei	① Flowers: medium, and a few larger ones	① No undeveloped twigs at the leaf axils		IGA0011348
		② Sepals: short, about 1/3 to 1/2 the length of the corolla, and the lobes are often shorter than the sepal tube, rarely as long as it			
		③ Most of the flower branches have flowers, corolla funnel-shaped, sparsely inverted conical, 4–6 cm long	② Leaves: sparse with finely linear in shape		
		④ The stem leaves in the middle and lower parts are narrow, linear-rectangular, or lanceolate, and the apex is acute			
		⑤ Corolla: light blue, with a yellowish white bottom and no spots			
	G. sino-ornata	① Flowers: medium, and a few larger ones	① Undeveloped branchlets at the leaf axils		SZ02027465
		② Sepals: short, about 1/3 to 1/2 the length of the corolla, and the lobes are often shorter than the sepal tube, rarely as long as it			
		③ Most of the flower branches have flowers, corolla funnel-shaped, sparsely inverted conical, 4–6 cm long	② Leaves: crowded with linear-lanceolate in shape		
		④ The stem leaves in the middle and lower parts are narrow, linear-rectangular, or lanceolate, and the apex is acute			
		⑤ Corolla: light blue, with a yellowish white bottom and no spots			
White flower	*G. szechenyii*	① Slightly fleshy fibrous roots	① Branches: few and straight extending, 2–3 cm long		SZ02029610
		② Short stems			
		③ Leaves: densely packed in a lotus shape	② Stem leaves: few and dense		
		④ Main root: thick, conical, or cylindrical	③ Calyx lobes: lanceolate, and not contracted at base		
		⑤ The lotus shaped leaf clusters of sterile stems are wider. Leaves and calyx lobes are broad with distinct cartilaginous edges	④ Corolla: deep blue gray wide stripes and spots, with white inside		
		⑥ Seeds: dark brown with light honeycomb reticulation on the surface			

(Continued on following page)

TABLE 1 (*Continued*) Summary of botanical characteristics and pictures of specimens of the dominant species of Bang Jian.

Classification	Species	Similarity	Differential	Herbarium pictures	Voucher no.
	G. stipitate	① Slightly fleshy fibrous roots	① A lot of flower branches, spreading out, with a length of 7–10 cm		SZ02031589
		② Short stems			
		③ Leaves: densely packed in a lotus shape	② Many pairs of stem leaves, with sparse lower parts and dense upper parts		
		④ Main root: thick, conical, or cylindrical	③ Calyx lobes: oblanceolate, and the base shrinks		
		⑤ Lotus shaped leaf clusters of sterile stems are wider. Leaves and calyx lobes are broad with distinct cartilaginous edges	④ Corolla: light blue gray		
		⑥ Seeds: dark brown with light honeycomb reticulation on the surface			
	G. algida	① Slightly fleshy fibrous roots	① Rhizomes or creeping stems, with well-developed rosette shaped leaf clusters		HNWP00026752
			② The shape of stem leaves is the same as the leaves of the lotus leaf cluster		
			③ The number of flowers are usually 1–3, sparsely up to 5, terminal		
			④ Calyx lobes: erect and neat		
			⑤ Corolla: blue spots and no stripes		
			⑥ Seeds: yellowish brown with spongy reticulation on the surface		

early time, mainly used for respiratory diseases. In Tibetan medicine, blue flowers are used to treat red eye headache, pharyngitis, damp heat, jaundice, and other symptoms. After investigation, the blue flower, Tibetan medicine called the *G. veitchiorum* combined with the investigation of clinical drug use, the varieties used by Tibetan hospitals and Tibetan medicine factories as *G. veitchiorum* are mainly *Gentian*, which are used as whole botanical drug.

It is also recorded in the annals of medicine of other ethnic minorities. The difference is that in other ethnic minorities, the medicinal parts are mainly concentrated on the whole botanical drug and the root (Table 2). It is recorded in "Kazak Pharmacy" that Alpine gentian *Gentiana. triflora* Pall., whole botanical drug into medicine, bitter, cold, with the function of clearing heat and detoxification, used for acute and chronic hepatitis, jaundice, cholecystitis, cystitis, hypertension, dizziness, tinnitus, and other diseases (BaHaErGuLi, 2009). "Uighur pharmacopoeia" contains gentian medicinal materials, also known as Jinti Yana, for *Gentiana scabra* Bunge., striped leaf *Gentiana manshurica* Kitag., three-flowered gentian *Gentiana triflora* Pall., strong *Gentiana crassa subsp. rigescens* (Franch. ex Hemsl.) Halda. are all rooted in medicine, with clear heat and dampness, diarrhea liver and eyes, mainly treating humid heat jaundice, scrotal itching, skin sores, eye redness and pain, high fever caused by liver fire, hand and foot spasm, seizures, and other symptoms. The roots and rhizomes of *Gentiana lutea* L. and the whole botanical drug of *G. algida* are also used for medicinal purposes (Liu and ShaWuTi, 1986). The Shangri-La Ethnic Medicine Guide contains: "*Gentiana aristata* Maxim.'s whole botanical drug is used as a Tibetan medicine" complete cloth, which has clear heat, dispel dampness, relieve cough, and mainly treat sore throat, cold fever, eczema and itching, lung fever, cough, bronchitis, and other symptoms. The root of *G. sino-ornata* Balf.f. cures nameless swelling and poison, huaqing heat and detoxification, treats influenza fever, lung fever, cough, and other symptoms (Liu and Zheng, 2008). In addition, one of the mainstream species of Bang Jian, *G. algida*, is also included in "The Haixi Mongol and Tibetan Materia Medica Resources." And it has a history of medicinal use in the Tibetan region of Qinghai. It can be seen that Bang Jian medicinal plants have rich medicinal plant resources with distinctive national medicinal characteristics, and are widely used in Tibet, Mongolia, Wei, Kazakh, and other ethnic minorities. In a word, Bang Jian have a wide range of traditional applications. The traditional uses of common species are summarized as the treatment of detoxification, various throat disorders including lung-heat cough, bronchitis, sore throat, and various other conditions. Therefore, Bang Jian as Tibetan medicine is worthy of further in-depth excavation and investigation, to provide a guidance for modern pharmacological studies.

5 Phytochemistry

The medicinal effects of botanical drugs are usually mediated by a variety of bioactive metabolites. Research into the chemical composition of Tibetan medicine Bang Jian began in 1980, focusing mainly on the mainstream species. To date, more than

TABLE 2 Traditional applications of Bang Jian in different ethnomedicines in China.

Ethnic groups/ areas	Medicinal parts	Source	Ethnomedicinal uses	References
Tibetan	flowers	*G. szechenyii*	Purification of lung heat, detoxification of toxin, relief of the throat, dipping of the actual fire in the gall bladder, treatment of seasonal fever; feverish cough, laryngitis and heat closure, poisonous diseases	Dimaer (1986)
		G. algida		
		G. stipitata		
		G. veitchiorum		
		G. sino-ornata		
		G. lawrencei		
Mongolian	flowers	*G. algida*	Heat clearing and toxin detoxification, cough relief and throat stimulation, sore throat, dullness, pulmonary fever, and toxic fever	Luo (1989)
		G. veitchiorum		
Kazak	Whole grass	*G. algida*	Heat and toxin removal, acute and chronic hepatitis, jaundice, cholecystitis, cystitis, hypertension, vertigo, and tinnitus	BaHaErGuLi (2009)
Uighur	Whole grass	*G. algida*	Clearing heat and drying dampness, diarrhea of the liver and clearing the eyes, damp-heat jaundice, redness and swelling of the eyes, constriction of the hands and feet, epilepsy etc.	Liu and ShaWuTi (1986)
Shangri-La	Roots; flowers	*G. sino-ornata*	Roots:tendon relaxation and blood revitalization to treat nameless swelling and toxicity; Flowers:Influenza, lung fever and cough, heat clearing and toxin detoxification	Liu and Zheng J. (2008)

ninety compounds have been isolated and identified by TLC, UV, IR, 1D and 2D NMR, HPLC, GC-MS, HRMS (Li, 2017; Li. et al., 2017), and other methods, mainly including iridoids (1–18), triterpenoids (19–47), flavonoids (47–73), xanthones (74–80), organic acids (81–87) and other compounds (87–92). Among them, the iridoids, flavonoids, triterpenoids, and xanthones have a strong physiological activity and are the main active metabolites in Bang Jian. A total of 92 were identified based on literature reports and chemical metabolites extracted from various medicinal parts. The names, classifications, formulation, botanical origin, and corresponding structures are shown in Table 3 and Figure 2.

5.1 Iridoids

Iridoids are primarily distributed among more than 50 plant families and are notably abundant in the Gentianaceae family. Within the genus *Gentiana*, a wealth of iridoids is found, with over 60 species of enol ether terpene glycosides identified to date. Moreover, across various plant species, the tally exceeds 1,000 isolated and identified iridoid compounds. In the realm of Tibetan medicine, the iridoids encompass iridoid glycosides, cleaved iridoid glycosides, strychnine, and several other significant compound groups (Xu et al., 2008). Iridoids play a pivotal role as key active metabolites in gentian plants and serve as characteristic metabolites within Tibetan pharmacopeia. Most of these metabolites are glycosides, with only a few exceptions. Among these, swertiamarin, sweroside, and β-sitosterol enjoy broader distribution in the plant kingdom (Li et al., 2016a; Li et al., 2016c; Olennikov and Chirikova, 2016). Structurally, these compounds exhibit distinctive features, including cyclopentane monoterpene derivative for iridoids. Iridoids glycosides commonly featured C_7 to C_8 bond breakage, forming 10 carbon atoms' compounds. Most of these are in the form of D-glucosides, with C_{11} undergoing oxidation to form carboxylic acids and esters.

Hence, iridoids emerge as the predominant metabolites in Bang Jian, with notable examples including gentiopicroside, sweroside, and swertiamarin. Among these, gentiopicroside assumes a pivotal role and is often utilized as the benchmark metabolite. Consequently, gentiopicroside finds its place in the Sichuan Chinese Materia Medica Standard (2010 edition) and Sichuan Tibetan Materia Medica Standard (2020 edition) as a quality standard for quantifying its content in both blue-flowered and white-flowered gentian species. It is worth noting that szechenyin A, gentizechenlioside A, gentiournosides D, depressine can be regarded as the characteristic metabolites of *G. szechenyii* and *G. stipitata* (Zhong et al., 2014; Ma et al., 2015). Because these characteristic components were isolated and identified only in this species. The next step should be further research on the characteristic metabolites, which can be incorporated into the standard-setting of this variety.

Besides, based on two iridoids metabolites, swertiamarin and gentiopicroside, our group established the first method of HPLC fingerprint analysis between Tibetan medicine Bang Jian, by comparing the fingerprint comparative analysis between different basal sources of Bang Jian (white-flowered gentian, blue-flowered gentian), mixed pseudo-products and traditional Chinese medicine gentian. It showed that the chemical composition of different interspecies differed greatly, which can be used as the basis for their species identification. Among them, the fingerprints of *G. szechenyii* and *G. stipitata* were more similar, with the values of 0.8–0.9, and both of them were less similar to *G. algida* and blue-flowered gentian (Ma, 2014). There are also studies on the quantitative analysis and fingerprinting of loganic acid, swertiamarin and gentiopicroside in the flowers and stems of *G. veitchiorum* and *G. lawrencei*, as well as in the flowers and stems of the same botanical drug, as determined by HPLC. The results showed that the content of gentiopicroside in flowers was higher than that in the

TABLE 3 Chemical compositions of Bang Jian.

No.	Name	Classification	Source	Formula	References
1	Gentiopicroside	Iridoids	*G. algida* *G. veitchiorum* *G. lawrencei*	$C_{16}H_{20}O_9$	Liu et al. (2006), Yang et al., 2008; Sun (2012)
2	Loganic acid	Iridoids	*G. veitchiorum* *G. lawrencei* *G. algida*	$C_{16}H_{24}O_{10}$	Yang et al. (2008), Bao et al. (2023)
3	Loganin	Iridoids	*G. szechenyii*	$C_{17}H_{26}O_{10}$	Zong (2015)
4	Swertiamarin	Iridoids	*G. sino-ornata* *G. lawrencei*	$C_{16}H_{22}O_{10}$	Sun, 2012; Ning et al. (2019)
5	Sweroside	Iridoids	*G. lawrencei* *G. sino-ornata* *G. algida*	$C_{16}H_{22}O_9$	Sun, 2012; Yang et al. (2014b), 2015; Bao et al. (2023)
6	Ridoside	Iridoids	*G. algida*	$C_{35}H_{42}O_{21}$	Yang et al. (2014b)
7	1-O-β-D-glucopyranosylamplexine	Iridoids	*G. algida*	$C_{16}H_{26}O_9$	Naonobu. et al. (2015)
8	6-O-β-D-glucopyranosyl gentiopicroside	Iridoids	*G. szechenyii*	$C_{22}H_{30}O_{14}$	Fu et al. (2018)
9	2'-(2,3-Dihydroxybenzoyl) sweroside	Iridoids	*G. algida*	$C_{23}H_{26}O_{12}$	Fu et al. (2018)
10	Gentiournoside E	Iridoids	*G. szechenyii* *G. stipitata*	$C_{29}H_{38}O_{18}$	Ma et al. (2015), Zong (2015)
11	Gentiournoside D	Iridoids	*G. szechenyii* *G. stipitata*	$C_{23}H_{28}O_{13}$	Ma et al. (2015), Fu et al. (2018)
12	Gentiournoside A	Iridoids	*G. szechenyii*	$C_{40}H_{52}O_{22}$	Zhang et al. (2022)
13	Depressine	Iridoids	*G. stipitata* *G. szechenyii*	$C_{30}H_{40}O_{18}$	Ma et al. (2015), Fu et al. (2018)
14	Gentizechenlioside A	Iridoids	*G. stipitata* *G. szechenyii*	$C_{46}H_{60}O_{26}$	Ma et al. (2015), Fu et al. (2018)
15	Szechenyin A	Iridoids	*G. stipitata* *G. szechenyii*	$C_{40}H_{50}O_{21}$	Ma et al. (2015), Fu et al. (2018)
16	Szechenyin B	Iridoids	*G. szechenyii*	$C_{24}H_{30}O_{13}$	Zhang et al. (2022)
17	10β-hydroxy-7 (11)-eremophilen-12,8α-olide	Iridoids	*G. algida*	$C_{15}H_{22}O_3$	Yang et al. (2013)
18	6β-hydroxy-7 (11)-eremophilen-12,8α-olide	Iridoids	*G. algida*	$C_{15}H_{22}O_3$	Yang et al. (2013)
19	Corosolic acid	Triterpenoids	*G. algida* *G. veitchiorum* *G. szechenyii*	$C_{30}H_{48}O_4$	Zou et al. (2010), Yang et al., 2015; Li (2017)
20	Roburic acid	Triterpenoids	*G. algida* *G. veitchiorum* *G. szechenyii*	$C_{30}H_{48}O_2$	Yang et al., 2015; Li (2017), Fu et al. (2018)

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TABLE 3 (*Continued*) Chemical compositions of Bang Jian.

No.	Name	Classification	Source	Formula	References
21	β-Sitosterol	Triterpenoids	*G. algida*	$C_{29}H_{50}O$	Yang et al. (2015), Bao et al. (2023)
			G. veitchiorum		
22	Oleanic acid	Triterpenoids	*G. algida*	$C_{30}H_{48}O_3$	Yang et al. (2015)
23	6'-(2,3-dihydroxybenzoyl) sweroside	Triterpenoids	*G. algida*	$C_{23}H_{26}O_{12}$	Tan et al. (1997)
24	6'-(2,3-dihydroxybenzoyl) swertiamarin	Triterpenoids	*G. algida*	$C_{23}H_{26}O_{13}$	Tan et al. (1997)
25	Taraxasterol	Triterpenoids	*G. algida*	$C_{30}H_{50}O$	Yang et al. (2011)
26	3β-palmitate	Triterpenoids	*G. algida*	$C_{46}H_{80}O_2$	Yang et al. (2011)
27	3β-palmitate-28-hydroxyl-α-amyrin	Triterpenoids	*G. algida*	$C_{46}H_{80}O_3$	Yang et al. (2011), Sun (2012)
			G. lawrencei		
28	3β-palmitate-28-hydroxyl-β-amyrin	Triterpenoids	*G. algida*	$C_{46}H_{80}O_3$	Zhang et al. (2009b), Yang et al. (2011)
			G. lawrencei		
29	24-hydroxy-α-amyrin	Triterpenoids	*G. algida*	$C_{30}H_{50}O_2$	Yang et al. (2011)
30	β-Amyrin	Triterpenoids	*G. algida*	$C_{30}H_{50}O$	Yang et al. (2011)
			G. lawrencei		
31	24-hydroxy-β-amyrin	Triterpenoids	*G. algida*	$C_{30}H_{50}O_2$	Yang et al. (2011)
32	Ursolic Acid	Triterpenoids	*G. algida*	$C_{30}H_{48}O_3$	Dou et al. (2020), Bao et al. (2023)
			G. veitchiorum		
33	28-hydroxy-α-amyrin	Triterpenoids	*G. algida*	$C_{30}H_{50}O_2$	Zhang et al. (2009c); Yang et al. (2011); Yang et al. (2015)
			G. lawrencei		
34	28-hydroxy-β-amyrin	Triterpenoids	*G. algida*	$C_{30}H_{50}O_2$	Yang et al. (2011)
			G. lawrencei		
35	α-Amyrin	Triterpenoids	*G. algida*	$C_{30}H_{50}O$	Yang et al. (2015)
			G. lawrencei		
36	Boehmerol	Triterpenoids	*G. algida*	$C_{29}H_{48}O$	Yang et al. (2015)
37	Arborinone	Triterpenoids	*G. algida*	$C_{30}H_{48}O$	Yang et al. (2015)
38	3β-Hydroxy-28-formaldehyde-12-en-ursane	Triterpenoids	*G. algida*	$C_{30}H_{48}O_2$	Yang et al. (2015)
39	28-hydroxy-lupinol	Triterpenoids	*G. algida*	$C_{30}H_{50}O_2$	Yang et al. (2015)
			G. lawrencei		
40	24-hydroxy-lupinol	Triterpenoids	*G. algida*	$C_{30}H_{50}O_2$	Yang et al. (2012), Yang et al. (2015)
			G. lawrencei		
41	Arborinol	Triterpenoids	*G. algida*	$C_{30}H_{50}O$	Yang et al. (2013)
42	Methyl 3-epimaslinate	Triterpenoids	*G. algida*	$C_{31}H_{50}O_4$	Yang et al. (2013)
43	Methy 2α,3α-dihydroxyurs-12-en-28-oate	Triterpenoids	*G. algida*	$C_{31}H_{50}O_4$	Yang et al. (2013)
44	3-Filicene	Triterpenoids	*G. algida*	$C_{30}H_{50}$	Yang et al. (2013)
45	9-Oxo-swerimuslactone	Triterpenoids	*G. veitchiorum*	$C_{37}H_{56}O_4$	Guo et al. (2021)
46	17-Hydroperoxide-28-norurs-12-en-3-one	Triterpenoids	*G. veitchiorum*	$C_{29}H_{46}O_3$	Guo et al. (2021)

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TABLE 3 (*Continued*) Chemical compositions of Bang Jian.

No.	Name	Classification	Source	Formula	References
47	28-O-(3,4-dihydroxyl-benzyl)-lupeol	Triterpenoids	*G. veitchiorum*	$C_{37}H_{56}O_4$	Guo et al. (2021)
48	Luteolin-6-C-glucoside	Flavonoids	*G. algida* *G. veitchiorum* *G. szechenyii*	$C_{21}H_{20}O_{11}$	Yang et al. (2008), Zou et al. (2010)
49	Isoorientin2″-O-rhamnoside	Flavonoids	*G. algida* *G. veitchiorum* *G. szechenyii*	$C_{27}H_{30}O_{15}$	Tozhiboev et al. (2011); Zong et al. (2015), Li et al. (2016b)
50	Isovitexin	Flavonoids	*G. veitchiorum*	$C_{21}H_{20}O_{10}$	Yang et al. (2008), Zou et al. (2010)
51	Isovitexin-2″-*O*-glucopyranoside	Flavonoids	*G. szechenyii*	$C_{27}H_{30}O_{15}$	Zhang (2017)
52	Gardenin A	Flavonoids	*G. algida*	$C_{21}H_{22}O_9$	Tozhiboev et al. (2011)
53	Flavocommelin	Flavonoids	*G. algida* *G. veitchiorum*	$C_{28}H_{32}O_{15}$	Tozhiboev et al. (2011), Li et al. (2016b)
54	Isoscoparin	Flavonoids	*G. algida* *G. veitchiorum* *G. lawrencei*	$C_{22}H_{22}O_{11}$	Zou et al. (2010), Sun (2012), Fu et al. (2018)
55	isoorientin-2″-*O*-glucopyranoside	Flavonoids	*G. veitchiorum* *G. sino-ornata* *G. lawrencei*	$C_{27}H_{30}O_{16}$	Sun (2012), Fu et al. (2018), Ning et al. (2019), Bao et al. (2023)
56	2,3-dihydroxybenzyl alcohol β-glucopyranoside	Flavonoids	*G. veitchiorum*	$C_{13}H_{18}O_8$	Bao et al. (2023)
57	3,6,4′-trimethoxy-5,7-Dihydroxyflavone	Flavonoids	*G. algida*	$C_{18}H_{16}O_7$	Tozhiboev et al. (2011)
58	Isoorientin3′-methyl ether	Flavonoids	*G. veitchiorum*	$C_{23}H_{24}O_{11}$	Yang et al. (2008)
59	Apigenin	Flavonoids	*G. veitchiorum* *G. algida*	$C_{15}H_{10}O_5$	Yang et al. (2014b)
60	Apigenin-6-C-β-D-glucopyranoside	Flavonoids	*G. veitchiorum*	$C_{21}H_{20}O_{10}$	Yang et al. (2008), Zou et al. (2010)
61	Apigenin 7-glucoside	Flavonoids	*G. veitchiorum* *G. szechenyii*	$C_{21}H_{20}O_{10}$	Zong et al. (2015), Li (2017)
62	Isoorientin-4′-diglucoside	Flavonoids	*G. veitchiorum*	$C_{33}H_{40}O_{21}$	Li et al. (2016b)
63	Isoorientin 4′-O-glucoside	Flavonoids	*G. algida* *G. veitchiorum* *G. lawrencei*	$C_{28}H_{32}O_{15}$	Yang et al. (2013), Li et al. (2016b), Wu et al. (2018)

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TABLE 3 (*Continued*) Chemical compositions of Bang Jian.

No.	Name	Classification	Source	Formula	References
64	Isosaponarin	Flavonoids	*G. veitchiorum*	$C_{27}H_{30}O_{15}$	Li. et al. (2017)
65	Swertisin	Flavonoids	*G. algida*	$C_{22}H_{22}O_{11}$	Yang et al. (2013)
66	Swertanone	Flavonoids	*G. algida*	$C_{30}H_{48}O$	Yang et al. (2013), Li et al. (2016b), Fu et al. (2018)
			G. veitchiorum		
			G. szechenyii		
67	Quercetagetin-7-O-glucoside	Flavonoids	*G. algida*	$C_{21}H_{20}O_{13}$	Yang et al. (2014b)
68	Swertiajaponin	Flavonoids	*G. algida*	$C_{22}H_{22}O_{12}$	Yang et al. (2014a), Fu et al. (2018)
			G. szechenyii		
69	Orientin-7-caffeate	Flavonoids	*G. veitchiorum*	$C_{30}H_{26}O_{14}$	Yang et al. (2014a), Li et al. (2016b), Fu et al. (2018)
			G. algida		
			G. szechenyii		
70	Mangiferin	Flavonoids	*G. szechenyii*	$C_{19}H_{18}O_{11}$	Fu et al. (2018)
71	Naringenin	Flavonoids	*G. szechenyii*	$C_{15}H_{12}O_5$	Fu et al. (2018)
72	7-O-feruloylorientin	Flavonoids	*G. veitchiorum*	$C_{31}H_{28}O_{14}$	Yang et al. (2014a), Li et al. (2016b)
			G. algida		
			G. szechenyii		
73	Isoscoparin-7-*O*-glucopyranoside	Flavonoids	*G. veitchiorum*	$C_{28}H_{32}O_{16}$	Li et al. (2016b)
74	Isobellidifolin	Xanthones	*G. algida*	$C_{14}H_{10}O_6$	Tozhiboev et al. (2011)
75	Swerchirin	Xanthones	*G. algida*	$C_{15}H_{12}O_6$	Yang et al. (2013)
76	1,5,8-Trihydroxy-3,4-dimethoxy xanthone	Xanthones	*G. algida*	$C_{15}H_{12}O_7$	Tozhiboev et al. (2011)
77	3-Methoxy-1,5,8-trihydroxy xanthone	Xanthones	*G. algida*	$C_{14}H_{10}O_6$	Tozhiboev et al. (2011)
78	Swertianolin	Xanthones	*G. algida*	$C_{20}H_{20}O_{11}$	Tozhiboev et al. (2011)
79	3-O-β-D-glucopyranosyl-1-hydroxy-7-methoxy xanthone	Xanthones	*G. algida*	$C_{20}H_{20}O_9$	Bao et al. (2023)
80	3-O-β-D-glucopyranosyl-1,6-dihydroxy xanthone glycoside	Xanthones	*G. algida*	$C_{19}H_{18}O_{10}$	Tozhiboev et al. (2011)
81	Oleic acid	Organic acids	*G. veitchiorum*	$C_{18}H_{34}O_2$	Huang and Zou (2013), Li (2017)
			G. szechenyii		
82	Linoleic acid	Organic acids	*G. algida*	$C_{18}H_{32}O_2$	Li (2013), Yang et al. (2014b)
83	Nonacosylic acid	Organic acids	*G. algida*	$C_{29}H_{58}O_2$	Li (2013)
84	2,3-Dihydroxybenzoic acid ethyl ester	Organic acids	*G. algida*	$C_9H_{10}O_4$	Li (2013)
85	Butyl isobutyl phthalate	Organic acids	*G. algida*	$C_{16}H_{22}O_4$	Li (2013)
86	L-Pyroglutamic acid	Organic acids	*G. algida*	$C_5H_7NO_3$	Bao et al. (2023)
87	Ethyl L-Pyroglutamate	Organic acids	*G. algida*	$C_7H_{11}NO_3$	Bao et al. (2023)

(Continued on following page)

TABLE 3 (*Continued*) Chemical compositions of Bang Jian.

No.	Name	Classification	Source	Formula	References
88	Daucosterol	Steroids	*G. veitchiorum*	$C_{35}H_{60}O_6$	Zou et al. (2010), Sun (2012), Naonobu. et al. (2015)
			G. algida		
			G. lawrencei		
89	5,8-dimethoxy-Furan coumarin	Coumarins	*G. algida*	$C_{13}H_{10}O_5$	Han (2012)
90	Acanthoside B	Lignans	*G. veitchiorum*	$C_{28}H_{36}O_{13}$	(Zong, 2015; Li, 2017)
			G. szechenyii		
91	Gentisuric acid	Hydroquinones	*G. szechenyii*	$C_9H_9NO_5$	Zong (2015)
92	Polygalacerebroside	Amides	*G. veitchiorum*	$C_{41}H_{79}NO_9$	Zou et al. (2010)

whole botanical drug and stems in different species. And the content of loganic acid, and swertiamarin did not have tissue specificity. When evaluating the content of *G. veitchiorum* and *G. lawrencei* with the content of gentiopicroside, the content of different species varied greatly, and the content of the flower part was better than that of the stem and leaf part. The content of the flower parts was better than that of the stem and leaf parts (Ma et al., 2018). This study provides data support for the traditional use of flower parts in Tibetan medicine, provides reference for the standardization of Tibetan medicine and the actual production of Tibetan medicine, and explores the necessity of establishing the standard of ethnomedicine for Daodi botanical drugs.

5.2 Flavonoids

Flavonoids have been studied in Chinese medicine and are the main active metabolites in Tibetan medicine. And research has shown that the occurrence of many diseases, such as tumors, cardiovascular and cerebrovascular disease, Alzheimer's disease, etc., are all associated to varying degrees with damage caused by free radicals (Tozhiboev et al., 2011; Ga et al., 2020). Flavonoids have the ability to scavenge disease-causing free radicals, as well as antibacterial, anti-aging, blood pressure and other health functions, and are a safe, effective, natural oxidant (Tadzhibaev. et al., 1992; Jiao et al., 2012). The basic backbone of flavonoids is C_6-C_3-C_6, and their metabolites can be broadly classified as follows: flavonoids, flavanols, dihydroflavonoids, chalcones, isoflavones, isoflavenes, dihydroisoflavones, etc (Li et al., 2016c). The Tibetan medicine Bang Jian contains a variety of flavonoid metabolites, mainly isoorientin, isoobtusin, sweretin, etc. Most of the flavonoids found in Bang Jian botanical drugs have hydroxyl and phenolic hydroxyl substituents. To date, only seven flavonoids have been isolated from the Bang Jian, and in addition to these compounds, eighteen other flavonoids have been detected or identified by LC-MS and HPLC. Besides, our group has also established the first fingerprint analysis method for the Tibetan medicine Bang Jian based on two flavonoid metabolites, isoorientin and Isovitexin, which can completely differentiate between the *G. szechenyii*, *G. algida* and the other blue-flowered Gentian (Ma L., 2014).

5.3 Xanthones

In addition to the flavonoids, a relatively large number of xanthones have been isolated. Xanthones are compounds with an oxygen-containing dibenzo-γ-pyrone heterocyclic scaffold, which are found in the form of mono- or polymethyl ethers and glycosides in this genus (M.M. et al., 2011). The parent nucleus of these compounds is a benzophenanthrone with eight substitutable positions in the parent nucleus. Xanthones exist mainly in the form of O-glycosides and C-glycosides. Xanthones are a class of compounds with a unique structure and are only found in a few families in nature, including Gentianaceae, Guttiferae, Moraceae, Clusiaceae, and Polygalaceae have inhibitory effects on a wide range of bacteria and fungi, and they have diuretic and cardiotonic activities. However, xanthones may not be characteristic metabolites of Bang Jian and the pharmacological activity of such constituents has been less studied.

5.4 Triterpenoids

In fact, triterpenoids are not the main active metabolites of Bang Jian, and the content is not high. At present, at least twenty saponin species have been isolated from gentian, and the triterpene saponin elements obtained by isolation are all derivatives of pentacyclic triterpenoids of the 3β-hydroxy oleanane type (Huang and Zou, 2013; Guo et al., 2021). In addition to the distribution of these compounds in the genus Swertia, ursolic acid, carotenosterol, oleanolic acid, and β-sitosterol have also been found in *G. algida* in recent years (Yang et al., 2015). In the 1980s, ursolic acid and oleanolic acid were shown to be effective anti-hepatitis and SGPT-lowering metabolites, and both have certain anti-tumor effects.

5.5 Other chemicals

There are other components in Tibetan botanical drug Bang Jian, such as coumarins, alkanols, volatile oils, polysaccharides, and many others (Su, 2004; Zhang, 2009; Xiao et al., 2018). Coniferin was isolated from the roots of Dauri gentian by Wang et al. and 5,8-dihydroxyfuranocoumarin was isolated from white flower gentian (Wang, 2022). Some scholars used gas chromatography-mass

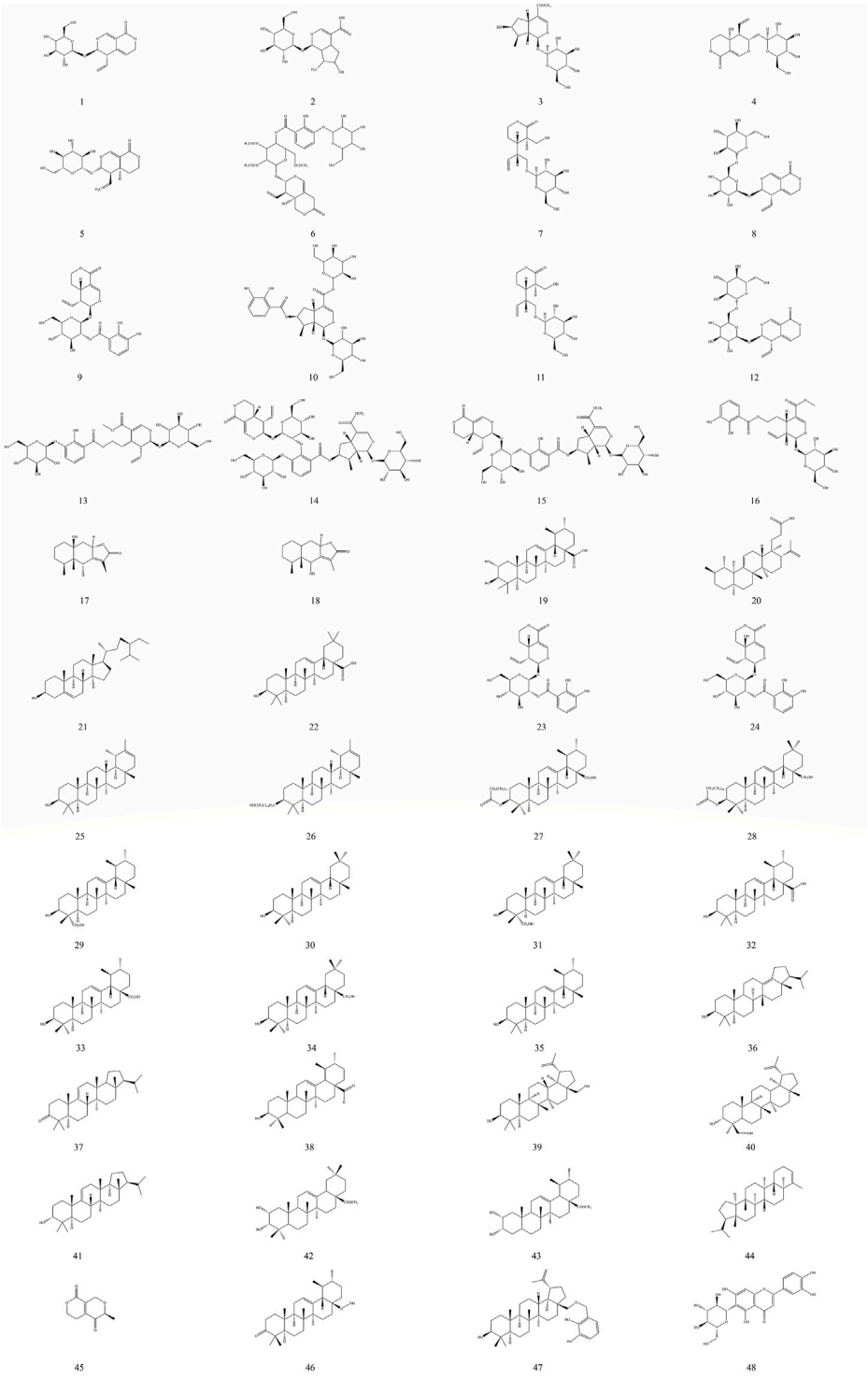

FIGURE 2
(Continued).

spectrometry (GC-MS) and the NIST mass spectrometry library to isolate and identify 83 metabolites from the volatile oil of *G. veitchiorum* flowers, mainly alkanes, with other alcohols, acids, esters, aldehydes, ketones, and phenolic compounds. In addition, it was indicated that Al, Fe, Pb, Cd, Be, Co, Tl, Rb, Sr, Se were the characteristic elements of *G. veitchiorum* (Kang et al., 2022). Moreover, there are extremely significant and significant positive correlations among characteristic elements.

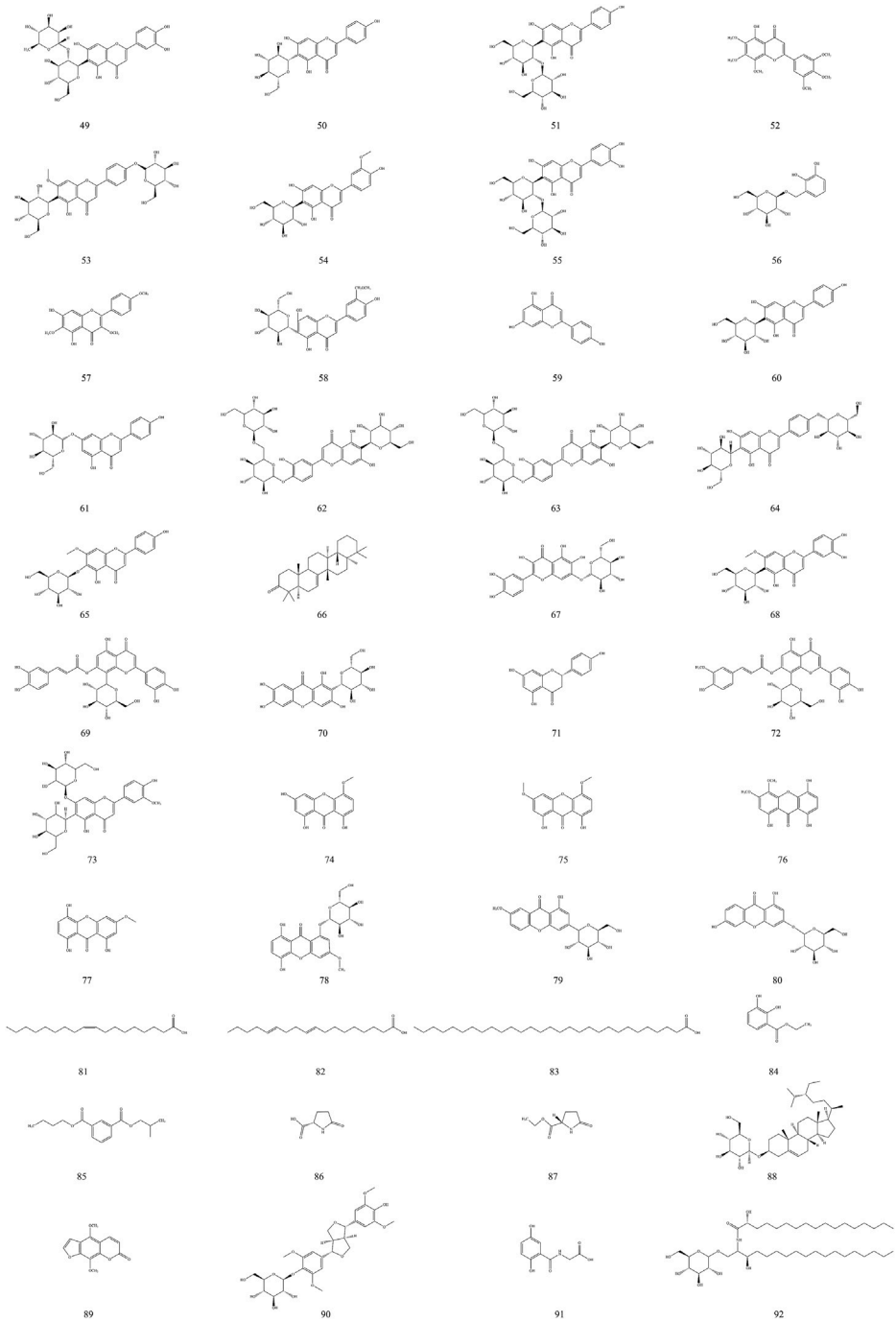

FIGURE 2
(Continued). Structures of the metabolites isolated from Bang Jian.

6 Pharmacology

As mentioned above, Bang Jian is a traditional Tibetan medicine with a variety of biologically active metabolites and is an ethnic medicine of great research value. Modern research has shown that extracts and isolates from different parts of the Bang Jian are rich in pharmacological activities, including respiratory protection, hepatoprotection, anti-tumor activity, anti-inflammatory activity, antibacterial activity, anti-viral activity, anti-oxidation, etc (Zhang, 2017). The specific pharmacological activities are summarized in Table 4. To date, relevant studies have focused on the anti-inflammatory and respiratory protective effects of *G. veitchiorum* and *G. szechenyii*, for which iridoids and flavonoids are the main active metabolites. Relevant pharmacological studies on other species are less frequent, and most of them are on crude extracts. In addition, research on the individual metabolites, including the identification of compounds, mechanisms of action, and molecular signaling pathways, have been lacking in the literature in recent

TABLE 4 Summary of the pharmacological activities of Bang Jian.

Pharmacological activity	Test substance/part	Test system	Tested method	Positive drug	Results	References
Respiratory system protection	*G. veitchiorum* was extracted by ethanol maceration, and the extract was concentrated and extracted by petroleum ether, chloroform, ethyl acetate, and n-butanol to obtain five products with different polarities	Chronic bronchitis model in mice due to ammonia stimulation	i.g 4 g/kg	GuiLongKeChuanNing Capsule	1) Compared with the model group, T-AOC activity in lung tissue of the n-butanol and aqueous layer groups was significantly higher ($p < 0.01$); SOD activity in lung tissue of the ethyl acetate and n-butanol groups was significantly higher ($p < 0.05$ or $p < 0.01$), and the serum levels of TNF-αand IL-10 of the ethyl acetate and n-butanol groups were significantly reduced ($p < 0.05$ or $p < 0.01$)	Geng et al. (2010)
					2) HE staining Pathological sections showed that the airway damage of chronic bronchitis in mice in the ethyl acetate and n-butanol extract treatment group was significantly improved after treatment with each polar metabolite of *G. veitchiorum* Hemsl	
	G. veitchiorum particles	mice inhaled NH_3 with YLS-8A equipment to induce the chronic bronchitis	i.g 4, 2, 1 g/kg	FuFangQiGuanYan Tablets	1) Compared with the model group, the contents of MDA in lung homogenate were decreased significantly and the contents of SOD were increased significantly in *G. veitchiorum* treatment groups	Hou et al. (2008), Hou (2011)
					2) HE staining showed the alleviation of injury from chronic bronchitis treated with *G. veitchiorum* particles	
	G. veitchiorum particles	the chronic asthmatic model was established with the ovalbumin sensitization and repeated inhalation of ovalbumin aerosols	i.g 0.33 g/kg	Dexamethasone	1) The difference of the dimensions, such as the positive staining of AB-PAS/Pbm, the positive staining of Bcl-2/Pbm between group B and group C were significant ($p < 0.01$), Aep/Pbm in B group was significantly higher than that in group C ($p < 0.01$), but no difference with group D	Zhang et al. (2009a)
					2) A close correlation between the positive staining of AB-PAS and the Bcl-2 content (r = 0.671, $p < 0.001$), and the r = 0.784 between the Aep/Pbm and the positive staining of Bcl-2 was significant, too	
	G. veitchiorum granules	30 male SD rats normal control group, 30 Gy irradiation group and Lanyuzan granules group	CT imagines and histopathological changes	—	1) The rats' lung CT images changed at different time periods and especially after 30 damaging showed fibrosis changes significantly. There was significant difference between Lanyuzan granules group and the 30 Gy irradiation group in pulmonary fibrosis imaging changes	Shi et al. (2016)
					2) Histopathology examinations showed rat lung tissue in Lanyuzan granules group were significantly alleviate than the 30 Gy irradiation group. Statistics results shows that there were significant different in hydroxyproline content between 30 Gy irradiation group and 30 Gy irradiation treatment group which accepted irradiation treatment with Lanyuzan granules (8 and 10 days b Post irradiation $p < 0.01$)	

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TABLE 4 (*Continued*) Summary of the pharmacological activities of Bang Jian.

Pharmacological activity	Test substance/part	Test system	Tested method	Positive drug	Results	References
Hepatoprotective effect	*G. veitchiorum*	Hepatic fibrosis induced by DMN	i.g, 2 g/kg, 1/d, 60 days	Predniso-ne Acetate Tablets	Regulation of lipid peroxidation *in vivo* and inhibition of hepatic stellate cell (HSC) activation	Li et al. (2008b)
	G. veitchiorum granule	Hepatic fibrosis induced by DMN	i.g, 2 g/kg, 1/d, 60 d	IFN-γ	1) In liver fibrosis model group, serum concentrations of ALT and AST were significantly higher, and concentration of ALB was significantly lower ($p < 0.01$), while significant increase in α-SMA levels and decrease in MMP-2 activity were observed compared with the control group ($p < 0.01$)	Fang (2009)
					2) Decrease ALT and AST serum concentrations, significantly increased ALB concentration, lowered α-SMA expression and elevated MMP-2 activity in liver tissue, were observed in Lanyuzan granule treatment group compared with model group ($p < 0.01$)	
					3) No notable difference was observed between low dosage group and model control group in MMP-2 activity. The pathological sections showed hepatic fibrosis was alleviated markedly	
	G. veitchiorum Methanol Extracts	CCl_4-induced acute liver injury	100, 200, 400 mg/kg	Silymarin	1) The levels of MAO and MDA were reduced and the levels of SOD and GSH were increased in the liver tissues of rats in the *G. veitchiorum* group	Zhang et al. (2014)
					2) Improvement in hepatic fibrosis was observed by HE and Masson staining	
Anti-inflammatory activity	*G. szechenyii* aqueous and ethanolic extracts	lene-induced acute ear swelling in mice and capillary permeability in the mouse abdomen	i.g 3.0, 1.5,0.8 g/kg, 14 days	—	1) The flowers of Gentiana alba also have good anti-inflammatory properties	Yang et al. (2010)
					2) Alcoholic extracts are significantly more effective than aqueous extracts	
	n-butanol, ethyl acetate, petroleum ether exacts of *G. szechenyii*	25% ammonia chemical stimulation to the rat acute pharyngitis model	i.g 0.5 g/kg, once/d, 7 days	QingHouLiYan Particle	The rats throat mucosa of model control group appeared obvious pathological changes, compared with model control group, *G. szechenyii* different polar parts group of rats with acute pharyngitis had different degrees of improvement, in which the pharyngeal lesions were repaired obviously, which could reduce the levels of IL-10, TNF-α in rat serum ($p < 0.01$)	Zhang et al. (2018)
	SanWeiLongDanHua Tablet (*G. szechenyii*)	Establishment of an allergic asthma model by intraperitoneal injection of ovalbumin sensitisation and nebulised inhalation provocation	High dose: 3.43 g/kg, medium dose 1.71 g/kg, low dose 0.86 g/kg groups	Dexamethasone	1) The latency of asthma in groups of the high, middle and low doses of Sanwei Longdanhua tablets were significantly longer than that of model group ($p < 0.01$)	Zhao et al. (2018)
					2) Compared with the model group, the levels of IL-4 and IFN-γ were significantly increased in dexamethasone group and Sanwei Longdanhua tablet high, middle, and low doses groups ($p < 0.05$)	

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TABLE 4 (*Continued*) Summary of the pharmacological activities of Bang Jian.

Pharmacological activity	Test substance/part	Test system	Tested method	Positive drug	Results	References
					3) The bronchial epithelial cells proliferated in model group with more mucus secretions in bronchial lumen, pulmonary interstitial edema, and increased cellular metabolites. A small amount of inflammatory exudate was found in the bronchial lumen of dexamethasone group and Sanwei Longdanhua tablet group	
Antibacterial activity	Ethanol, chloroform, n-butanol, distilled water extracts of *G. veitchiorum*	Methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-sensitive *Staphylococcus aureus* (MSSA) strains	Vitro: bouillon dilution method	YinHuangCapsule	The n-butanol fraction showed the highest inhibitory effect, followed by ethanol, aqueous layer fraction	Liu et al. (2011)
			Vivo:Establishment of a mouse infection model using intraperitoneal MRSA injection method			
	Extracts of *G. veitchiorum*	*Staphylococcus aureus* and *Escherichia coli*	Bacterial ring method	—	*Staphylococcus aureus* had the most pronounced inhibitory effect with a MIC value of 99.3 mg/L, followed by *Escherichia coli* (MIC value 225.5 mg/L) and *Candida albicans* (MIC value 126.8 mg/L), and *Salmonella* and *Shigella* dysenteriae	Geng et al. (2019)
	Petroleum ether, ethyl acetate, n-butanol extracts of *G. algida*	*Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Bacillus licheniformis*	Oxfordshire cup method	Penicillin/Streptomycin	1) The ethyl acetate and n-butanol extracts of *G. algida* were found to be bacteriostatic against five species of bacteria, and their bacteriostatic effect increased with the increase in the concentration of the extracts	Han (2012)
					2) Whereas the petroleum ether extract of *G. algida* showed antibacterial effect only against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus licheniformis*	
Antitumor activity	Oleanolic acid and ursolic acid of *G. algida*	Human leukaemia cells	—	—	Oleanolic acid and ursolic acid may also enhance immunity to splenoma cells by promoting the body's immune organs	Huang and Zou (2013)
	3-Filicene, arborinone, boehmerol, carotenoside, 3β-acetoxy-28-hydroxy-12-en-ursane and swertisin from *G. algida*	Human cervical cancer Hela cells	MTT	—	The activity of isoscoparin was the strongest, with an IC_{50} value of 45.75 μg/mL. Boehmerol was the next most active, with an IC_{50} value of 51.15 μg/mL. Ursolic acid, 3β-acetoxy-28-hydroxy-12-en-ursane, arborinone with an IC_{50} value of 115.52, 117.01, 235.25 μg/mL, respectively. 3-Filicene was the worst with an IC_{50} value of 280.07 μg/mL	Han (2012)
	Patulitrin, oscoparin, swertisin, Swertiajaponin of G. algida	Hepatocellular carcinoma (HepG2) cells	MTT	—	Oscoparin has high cellular activity with an IC_{50} of 47.25 g/mL	Khobrakova et al. (2017)
Antiviral activity	Aqueous decoction and n-butanol, ethyl acetate, chloroform extracts of *G. veitchiorum*	Vitro: Hep-2 cells CPE and MTT	i.g	Penicillin/Streptomycin	Vitro: Compared with the virus control group, the viability of RSV-infected cells increased to 77.3% ± 7.1%, 8.4% ± 6.5%, 50.4% ± 3.8% and 47.5% ± 5.2% with the decoction of *G. veitchiorum* and the extracts of n-butanol, ethyl acetate and chloroform, respectively; the inflammatory reaction was significantly milder in the treatment group than in the virus control group	Wei (2011), Wei et al. (2011)

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TABLE 4 (*Continued*) Summary of the pharmacological activities of Bang Jian.

Pharmacological activity	Test substance/part	Test system	Tested method	Positive drug	Results	References
		Vivo: Respiratory syncytial virus (RSV) infected	0.5 mL, 7 days		Vivo: Compared with the virus control group, the inflammatory reaction of the *G. veitchiorum* decoction treatment group was significantly milder than that of the virus control group	
	Chinese gentian	The anti-RSV effect of Chinese Gentian in HeLa cell culture was observed by means of the cytopathic effect inhibition assay	—	Bingduzuo Injection	In HeLa cell culture, Chinese Gentian was found to be an inhibitor of RSV in a concentration-dependent manner, and the TC_{50} was 15.25 mg/mL, and the EC_{50} was 1.07 mg/mL, and the TI was 14.25	Li and Li (2006)
Antioxidant	Isoorientin, isoscoparin, gentiournosides E, szechenyin B and gentiournosides A isolated from *G. szechenyii*	HPLC-DPPH-MS/MS	—	L-Ascorbic acid	The strongest antioxidant activity was found in gentiournosides E, and the weakest was found in szechenyin B	Zhang et al. (2022)
	Apigenin isolated from *G. veitchiorum*	DPPH	—	—	Not only does it reduce the production of reactive oxygen species, but it also regulates cholesterol metabolism	Dou et al. (2020)
	G. veitchiorum particles	DPPH	—	—	Enhance the vitality of antioxidant enzymes in the body, regulate oxidation and antioxidant balance	Hou (2011)

years. Therefore, there is still a need for more in-depth and comprehensive studies on the material basis and mechanism of action of the pharmacological effects.

6.1 Protection of the respiratory system

Bang Jian has a long history of medicinal use, with the effects of detoxification and the treatment of various kinds of fever, laryngitis, and heat closure. As a commonly used in Tibetan medicine, *G. szechenyii* is used as the main raw material in many compound preparations of Tibetan medicines under the State Pharmaceutical Approval Number (Table 5), which is used for relieving coughs and asthma, and treating respiratory system diseases such as acute and chronic bronchitis clinically. In addition, in the Tibetan areas of Qinghai province and Tibet of China, *G. veitchiorum*, *G. szechenyii*, and *G. algida* are often administered separately to treat respiratory diseases such as bronchitis (Yang, 1991). At present, research on the respiratory system mainly exists in two aspects: chronic bronchitis and pulmonary fibrosis.

Firstly, chronic bronchitis is one of the most common respiratory diseases, which mainly refers to the chronic non-specific inflammation of the respiratory system trachea, tracheal mucosa, and surrounding tissues (Lahousse et al., 2017). It is clinically characterized by cough, sputum, or wheezing, and can be complicated by emphysema, pulmonary hypertension, and pulmonary heart disease (Raju et al., 2016). At present, antibacterial drugs are mainly used in clinical treatment. However, the toxic side effects are serious, and it is easy to produce drug resistance. Long-term use of antibacterial drugs can also cause a bacterial imbalance, increasing the possibility of developing other diseases.

The second is pulmonary fibrosis, which is also a common chronic, interstitial lung disease in respiratory diseases. It is characterized by progressive dyspnea, which may further develop respiratory failure and lead to death (Mathai and Schwartz, 2019; Liu et al., 2022). Currently, the treatment options for pulmonary fibrosis are also very limited, and the main clinical tools are anti-pulmonary fibrosis drugs and lung transplantation. However, the clinical prognosis is poor, the incidence rate is increasing year by year, which seriously threatens human health and life, and the clinical lack of effective treatment methods.

Due to the advantages of multi-targets, multiple efficacy and low side effects, the research of traditional Chinese medicine in treating pulmonary fibrosis has attracted wide attention. Currently, according to the results of existing studies, the ethyl acetate site and the n-butanol site are the effective sites for the treatment of lung diseases by the *G. veitchiorum*. Some studies have shown that *G. veitchiorum* extract has a certain effect on the treatment of chronic bronchitis mice. The results showed an increase in T-AOC and SOD activity and a decrease in serum TNF-α and IL-10 levels in the lung tissue of mice in the ethyl acetate and n-butanol groups. Pathological sections showed significant improvement in the symptoms of chronic bronchitis airway damage in mice. Similarly, several studies have investigated the therapeutic effect of nebulised inhalation of *G. veitchiorum* in a model of TGF-β1-induced pulmonary fibrosis. It can be considered to have an antagonistic effect on the development of pulmonary fibrosis induced by TGF-β1. From the HPLC of the alcoholic extracts of *G. veitchiorum*, we could find that the substances in the ethyl acetate and n-butanol fractions

TABLE 5 Clinical application of preparations of Bang Jian.

No.	Name	Main compositions	Clinical application	Standards	References
1	SanWeiLongDanHuaPian	*G. szechenyii,* Licorice, honey balm	Clears heat and moistens the lungs. For heat and asthma of the lungs and pharyngitis	Ministry of Health Drug Standard Tibetan Medicine Book I	Chinese Pharmacopoeia Commission (1995)
2	ShiWeiLongDanHua Keli	*G. szechenyi, Rhododendron anthopogonoides*, Licorice, *Corydalis hendersonii*, *Fritillaria cirrhosa*, Xiaobopi, Jidanshen, *Phlomoides younghushandii*, *Inula racemosa, Przewalskia tangutica*	Relieves cough and asthma, clears heat, and dispels phlegm	Ministry of Health Drug Standard Tibetan Medicine Book I	Chinese Pharmacopoeia Commission (1995)
3	ShiWeiLongDanHua Jiaonang	*G. szechenyii, Rhododendron anthopogonoides*, Licorice, *Corydalis hendersonii*, *Fritillaria cirrhosa*, Xiaobopi, Jidanshen, *Phlomoides younghushandii*, *Inula racemosa, Przewalskia tangutica*	Relieves cough and asthma, clears heat, and dispels phlegm	Ministry of Health Drug Standard Tibetan Medicine Book I	Chinese Pharmacopoeia Commission (1995)
4	ShiwuWeiLongDanHua Jiaonang	*G. szechenyii*, *Santalum album*, *Terminalia chebula*, *Terminalia billerica*, *Phyllanthus emblica*, Shihuihua, Muxiang, guangzao, *Eugenia caryophyllata*, *Myristica fragrans*, *Tinospora sinensis*, *Aquilaria sinensis*, Baxiaga, Wujingji, Licorice	Cleanses heat and regulates the lungs, relieves cough, and dissolves phlegm. For bronchitis and emphysema, cough and asthma, hoarseness, and huskiness	Ministry of Health Drug Standard Tibetan Medicine Book I	Chinese Pharmacopoeia Commission (1995)
5	ShiwuWeiLongDanHua Wan	*G. szechenyii*, *Santalum album*, *Terminalia chebula*, *Terminalia billerica*, *Phyllanthus emblica*, Shihuihua, Muxiang, guangzao, *Eugenia caryophyllata*, *Myristica fragrans*, *Tinospora sinensis*, *Aquilaria sinensis*, Baxiaga, Wujingji, Licorice	Cleanses heat and regulates the lungs, relieves cough, and dissolves phlegm. For bronchitis and emphysema, cough and asthma, hoarseness, and huskiness	Ministry of Health Drug Standard Tibetan Medicine Book I	Chinese Pharmacopoeia Commission (1995)

have a higher likelihood of containing glycosides, and one of the substances, identified by HPLC comparison, is gentiopicroside. Gentiopicroside has been proven to have anti-inflammatory activity in previous studies, so it may also be one of the active metabolites in the treatment of respiratory diseases by *G. veitchiorum*.

In addition to the examination of the activity of *G. veitchiorum*, there is also the study of *G. veitchiorum* granules. And *G. veitchiorum* granules have better efficacy in chronic bronchitis. After the administration of *G. veitchiorum* granules, both SOD and MDA were restored to normal levels in mice suffering from chronic bronchitis. It may be possible to treat chronic bronchitis through antioxidant damage, enhancement of the organism's antioxidant enzyme activity, and regulation of the oxidative and antioxidant balance (Hou, 2011). In addition, in mice with pulmonary fibrosis, the levels of SOD and HP in lung tissue were measured and showed good control and relief. The reason may be the inhibition of airway inflammation and airway reconstruction in chronic asthma mice, and the mechanism may be related to the inhibition of TGF-β expression (Zhang, 2009). It forms a certain foundation for future in-depth research on the active metabolites of *G. veitchiorum* in the treatment of lung diseases.

6.2 Hepatoprotective effect

There are many causes of liver system disorders, among which liver fibrosis is a common liver disease. Liver fibrosis is a process of excessive repair after liver parenchymal injury in chronic liver disease, which is mainly characterized by excessive deposition of extracellular matrix, and further develops into cirrhosis or even liver cancer, which is an important public health problem threatening human health. At present, there is no effective medicine for liver fibrosis in Western medicine, and in recent years, studies have found that traditional Chinese medicine can control or even reverse liver fibrosis, which is worthy of in-depth exploration. It has been found that *G. veitchiorum* can prevent DMN-induced hepatic fibrosis by regulating lipid peroxidation and inhibiting the activation of hepatic stellate cells (Li et al., 2008a; Li, 2008). Through *in vivo* animal experiments, it was found that the methanol extract of *G. veitchiorum* had a protective effect against carbon tetrachloride-induced liver injury in mice, which was partly attributed to its ability to scavenge carbon tetrachloride-associated free radicals via scavenging (Li et al., 2008b; Zhang et al., 2014). Besides, except the normal group, all the rats were injected with dimethyl nitrosamine to establish the fibrosis model. And the results showed that compared with the model group, the level of MAO and MDA decreased in the liver tissues of rats in the G. veitchiorum group. It was found that MAO and MDA levels were reduced and SOD and GSH levels were increased in the liver tissues of rats in the G. veitchiorum group compared with the model group, and the degree of hepatic fibrosis was improved as observed by HE and Masson staining (Li et al., 2008a).

6.3 Antitumor activity

Malignant tumors are major diseases that pose a serious threat to human health. Studies have reported that as of 2020, malignant

neoplasms are responsible for more than 10 million deaths, accounting for one-sixth of global deaths (Sung et al., 2021). Among them, breast cancer, lung cancer, liver cancer, colon cancer, and cervical cancer have higher incidence rates (Arranja et al., 2017). At present, traditional Chinese medicine and ethnomedicine have special advantages and remarkable efficacy in the treatment of tumors, and their main anti-tumor pathways include inhibition of tumor cell proliferation, differentiation, and induction of apoptosis of hepatoma cells (Cheng et al., 2020; Xu et al., 2020).

The current research on the antitumor activity of Bang Jian is mainly focused on the study of flavonoids, iridoids, and triterpenoids, which are the monomer metabolites in *G. algida*. It mainly targets leukaemia and human cervical cancer cells and hepatocellular carcinoma cells. Specifically, inhibition of tumor cell proliferation and unlimited proliferation of malignant tumor cells are closely related to the activation of telomerase, which is a ribonucleoprotein complex that can sustain the proliferation of malignant cells, and there is no activated telomerase in normal tissues. It was found that oleanolic acid and ursolic acid in *G. algida* can be used in human leukemia cells for a period of time, the activity of telomerase within the malignant cells is reduced, thus contributing to the decline in cell growth rate apoptosis rate increases. Oleanolic acid and ursolic acid can significantly inhibit the growth of human cervical cancer cells, but also through the promotion of the body's immune organs such as the growth of the spleen and lymphocyte proliferation to improve the immunity to splenic tumor cells and enhance the body's anti-splenic tumor immune response to achieve the effect of anti-tumor. The compounds 3-Filicene, arborinone, boehmerol, carotenoside, 3β-acetoxy-28-hydroxy-12-en-ursane and swertisin from *G. algida* (Han, 2012). And these have different degrees of anti-tumor activity against human cervical cancer Hela cells. Similarly, some scholars found that four flavonoids, including patulitrin, oscoparin, swertisin, and swertiajaponin, were isolated from *G. algida*. The cytotoxicity of four flavonoids on the growth of HepG2 cells was investigated, and the results showed that oscoparin possessed high cellular activity with an IC_{50} of 47.25 g/mL (Yang et al., 2014a).

6.4 Anti-inflammatory activity

Inflammation is involved in many complex diseases and organismal disorders, including autoimmune diseases, metabolic syndrome, neurodegenerative diseases, cardiovascular diseases, and cancer (Felger and Treadway, 2017). Studies on the anti-inflammatory effects of Bang Jian mainly include the pharmacological studies of different solvent extracts of *G. algida*, *G. szechenyii*, and *G. veitchiorum* on model animals and the prediction of their mechanisms of action (Li et al., 2001; Chen et al., 2008; Yang et al., 2010). It was found that the aqueous and ethanol extracts of *G. algida* had inhibitory effects on xylene-induced acute ear swelling and abdominal capillary permeability in mice in both the medium and high dose groups, indicating that *G. algida* has anti-inflammatory activity (Yang et al., 2010). It was also found that the alcoholic extract of *G. purdomii* had anti-inflammatory effects at a certain concentration through mouse ear swelling experiments and granuloma experiments (Ga et al., 2020). It was experimentally confirmed that *G. szechenyii* extracts had ameliorative effects on ammonia-induced acute pharyngitis model in rats, and its mechanism of action may be related to the reduction of TNF-α, IL-1β, and IL-6 levels (Liu et al., 2018). Our group also confirmed the anti-inflammatory effects of *G. szechenyii* and *G. veitchiorum* through the experiments of ear swelling and foot swelling in mice in the previous period. And it was concluded that the combined anti-inflammatory and antibacterial efficacy of the flavonoid metabolites of *G. szechenyii* was stronger than that of the iridoids parts. The combined efficacy of iridoids such as loganic acid, gentiopicroside and sweroside in *G. veitchiorum* was found to be stronger than that of the flavonoids such as isoorientin, isoscoparin-2″-β-D-glucopyranoside and isoscoparin.

6.5 Antibacterial activity

Antibiotic resistance is a growing medical problem. The Centers for Disease Control and Prevention estimates that drug-resistant bacteria cause 23,000 deaths and 2 million illnesses in the United States each year (Khobrakova et al., 2017). Therefore, it is urgent to find new antibacterial drugs that are effective against drug-resistant strains of bacteria. As a natural active product, Bang Jian has antibacterial activity with iridoids, flavonoids, triterpenoids, and xanthones, etc. Among them, the strongest antibacterial activity is xanthones and iridoids, such as demethylbellidifolin, mangiferin, swertiamarin and gentiopicrin (Lu et al., 2018). The research about the Tibetan medicine Bang Jian found that the extracts (ethanol, chloroform, n-butanol, distilled water) of different polarities of *G. veitchiorum* had different degrees of bacteriostatic effects on methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-sensitive *S. aureus* (MSSA) strains, among which the n-butanol fraction had the strongest bacteriostatic effect, followed by ethanol and aqueous-layer fractions (Liu et al., 2011). In addition, it was found that the extract of *G. veitchiorum* had inhibitory effects on both *S. aureus* and *Escherichia coli* (Geng et al., 2019). Moreover, the solid-liquid extraction method was used to extract the active metabolites from the *G. veitchiorum* flowers, and the results of *in vitro* bacteriostatic experiments showed that the extract of *G. veitchiorum* flowers had the most obvious inhibitory effect on *S. aureus*, with a MIC value of 99.3 mg/L, and the second most obvious inhibitory effect on *E. coli* (MIC value 225.5 mg/L) and *Candida albicans* (MIC value 126.8 mg/L), and the inhibitory effect on *Salmonella* and *Shigella dysenteriae* was insignificant (MIC value 126.8 mg/L). The inhibitory effects on *Salmonella* and *S. dysenteriae* were not significant (Geng et al., 2020). And Han et al. found that the ethyl acetate isolates of *G. algida* had good inhibitory activity against five curative bacteria including *E. coli*, *S. aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa* and *Bacillus licheniformis* (Han, 2012).

6.6 Antiviral activity

G. veitchiorum decoction and n-butanol, ethyl acetate, and chloroform extracts inhibited respiratory syncytial virus (RSV) *in vitro* and *in vivo*. It was found that the decoction of *G. veitchiorum* was more effective than n-butanol, chloroform,

and ethyl acetate extracts in inhibiting the entry of the virus into the cells. Meanwhile, pathological sections of mice infected with RSV treated by gavage with the decoction of *G. veitchiorum* showed a significant reduction of inflammatory exudation in the group of mice compared with that in the control group. Immunofluorescence experiments showed that the fluorescence brightness and the number of particles were reduced in the drug-treated sections, indicating that *G. veitchiorum* showed certain effects in both anti-RSV value-added and anti-RSV-induced inflammation (Wei et al., 2011). Moreover, the aqueous extract of Gentian, RG2-1 and RG3-1, the effective antiviral parts of Gentian, inhibited respiratory syncytial virus *in vitro*, and the quantitative effect relationship was obvious.

6.7 Antioxidant

Oxidative stress predisposes to lipid membrane peroxidation, which damages membrane integrity and leads to cell death, causing major diseases such as atherosclerosis, diabetes, cancer, and respiratory diseases (Hayes et al., 2020; Griendling et al., 2021). Studies have shown that flavonoids and iridoids are the main antioxidant active metabolites of Bang Jian species. The main studies have focused on the evaluation of antioxidant properties *in vitro*. Bang Jian botanical drugs are mainly used in the treatment of the respiratory system.

Since the abnormal activation of macrophages in upper respiratory tract infections is closely related to reactive oxygen species (ROS) signaling in cells, and the mechanism of cellular damage caused by viral infection and hypoxia is also related to oxidative damage caused by the accumulation of reactive oxygen species, treatment is often supplemented with antioxidants. It has been found that antioxidants may prevent hypoxic tissue damage and reduce viral cellular damage by inhibiting ROS signaling-dependent macrophage overactivation. Therefore, HPLC-DPPH-MS/MS was used for rapid screening and identification of antioxidant active metabolites in *G. szechenyii*. The HP online antioxidant screening system was used to carry out the screening of active compounds, the active products were separated and structurally identified using preparative chromatography. Besides, the antioxidant capacity of the five main active metabolites was determined using DPPH. The results showed that five antioxidant active compounds were screened from *G. szechenyii*, namely, isoorientin, isoscoparin, gentiournosides E, szechenyin B and gentiournosides A. In addition, gentiournosides E had the strongest antioxidant activity and szechenyin B was the weakest of the five compounds (Zhang et al., 2022). Similarly, it was found that apigenin, extracted and isolated from *G. veitchiorum*, is a flavonoid compound with antioxidant activity that not only reduces the production of reactive oxygen species but also regulates cholesterol metabolism (Dou et al., 2020). Therefore, tapping the natural antioxidant active metabolites in clinically applied Chinese and Tibetan botanical drugs for the treatment of upper respiratory tract infections is expected to produce desirable adjuvant therapeutic effects on respiratory system diseases and provide new ideas for clinical treatment.

7 Discussion

*Gentiana i*s an annual or perennial botanical drug. In Gentianaceae, the genus has the largest number of plant species, *Gentiana* plants can be used for ornamental, medicinal and other purposes, and most of them are traditional Tibetan medicine. Tibetan medicine Bang Jian is the generic name for a variety of medicinal plants in the genus *Gentiana*, which is representative of one of the commonly used medicines in Tibetan medicine. It is one of the commonly used medicines in Tibetan medicine. It has the efficacy of treating poisonous diseases, various kinds of fever, laryngitis, heat paralysis and so on. The efficacy is exact and widely used.

This review provides an overview of the botanical characteristics, traditional ethnomedicinal applications, chemical composition, and pharmacological properties of Bang Jian. Tibetan medicine boasts a rich historical lineage, utilizing various parts of botanical drugs, each yielding distinct therapeutic benefits. Among the frequently employed the whole grass in Tibetan medicine, Bang Jian is predominantly administered in the form of dried flowers. These flowers are readily available and renowned for their remarkable therapeutic efficacy. Multiple studies have corroborated the global utilization of various metabolites of Tibetan medicine Bang Jian in ethnobotanical practices. Pharmacological research has shed light on the biological activities of Tibetan medicine Bang Jian, enriching our understanding of its medicinal properties. Despite significant advancements in Bang Jian research, there are still some gaps in our knowledge, particularly in uncovering novel discoveries in this field.

Currently, the complexity of the origins of the Tibetan medicine Bang Jian, according to botanical drug records are used to classify type, according to the flower color is divided into white, black, blue, miscellaneous. The classification is confusing, and the varieties are named differently, which is not conducive to the standardization of the varieties and the accuracy of the medication. It is necessary to classify the varieties through resource science, molecular biology and other methods, and molecular biology is an effective means of identifying species that are easily confused with their relatives, and genomics, transcriptomics and gene barcode identification can be considered, which makes it possible to provide more reasonable and standardized guidance for clinical use of medicines.

Besides, Bang Jian botanical drugs are primarily utilized within ethnic medicine, with a majority of studies conducted domestically and limited involvement from foreign scholars. Through the summary of the chemical composition of Bang Jian, it was found that there were some differences in the chemical composition between the white flower Gentian and the blue flower Gentian. The iridoids of gentiopicroside and swertiamarin are the main characteristic metabolites of blue flowers. However, szechenyin A, gentizechenlioside A, depressine, and gentiournoside D were the main iridoid metabolites identified in white flowers, while these metabolites are not identified in blue-flowered gentian. It is well known that differences in ingredients can lead to differences in efficacy. Therefore, it is necessary to combine the characteristic metabolites with pharmacodynamics research in the next step to find pharmacodynamic markers. It further provides guidance for clinical medication.

Furthermore, current pharmacological research into the effectiveness of Tibetan botanical drugs is predominantly focused on just two or three species, neglecting other valuable species. And the research on the pharmacological activity of mainstream species is still in the primary stage. The main research is in some simple pharmacodynamic experiments. Expanding the scope of research on Bang Jian represents a logical next step. It is crucial to highlight that research into the metabolites and mechanisms of action of Tibetan medicine Bang Jian is somewhat superficial. Pharmacological investigations often center on crude extracts, with limited exploration of the effects of individual metabolites. Therefore, it is necessary to carry out a comprehensive study on the chemical composition of Bang Jian and the differences between different medicinal parts. The pharmacological effect studies of single compound and clinical efficacy studies should be strengthened for a more rational utilisation of Bang Jian.

Tibetan medicine Bang Jian is not only a medicinal plant, but also a very ornamental flowering plant, which occupies an important position in the vegetation of the plateau and in the system of Tibetan medicine. However, due to the wide application of Tibetan medicine and its unique therapeutic efficacy in recent years, the number of wild resources has been declining year by year, and the regeneration capacity of the species involved has been decreasing year by year in the harsh growing environment of the plateau. In order to effectively solve the problem of the resource quantity of Bang Jian, in addition to the research on the origin, pharmacological activity and chemical composition of Bang Jian species, people should be called upon to protect its wild resources and carry out the related artificial nursery technology. While expanding the resources of Bang Jian, it can also provide an effective reference for the development of the local tourism industry, so as to improve the economic and ecological benefits. Also, to preserve these invaluable resources and fully harness their potential, there is an urgent need to bolster research efforts into the pharmacological foundation and clinical applications of Bang Jian.

8 Conclusion and perspectives

This review provides a comprehensive account of the traditional uses, botanical characteristics, phytochemistry, and pharmacological activities of the Bang Jian group of botanical drugs in Tibetan medicine. The Tibetan medicine Bang Jian botanical drug have a long recorded history and are receiving increasing attention as a commonly used botanical species in traditional Tibetan medicine. In summary, in terms of traditional medicinal use, it is interesting to note that unlike the traditional Chinese medicine of gentian, which is administered from the root, traditional Tibetan medicine usually uses the dried flowers of Bang Jian in medicine. It is mainly used to treat fever, laryngitis, and pyrexia. The main active metabolites of Bang Jian are iridoids, flavonoids, triterpenoids, and xanthones. A total of 92 compounds have been isolated and identified, including 18 iridoids, 26 flavonoids, 29 triterpenoids, 7 xanthones, and 12 other compounds. According to modern pharmacological studies, Bang Jian has a wide range of pharmacological activities. These include respiratory protection, hepatoprotection, antitumor, anti-inflammatory, antibacterial, antiviral, and antioxidant activities. It provides a reference for the further development and utilization of Tibetan medicine Bang Jian.

Despite the extensive medicinal value of Bang Jian as a traditional Tibetan medicine. However, there are some limitations as well. Firstly, at present, Bang Jian botanical drugs contain a rich variety of metabolites, such as iridoids, flavonoids, triterpenoids and so on, which are found in the flowers and above-ground parts of the Bang Jian group of medicinal plants. However, the current quality standard of Bang Jian only uses gentiopicroside and isoorientin as indicator metabolites. More importantly, it is incomplete to measure the quality of botanical drugs only by a single or two indicator metabolites. Secondly, there is a wide range of pharmacological studies, including anti-inflammatory, antitumor, antiviral and antioxidant aspects, but most of them are focused on three species, *G. veitchiorum*, *G. algida* and *G. szechenyii*, with fewer studies on the pharmacological activities of other species. In addition, research on the pharmacological activity of Bang Jian botanical drug is still at a preliminary stage. The pharmacological substance basis of the Bang Jian botanical drug is not clear enough. Most of the studies were on crude extracts, and there were fewer studies on single pharmacodynamic components, as well as studies on pharmacodynamic mechanisms still need to be further explored and expanded.

Therefore, in order to further protect the resources and develop their medicinal value, it is necessary to strengthen the research on the pharmacological material basis and clinical application of Bang Jian botanical drugs. Specifically, it is necessary to carry out research on the phytochemistry of the various parts of Bang Jian botanical drugs of Tibetan medicine and to strengthen the research on the pharmacological effects of monomer phytochemistry, pharmacodynamic mechanism and clinical efficacy. In order to lay a certain foundation for the more reasonable and effective use of the plant resources of Tibetan medicinal botanical drugs, it is necessary to carry out research on the chemical composition of each part of Tibetan medicinal drugs. Then, in order to ensure the safety and efficacy of clinical use of drugs. It is necessary to establish a more perfect theoretical system of quality standards and control of traditional Chinese medicine and Tibetan medicine through multi-indicator quantitative composition analysis combined with pharmacodynamic experiments. In addition, further development of applications other than medicinal plants of Bang Jian can also be carried out. It is known through pharmacological studies that Bang Jian botanical drugs also have anti-inflammatory and antioxidant effects, which can be considered to be developed into healthcare products such as tea. In addition, with its bright color and graceful appearance, it has high ornamental value. It can be domesticated and cultivated as an ornamental plant. In summary, we can make fuller and more comprehensive use of Bang Jian and realize its great potential.

Author contributions

YL: Formal Analysis, Methodology, Writing–original draft. JZ: Formal Analysis, Writing–original draft. J-yF: Methodology, Writing–original draft. S-hZ: Writing–review and editing. RG: Funding acquisition, Writing–review and editing.

Funding

The authors declare financial support was received for the research, authorship, and/or publication of this article. This work was supported by the Ministry of Science and Technology of the People's Republic of China (No. 2019YFC1712305), the National Natural Science Foundation of China (No. 82073964), and Sichuan Provincial Administration of Traditional Chinese Medicine Project (No. 2023MS616).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2023.1295789/full#supplementary-material

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frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 23 January 2024
DOI 10.3389/fphar.2024.1290888

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OPEN ACCESS

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RECEIVED 08 September 2023
ACCEPTED 10 January 2024
PUBLISHED 23 January 2024

CITATION
Tang S, Wang M, Peng Y, Liang Y, Lei J, Tao Q, Ming T, Shen Y, Zhang C, Guo J and Xu H (2024), *Armeniacae semen amarum*: a review on its botany, phytochemistry, pharmacology, clinical application, toxicology and pharmacokinetics. *Front. Pharmacol.* 15:1290888. doi: 10.3389/fphar.2024.1290888

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Armeniacae semen amarum: a review on its botany, phytochemistry, pharmacology, clinical application, toxicology and pharmacokinetics

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Armeniacae semen amarum—seeds of *Prunus armeniaca* L. (Rosaceae) (ASA), also known as Kuxingren in Chinese, is a traditional Chinese herbal drug commonly used for lung disease and intestinal disorders. It has long been used to treat coughs and asthma, as well as to lubricate the colon and reduce constipation. ASA refers to the dried ripe seed of diverse species of Rosaceae and contains a variety of phytochemical components, including glycosides, organic acids, amino acids, flavonoids, terpenes, phytosterols, phenylpropanoids, and other components. Extensive data shows that ASA exhibits various pharmacological activities, such as anticancer activity, anti-oxidation, antimicrobial activity, anti-inflammation, protection of cardiovascular, neural, respiratory and digestive systems, antidiabetic effects, and protection of the liver and kidney, and other activities. In clinical practice, ASA can be used as a single drug or in combination with other traditional Chinese medicines, forming ASA-containing formulas, to treat various afflictions. However, it is important to consider the potential adverse reactions and pharmacokinetic properties of ASA during its clinical use. Overall, with various bioactive components, diversified

Abbreviations: ABTS 2′-Azinobis-(3-ethylbenzthiazoline-6-sulphonate); AchE acetyl cholinesterase; ASA Armeniacae semen amarum; ACE angiotensin-converting enzyme; ALI acute lung injury; ALT alanine aminotransferase; AST aspartate aminotransferase; AUC_{0-t} area under the plasma concentration curve (0-t); $AUC_{0-\infty}$ area under the plasma concentration curve (0-∞); CAT catalase; CL body clearance; CLz/F clearance/bioavailability; C_{max} maximum concentration; CNKI China National Knowledge Infrastructure; COPD chronic obstructive pulmonary disease; COX-2 cyclooxygenase-2; DPPH 2,2-diphenyl-1-picrylhydrazyl; DM diabetes mellitus; DMBA 2,2′-Bis (hydroxymethyl)butyric; EMT epithelial-mesenchymal transition; F (%) bioavailability; FRAP ferric reducing anti-oxidant power; GAE gallic acid equivalent; GGT gamma-glutamyl transferase; GSH glutathione; GST glutathione S-transferase; HbA1c hemoglobin A1C; HDL-C high-density lipoprotein cholesterol; IC50 half maximal inhibitory concentration; iNOS inducible nitric oxide synthase; ke elimination rate constant; LD50 median lethal dose; LDL-C low-density lipoprotein cholesterol; LPO lipid peroxide; MDA malondialdehyde; MRT mean residence time; PGE2 prostaglandin E2; ROS reactive oxygen species; SOD superoxide dismutase; $t_{1/2}$ elimination half-life; TAC total anti-oxidant capacity; T_{max} time to peak concentration; Vd volume of distribution; Vz/F distribution/bioavailability.

pharmacological actions and potent efficacies, ASA is a promising drug that merits in-depth study on its functional mechanisms to facilitate its clinical application.

KEYWORDS

***Armeniacae semen amarum*, *Prunus armeniaca* L., traditional Chinese medicine, ethnopharmacology, phytochemistry, pharmacology, clinical application, toxicology**

1 Introduction

Armeniacae semen amarum—seeds of *Prunus armeniaca* L. (Rosaceae) (ASA), also known as bitter almond or apricot kernel and Kuxingren in Chinese, is a widely used traditional Chinese herbal drug. It is renowned for its effectiveness in treating lung and intestinal diseases (Wei et al., 2023). In traditional Chinese medicine, it is commonly prescribed for relieving cough and asthma, as well as moisturizing the intestine to alleviate constipation (Gao et al., 2014). Modern studies have shown that ASA has a diverse range of pharmacological effects, including alleviating cough and resolving phlegm, as well as immunomodulation and anti-inflammatory properties (Ma et al., 2021; Zhao Y. et al., 2022). Meanwhile, both clinical and animal experiments have demonstrated that the effective components and prescriptions of ASA have significant therapeutic effects on respiratory diseases (Si and Zhang, 2021; Wang et al., 2023).

ASA is composed of various chemical components including glycosides, organic acids, amino acids, flavonoids, terpenes, phytosterols, phenylpropanoids, and other substances. The abundance of these active components makes ASA a valuable subject for research and application. Amygdalin, as the main active ingredient in ASA, has been found to have beneficial effects in relieving cough and asthma, as well as exhibiting anti-inflammatory and anti-fibrotic properties, which makes it a promising candidate for the treatment of respiratory diseases, with significant potential for disease management (Wang et al., 2021). Numerous studies have demonstrated the positive effects of ASA and its active ingredients on various respiratory conditions, including cough, asthma, chronic obstructive pulmonary disease (COPD), pulmonary heart disease, and lung function injury. Moreover, recent research has also suggested its potential role in treating COVID-19 (Luo et al., 2020; Zhou et al., 2020). Furthermore, ASA can be combined with other treatments to enhance its efficacy (Li et al., 2021; Noureen et al., 2022).

Although considerable studies have been performed on the ASA (Wei et al., 2023), there is still a lack of comprehensive and in-depth review of ASA. Herein, we conducted a comprehensive literature search using online databases such as PubMed, Web of Science, China National Knowledge Infrastructure (CNKI), and Google Scholar, with the keywords including ASA, its bioactive components, or ASA-containing formulas, up to December 2023. Then, we systematically summarize and highlight the botanical features and traditional uses, phytochemical components, pharmacological activities, clinical applications, toxicological effects including adverse reactions and detoxification methods, and pharmacokinetic characteristics of ASA, attempting to lay a foundation for the in-depth basic research on ASA and expanding its application in the clinical settings.

2 Botanical features and traditional uses

ASA, as defined in the 2020 edition of Chinese Pharmacopoeia, refers to the dried ripe seeds of various species of Rosaceae, namely, *P. armeniaca* L.var.*ansu* Maxim., *Prunus sibirica* L., *Prunus mandshurica* (Maxim.) Koehne, or *P. armeniaca* L.

It is recommended to harvest fully ripe fruits in the summer and extract their seeds by removing the pulp and core shell. The seeds should then be dried under the Sun. ASA, which contains cyanogenic components (Kovacikova et al., 2019), is known to have beneficial properties and minor toxicity. In traditional Chinese medicine, it is believed that ASA affects the lung and large intestine meridian. The Chinese Pharmacopoeia 2020 states that ASA has therapeutic effects such as lowering Qi, relieving cough and asthma, moisturizing the intestine, and relaxing the bowels (Wei et al., 2023) (Figure 1).

ASA was first documented in *Shennong's Herbal* (Shen Nong Ben Cao Jing). It has a sweet taste and warm nature, primarily used for alleviating coughs caused by Qi. However, according to *Miscellaneous Records of Famous Physicians* (Ming Yi Bie Lu), ASA is described as having a bitter and toxic taste, commonly used to treat distress below the heart, abdominal fullness and distention, and occasionally headaches (Xue et al., 2022). The *essentials of Materia Medica* (Ben Cao Bei Yao) states that ASA is bitter in taste and warm in nature, with the ability to dissipate cold and alleviate irritable heat and shortness of breath. The *Compendium of Materia Medica* (Ben Cao Gang Mu) further indicates that ASA has various effects such as dispersing and reducing energy, relieving muscle and dispelling wind, reducing the Qi and moistening dryness, eliminating food stagnation, and treating injuries. Additionally, ASA has been found to have the potential of treating sores and repelling insects due to its toxicity. The book *Materia Medica Companion* (Ben Cao Meng Quan) describes its properties in further detail. However, it is important to note that ASA should not be used in conjunction with *Astragali radix*—roots of *Astragalus mongholicus* Bunge (Fabaceae), *Scutellariae radix*—roots of *Scutellaria baicalensis* Georgi (Lamiaceae), and *Puerariae lobatae radix*—roots of *Pueraria lobata* Ohwi (Fabaceae). ASA is commonly used for coughs with phlegm, constipation, and insect bites. It is worth mentioning that the treatment for constipation varies depending on whether it is related to Qi or blood deficiency. ASA is used for addressing Qi deficiency, while *Persicae semen*—seeds of *Prunus persica* (L.) Batsch (Rosaceae) is employed to promote blood circulation. In cases of Qi deficiency and a floating pulse, a combination of ASA and *Citri reticulatae pericarpium*—epicarps of *Citrus reticulata* Blanco (Rutaceae) is recommended. On the other hand, combining *P. semen* with *C. reticulatae pericarpium* is advised for addressing blood deficiency and a sinking pulse (Du and Yu, 2023).

3 Phytochemical components

Numerous studies have shown that ASA contains a variety of bioactive components and nutrients including glycosides, organic acids, amino acids, flavonoids, terpenes, phytosterols, phenylpropanoids, and other compounds. This section presents a compilation of literature on the chemical composition of ASA, providing detailed information on 170 major chemical components that have been isolated from it (Table 1). Furthermore, we have depicted the chemical structures of the main active components found in ASA (Figure 2).

3.1 Glycosides

The glycosides found in ASA primarily consist of cyanogenic glycosides, which serve as both its main toxic components and its primary pharmacologically active ingredients. The principal glycoside in ASA is amygdalin **(1)**. It is important to note that consuming a large amount of amygdalin within a short period of time may lead to cyanide poisoning. This occurs due to the hydrolysis of amygdalin by β-D-glucosidase, leading to the production of benzaldehyde and hydrocyanic acid, which can cause respiratory depression (Song and Xu, 2014). Pharmacological studies have demonstrated that amygdalin exhibits significant anti-tumor activity, as well as antinociceptive and antiphlogistic effects, making it a promising candidate for various applications (Park et al., 2005; Hwang et al., 2008; Figurová et al., 2021; Guo et al., 2023; Zhang et al., 2023). In addition, another cyanogenic glycoside called neoamygdalin **(2)** has been isolated and identified from ASA. Neoamygdalin is an epimorphous isoform of amygdalin and shows great potential in the treatment of cough and asthma (Xu et al., 2017). Besides, mass spectrometry analysis has revealed the presence of amygdalin metabolites and its glycosides in ASA extracts, including prunasin **(3)**, mandelic acid-β-glucopyranoside **(5)**, mandelic acid-β-gentiobioside **(6)**, mandelic acid amide-β-glucopyranoside **(7)**, mandelic acid amide-β-gentiobioside **(8)**, and benzyl-β-gentiobioside **(9)**. Furthermore, ASA methanol extracts also contain propyl-β-gentiobioside **(4)**, adenosine **(10)** and cytarabine **(11)** (Chen Y. et al., 2022). The information of these glycosides is listed in Table 1, and the chemical structures were drawn by ChemDraw 20.0 and presented in Figure 2.

3.2 Organic acids

Currently, a total of 39 organic acids have been isolated and identified in ASA. Among them, **(12–27)** are fatty acids, accounting for approximately 50% of ASA (Jin et al., 2018), which can be divided into saturated fatty acids **(12–17)**, monounsaturated fatty acids **(18–23)**, and polyunsaturated fatty acids **(24–27)**. Notably, unsaturated fatty acids such as oleic acid **(20)**, linoleic acid **(24)**, and linolenic acid **(25)** are essential for the human body as they cannot be synthesized internally and must be obtained from food (Spector and Kim, 2015). Pharmacological studies have demonstrated that unsaturated fatty acids possess various beneficial effects such as regulation of thrombosis, immune modulation, and anti-fibrosis (Khosla and Fungwe, 2001; Vangaveti et al., 2016; Turolo et al., 2021), making them of significant medicinal value. In addition, ASA contains a range of phenolic acids **(28–36)**, which have antibacterial, anti-inflammatory, anti-oxidation and other pharmacological effects (Bak et al., 2013; Thakare et al., 2017). Furthermore, mandelic acid **(42)**, a metabolite of amygdalin, has been investigated for its antimicrobial activity and low vaginal irritation, particularly in the context of urinary tract infections and vaginal trichomoniasis (Xia et al., 2020). Other organic acids, including fumaric acid **(47)**, malic acid **(48)**, citric acid **(49)**, and gluconic acid **(50)**, have also been isolated and identified from ASA. Information of these organic acids is listed in Table 1. The chemical structures were drawn by ChemDraw 20.0 and shown in Figure 2.

3.3 Amino acids

Protein is a crucial component of human cells and tissues. The human body contains numerous proteins with diverse functions, all of which are formed through the dehydration and condensation of

FIGURE 1
P. armeniaca and processed products of *Armeniacae semen amarum*.

TABLE 1 Chemical components isolated and structurally identified from ASA.

No.	Chemical constituent	Molecular formula	Extract	Method	Reference
Glycosides					
1	Amygdalin	$C_{20}H_{27}NO_{11}$	Ethylacetate; ethanol	HPLC-PDA-ESI/MS	Hrichi et al. (2020)
			Methanol	HPLC-ESI-MS/MS	Xu et al. (2017)
			Methanol/water (7:3)	HPLC-ESI-MS	Senica et al. (2017)
2	Neoamygdalin	$C_{20}H_{27}NO_{11}$	Methanol	HPLC-ESI-MS/MS	Xu et al. (2017)
			Methanol/water (7:3)	HPLC-ESI-MS	Senica et al. (2017)
3	Prunasin	$C_{14}H_{17}NO_6$	Methanol	UPLC-MS/MS	Chen et al. (2022b)
			Methanol	HPLC-Q-TOF MS	Zhou et al. (2021)
			Methanol/water (7:3)	HPLC-ESI-MS	Senica et al. (2017)
4	Propyl-β-gentiobioside	$C_{15}H_{28}O_{11}$	Methanol	HPLC-Q-TOF MS	Zhou et al. (2021)
5	Mandelic acid-β-glucopyranoside	$C_{14}H_{18}O_8$	Methanol	HPLC-Q-TOF MS	Zhou et al. (2021)
6	Mandelic acid-β-gentiobioside	$C_{20}H_{28}O_{13}$	Methanol	UPLC-MS/MS	Chen et al. (2022b)
				HPLC-Q-TOF MS	Zhou et al. (2021)
7	Mandelic acid amide-β-glucopyranoside	$C_{14}H_{19}NO_7$	Methanol	UPLC-MS/MS	Chen et al. (2022b)
				HPLC-Q-TOF MS	Zhou et al. (2021)
8	Mandelic acid amide-β-gentiobioside	$C_{20}H_{29}NO_{12}$	Methanol	UPLC-MS/MS	Chen et al. (2022b)
				HPLC-Q-TOF MS	Zhou et al. (2021)
9	Benzyl-β-gentiobioside	$C_{19}H_{28}O_{11}$	Methanol	UPLC-MS/MS	Chen et al. (2022b)
				HPLC-Q-TOF MS	Zhou et al. (2021)
10	Adenosine	$C_{10}H_{13}N_5O_4$	Methanol	UPLC-MS/MS	Chen et al. (2022b)
				HPLC-Q-TOF MS	Zhou et al. (2021)
11	Cytarabine	$C_9H_{13}N_3O_5$	Methanol	UPLC-MS/MS	Chen et al. (2022b)
Organic acids					
Fatty acids					
12	Myristic acid	$C_{14}H_{28}O_2$	Ethylacetate; ethanol; Dichloromethane; chloroform	GC-FID/MS	Hrichi et al. (2020)
13	Palmitic acid	$C_{16}H_{32}O_2$	Ethylacetate; ethanol; Dichloromethane; chloroform	GC-FID/MS	Hrichi et al. (2020)
14	Heptadecanic acid	$C_{17}H_{34}O_2$	Ethylacetate; ethanol; Dichloromethane; chloroform	GC-FID/MS	Hrichi et al. (2020)
15	Stearic acid	$C_{18}H_{36}O_2$	Ethylacetate; ethanol; Dichloromethane; chloroform	GC-FID/MS	Hrichi et al. (2020)
16	Arachidic acid	$C_{20}H_{40}O_2$	Ethylacetate; ethanol; Dichloromethane; chloroform	GC-FID/MS	Hrichi et al. (2020)
17	Behenic acid	$C_{22}H_{44}O_2$	Ethylacetate; ethanol; Dichloromethane; chloroform	GC-FID/MS	Hrichi et al. (2020)
18	Palmitoleic acid	$C_{16}H_{30}O_2$	Ethylacetate; ethanol; Dichloromethane; chloroform	GC-FID/MS	Hrichi et al. (2020)
19	Heptadecenoic acid	$C_{17}H_{32}O_2$	Ethylacetate; ethanol	GC-FID/MS	Hrichi et al. (2020)
			Dichloromethane; chloroform		

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TABLE 1 (*Continued*) Chemical components isolated and structurally identified from ASA.

No.	Chemical constituent	Molecular formula	Extract	Method	Reference
20	Oleic acid	$C_{18}H_{34}O_2$	Ethylacetate; ethanol; Dichloromethane; chloroform	GC-FID/MS	Hrichi et al. (2020)
21	Eicosenoic acid	$C_{20}H_{38}O_2$	Ethylacetate; ethanol; Dichloromethane; chloroform	GC-FID/MS	Hrichi et al. (2020)
22	cis-13-Octadecenoic acid	$C_{18}H_{34}O_2$	H_2O	GC-MS	Shao et al. (2022)
23	9-Hexadecenoic acid	$C_{16}H_{30}O$	Aether	GC-MS	Zhang et al. (2007)
24	Linoleic acid	$C_{18}H_{32}O_2$	Ethylacetate; ethanol; Dichloromethane; chloroform	GC-FID/MS	Hrichi et al. (2020)
25	Linolenic acid	$C_{18}H_{30}O_2$	Ethylacetate; ethanol; Dichloromethane; chloroform	GC-FID/MS	Hrichi et al. (2020)
26	10-Octadecadienoic acid	$C_{18}H_{32}O_2$	Aether	GC-MS	Zhang et al. (2007)
27	Hydroxy-octadecatrienoic acid	$C_{18}H_{32}O_3$	Methanol	HPLC-Q-TOF MS	Zhou et al. (2021)
phenolic acids					
28	Protocatechuic acid	$C_7H_6O_4$	Ethylacetate; ethanol	HPLC–PDA–ESI/MS	Hrichi et al. (2020)
29	Gallic acid	$C_7H_6O_5$	Ethylacetate; ethanol	HPLC–PDA–ESI/MS	Hrichi et al. (2020)
30	Syringic acid	$C_9H_{10}O_5$	Methanol and then n-hexane	HPLC	Qin et al. (2019)
31	Salicylic acid	$C_7H_6O_3$	Ethanol	LC-ESI/MS	Cecarini et al. (2022)
32	Gentisic acid	$C_7H_6O_4$	Ethanol	LC-ESI/MS	Cecarini et al. (2022)
33	Vanillic acid	$C_8H_8O_4$	Ethanol	LC-ESI/MS	Cecarini et al. (2022)
34	Homovanillic acid	$C_9H_{10}O_4$	Ethanol	LC-ESI/MS	Cecarini et al. (2022)
35	Shikimic acid	$C_7H_{10}O_5$	H_2O	LC-ESI/MS	Al-Juhaimi et al. (2021)
36	Loganic acid	$C_{16}H_{24}O_{10}$	H_2O	LC-ESI/MS	Al-Juhaimi et al. (2021)
Other acids					
37	Quinic acid	$C_7H_{12}O_6$	Methanol	UPLC-MS/MS	Chen et al. (2022b)
38	2-Furoic acid	$C_5H_4O_3$	Methanol	UPLC-MS/MS	Chen et al. (2022b)
39	Orotic acid	$C_5H_4N_2O_4$	Methanol	UPLC-MS/MS	Chen et al. (2022b)
40	Nicotinic acid	$C_6H_5NO_2$	Methanol	UPLC-MS/MS	Chen et al. (2022b)
41	Pipecolic acid	$C_6H_{11}NO_2$	Methanol	UPLC-MS/MS	Chen et al. (2022b)
42	Mandelic acid	$C_8H_8O_3$	Methanol	UPLC-MS/MS	Chen et al. (2022b)
43	Indoleacrylic acid	$C_{11}H_9NO_2$	Methanol	UPLC-MS/MS	Chen et al. (2022b)
44	Benzoic acid	$C_7H_6O_2$	H_2O	GC-MS	(Geng et al., 2016; Li et al., 2016)
45	Benzeneacetic acid, alpha-hydroxy-, (S)	$C_8H_8O_3$	H_2O	GC-MS	Shao et al. (2022)
46	3-Pyrrolidineacetic acid or isomer	$C_6H_{11}NO_2$	Methanol	HPLC-Q-TOF MS	Zhou et al. (2021)
47	Fumaric acid	$C_4H_4O_4$	Methanol	UPLC-MS/MS	Chen et al. (2022b)
48	Malic acid	$C_4H_6O_5$	Methanol	UPLC-MS/MS	Chen et al. (2022b)
49	Citric acid	$C_6H_8O_7$	Methanol	UPLC-MS/MS	Chen et al. (2022b)
50	Gluconic acid	$C_6H_{12}O_7$	Methanol	HPLC-Q-TOF MS	Zhou et al. (2021)
Amino acids					
51	Aspartic acid	$C_4H_7NO_4$	Methanol	UPLC-MS/MS	Chen et al. (2022b)

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TABLE 1 (*Continued*) Chemical components isolated and structurally identified from ASA.

No.	Chemical constituent	Molecular formula	Extract	Method	Reference
52	Glutamic acid	$C_5H_9NO_4$	Methanol	UPLC-MS/MS	Chen et al. (2022b)
53	Proline	$C_5H_9NO_2$	Methanol	UPLC-MS/MS	Chen et al. (2022b)
				HPLC-Q-TOF MS	Zhou et al. (2021)
54	Leucine	$C_6H_{13}NO_2$	Methanol	HPLC-Q-TOF MS	Zhou et al. (2021)
55	Isoleucine	$C_6H_{13}NO_2$	Methanol	UPLC-MS/MS	Chen et al. (2022b)
				HPLC-Q-TOF MS	Zhou et al. (2021)
56	Phenylalanine	$C_9H_{11}NO_2$	Methanol	UPLC-MS/MS	Chen et al. (2022b)
				HPLC-Q-TOF MS	Zhou et al. (2021)
57	Tryptophan	$C_{11}H_{12}N_2O_2$	Methanol	UPLC-MS/MS	Chen et al. (2022b)
				HPLC-Q-TOF MS	Zhou et al. (2021)
58	Threonine	$C_4H_9NO_3$	HCL	Automatic amino acid analyzer	Li et al. (2004)
59	Serine	$C_3H_7NO_3$	HCL	Automatic amino acid analyzer	Li et al. (2004)
60	Glycine	$C_2H_5NO_2$	HCL	Automatic amino acid analyzer	Li et al. (2004)
61	Alanine	$C_3H_7NO_2$	HCL	Automatic amino acid analyzer	Li et al. (2004)
62	Cysteine	$C_3H_7NO_2S$	HCL	Automatic amino acid analyzer	Li et al. (2004)
63	Valine	$C_5H_{11}NO_2$	HCL	Automatic amino acid analyzer	Li et al. (2004)
64	Methionine	$C_5H_{11}O_2NS$	HCL	Automatic amino acid analyzer	Li et al. (2004)
65	Tyrosine	$C_9H_{11}NO_3$	HCL	Automatic amino acid analyzer	Li et al. (2004)
66	Lysine	$C_6H_{14}N_2O_2$	HCL	Automatic amino acid analyzer	Li et al. (2004)
67	Histidine	$C_6H_9N_3O_2$	HCL	Automatic amino acid analyzer	Li et al. (2004)
68	Arginine	$C_6H_{14}N_4O_2$	HCL	Automatic amino acid analyzer	Li et al. (2004)
Flavonoids					
69	Catechin	$C_{15}H_{14}O_6$	Ethylacetate; ethanol	HPLC–PDA–ESI/MS	Hrichi et al. (2020)
			Methanol/water (7:3)	HPLC-ESI-MS	Senica et al. (2017)
70	Epicatechin	$C_{15}H_{14}O_6$	Ethylacetate; ethanol	HPLC–PDA–ESI/MS	Hrichi et al. (2020)
			Methanol/water (7:3)	HPLC-ESI-MS	Senica et al. (2017)
71	Dimethoxyflavone	$C_{17}H_{14}O_4$	Ethylacetate; ethanol	HPLC–PDA–ESI/MS	Hrichi et al. (2020)
72	Acetylgenistin	$C_{23}H_{22}O_{11}$	Ethylacetate; ethanol	HPLC–PDA–ESI/MS	Hrichi et al. (2020)
73	Daidzein	$C_{15}H_{10}O_4$	Methanol	UPLC-MS/MS	Chen et al. (2022b)
74	Genistein	$C_{15}H_{10}O_5$	Methanol	UPLC-MS/MS	Chen et al. (2022b)
75	Neobavaisoflavone	$C_{20}H_{18}O_4$	Methanol	UPLC-MS/MS	Chen et al. (2022b)
76	Bavachinin	$C_{21}H_{22}O_4$	Methanol	UPLC-MS/MS	Chen et al. (2022b)

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TABLE 1 (*Continued*) Chemical components isolated and structurally identified from ASA.

No.	Chemical constituent	Molecular formula	Extract	Method	Reference
77	Naringenin hexoside	$C_{27}H_{32}O_{14}$	Methanol/water (7:3)	HPLC-ESI-MS	Senica et al. (2017)
78	Procyanidin dimer	$C_{30}H_{26}O_{12}$	Methanol/water (7:3)	HPLC-ESI-MS	Senica et al. (2017)
79	Phloridzin	$C_{21}H_{24}O_{10}$	Methanol/water (7:3)	HPLC-ESI-MS	Senica et al. (2017)
80	Quercetin-3-xyloside	$C_{20}H_{18}O_{11}$	Methanol/water (7:3)	HPLC-ESI-MS	Senica et al. (2017)
81	Quercetin-3-rhamnoside	$C_{21}H_{20}O_{11}$	Methanol/water (7:3)	HPLC-ESI-MS	Senica et al. (2017)
82	Quercetin-3-galactoside	$C_{21}H_{20}O_{12}$	Methanol/water (7:3)	HPLC-ESI-MS	Senica et al. (2017)
83	Quercetin-3-glucoside	$C_{21}H_{20}O_{12}$	Methanol/water (7:3)	HPLC-ESI-MS	Senica et al. (2017)
84	Quercetin-3-rutinoside	$C_{27}H_{30}O_{16}$	Methanol/water (7:3)	HPLC-ESI-MS	Senica et al. (2017)
85	Rutin trihydrate	$C_{27}H_{36}O_{19}$	Methanol and then n-hexane	HPLC	Qin et al. (2019)
86	Apigenin-7-glucoside	$C_{21}H_{20}O_{10}$	Methanol and then n-hexane	HPLC	Qin et al. (2019)
87	Naringenin	$C_{15}H_{12}O_5$	Methanol and then n-hexane	HPLC	Qin et al. (2019)
88	Quercetin	$C_{15}H_{10}O_7$	Methanol and then n-hexane	HPLC	Qin et al. (2019)
89	Isorhamnetin	$C_{16}H_{12}O_7$	Methanol and then n-hexane	HPLC	Qin et al. (2019)
90	Kaempferol	$C_{15}H_{10}O_6$	Methanol and then n-hexane	HPLC	Qin et al. (2019)
91	Luteolin 7-xyloside	$C_{20}H_{18}O_{10}$	Ethanol	LC-ESI/MS	Cecarini et al. (2022)
92	Apigenin	$C_{15}H_{10}O_5$	Ethanol	LC-ESI/MS	Cecarini et al. (2022)
93	Tricetin 3′-xyloside	$C_{20}H_{18}O_{11}$	Ethanol	LC-ESI/MS	Cecarini et al. (2022)
94	Quercitrin	$C_{21}H_{20}O_{11}$	Ethanol	LC-ESI/MS	Cecarini et al. (2022)
95	Rutin	$C_{27}H_{30}O_{16}$	Ethanol	LC-ESI/MS	Cecarini et al. (2022)
96	(±)Taxifolin	$C_{15}H_{12}O_7$	Ethanol	LC-ESI/MS	Cecarini et al. (2022)
97	Quercetin 3-(3″-sulfatoglucoside)	$C_{21}H_{20}O_{15}S$	Ethanol	LC-ESI/MS	Cecarini et al. (2022)
98	Isoliquiritigenin	$C_{15}H_{12}O_4$	Ethanol	LC-ESI/MS	Cecarini et al. (2022)
99	Petunidin	$C_{16}H_{13}O_7$	Ethanol	LC-ESI/MS	Cecarini et al. (2022)
100	Petunidin 3-rutinoside	$C_{28}H_{33}O_{16}$	Ethanol	LC-ESI/MS	Cecarini et al. (2022)
101	Petunidin 3-galactoside	$C_{22}H_{23}O_{12}$	Ethanol	LC-ESI/MS	Cecarini et al. (2022)
102	Cyanidin 3-O-galactoside	$C_{21}H_{21}O_{11}$	Ethanol	LC-ESI/MS	Cecarini et al. (2022)
103	Cyanidin 3-rutinoside	$C_{27}H_{31}O_{15}$	Ethanol	LC-ESI/MS	Cecarini et al. (2022)
104	Cyanidin 3-glucogalactoside	$C_{27}H_{31}O_{16}$	Ethanol	LC-ESI/MS	Cecarini et al. (2022)
105	Cyanidin 3-(6-acetylgalactoside)	$C_{23}H_{23}O_{12}$	Ethanol	LC-ESI/MS	Cecarini et al. (2022)
106	Cyanidin 3-(4″- acetylrutinoside)	$C_{29}H_{33}O_{16}$	Ethanol	LC-ESI/MS	Cecarini et al. (2022)
107	Pelargonidin 3-arabinoside	$C_{20}H_{19}O_9$	Ethanol	LC-ESI/MS	Cecarini et al. (2022)
108	Pelargonidin 3-lathyroside	$C_{26}H_{29}O_{14}$	Ethanol	LC-ESI/MS	Cecarini et al. (2022)
109	Pelargonidin 3-p-coumarylglucoside	$C_{30}H_{27}O_{12}$	Ethanol	LC-ESI/MS	Cecarini et al. (2022)
110	Malvidin 3-glucoside-pyruvate	$C_{26}H_{25}O_{14}$	Ethanol	LC-ESI/MS	Cecarini et al. (2022)
111	Delphinidin-3,5-diglucoside	$C_{27}H_{30}O_{17}$	H_2O	LC-ESI/MS	Al-Juhaimi et al. (2021)
112	Kaempferol-3- glucoside	$C_{21}H_{20}O_{11}$	H_2O	LC-ESI/MS	Al-Juhaimi et al. (2021)

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TABLE 1 (*Continued*) Chemical components isolated and structurally identified from ASA.

No.	Chemical constituent	Molecular formula	Extract	Method	Reference
Terpenoids					
Monoterpenoids					
113	3-Carene	$C_{10}H_{16}$	H_2O	GC-MS	Shao et al. (2022)
114	Cyclohexene, 1-methyl-4-(1-methylethylidene)	$C_{10}H_{16}$	H_2O	GC-MS	Shao et al. (2022)
115	1-Cyclohexene-1-methanol, 4-(1-methylethenyl)	$C_{10}H_{16}O$	H_2O	GC-MS	Shao et al. (2022)
116	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)	$C_{10}H_{18}O$	H_2O	GC-MS	Shao et al. (2022)
117	Alpha-Pinene	$C_{10}H_{16}$	1,2,3-trichloropropane	GC-MS	Jin et al. (2018)
118	2-Isopropyl-5-methylhexan-1-ol	$C_{10}H_{22}O$	1,2,3-trichloropropane	GC-MS	Jin et al. (2018)
119	Camphene	$C_{10}H_{16}$	1,2,3-trichloropropane	GC-MS	Jin et al. (2018)
120	Borneol	$C_{10}H_{18}O$	Deionized water	GC-MS	Hui et al. (2003)
121	Menthol	$C_{10}H_{20}O$	Deionized water	GC-MS	Hui et al. (2003)
122	Camphor	$C_{10}H_{16}O$	Deionized water	GC-MS	Hui et al. (2003)
			Not mentioned	HS-GC-MS	Chen et al. (2023)
123	Cinene	$C_{10}H_{16}$	Not mentioned	HS-GC-MS	Chen et al. (2023)
124	Linalool	$C_{10}H_{18}O$	Not mentioned	HS-GC-MS	Chen et al. (2023)
125	Terpineol	$C_{10}H_{18}O$	Not mentioned	HS-GC-MS	Chen et al. (2023)
Sesquiterpenes					
126	Copaene	$C_{15}H_{24}$	H_2O	GC-MS	Shao et al. (2022)
127	Caryophyllene	$C_{15}H_{24}$	Deionized water	GC-MS	Hui et al. (2003)
128	α-Caryophyllene	$C_{15}H_{24}$	Deionized water	GC-MS	Hui et al. (2003)
Diterpenoids					
129	trans-Geranylgeraniol	$C_{20}H_{34}O$	H_2O	GC-MS	Li et al. (2016)
130	Phytol	$C_{20}H_{40}O$	H_2O	GC-MS	Shao et al. (2022)
Triterpenoids					
131	Squalene	$C_{30}H_{50}$	n-hexane	TLC and capillary GLC	Rudzińska et al. (2017)
132	Amarogentin	$C_{29}H_{30}O_{13}$	H_2O	LC-ESI/MS	Al-Juhaimi et al. (2021)
Phytosterols					
133	Cholest-4-ene	$C_{27}H_{46}$	H_2O	GC-MS	Li et al. (2016)
134	cholesterol	$C_{27}H_{46}O$	n-hexane	TLC and capillary GLC	Rudzińska et al. (2017)
135	campesterol	$C_{28}H_{48}O$	n-hexane	TLC and capillary GLC	Rudzińska et al. (2017)
136	gramisterol	$C_{29}H_{48}O$	n-hexane	TLC and capillary GLC	Rudzińska et al. (2017)
137	Δ5-avenasterol	$C_{29}H_{48}O$	n-hexane	TLC and capillary GLC	Rudzińska et al. (2017)
138	Δ7-stigmasterol	$C_{29}H_{48}O$	n-hexane	TLC and capillary GLC	Rudzińska et al. (2017)
139	Δ7-Avenasterol	$C_{29}H_{48}O$	n-hexane	TLC and capillary GLC	Rudzińska et al. (2017)
140	β-sitosterol	$C_{29}H_{50}O$	n-hexane	TLC and capillary GLC	Rudzińska et al. (2017)
141	citrostadienol	$C_{30}H_{50}O$	n-hexane	TLC and capillary GLC	Rudzińska et al. (2017)
142	24-methylene-cycloartanol	$C_{31}H_{52}O$	n-hexane	TLC and capillary GLC	Rudzińska et al. (2017)

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TABLE 1 (*Continued*) Chemical components isolated and structurally identified from ASA.

No.	Chemical constituent	Molecular formula	Extract	Method	Reference
Phenylpropanoids					
143	Ferulic acid	$C_{10}H_{10}O_4$	Ethylacetate; ethanol	HPLC–PDA–ESI/MS	Hrichi et al. (2020)
144	Chlorogenic acid	$C_{16}H_{18}O_9$	Ethylacetate; ethanol	HPLC–PDA–ESI/MS	Hrichi et al. (2020)
			Methanol/water (7:3)	HPLC-ESI-MS	Senica et al. (2017)
145	Neochlorogenic acid	$C_{16}H_{18}O_9$	Ethylacetate; ethanol	HPLC–PDA–ESI/MS	Hrichi et al. (2020)
			Methanol/water (7:3)	HPLC-ESI-MS	Senica et al. (2017)
146	p-Coumaric acid	$C_9H_8O_3$	Methanol/water (7:3)	HPLC-ESI-MS	Senica et al. (2017)
147	3-Feruloylquinic acid	$C_{17}H_{20}O_9$	Methanol/water (7:3)	HPLC-ESI-MS	Senica et al. (2017)
148	5-Feruloylquinic acid	$C_{17}H_{20}O_9$	Methanol/water (7:3)	HPLC-ESI-MS	Senica et al. (2017)
149	p-Coumaric acid hexoside	$C_{15}H_{18}O_8$	Methanol/water (7:3)	HPLC-ESI-MS	Senica et al. (2017)
150	Caffeic acid hexoside	$C_{15}H_{18}O_9$	Methanol/water (7:3)	HPLC-ESI-MS	Senica et al. (2017)
151	Dicaffeoylquinic acid	$C_{25}H_{24}O_{12}$	Methanol/water (7:3)	HPLC-ESI-MS	Senica et al. (2017)
152	Coumarin	$C_9H_6O_2$	Ethylacetate; ethanol	HPLC–PDA–ESI/MS	Hrichi et al. (2020)
153	Psoralen	$C_{11}H_6O_3$	Methanol	UPLC-MS/MS	Chen et al. (2022b)
154	Schisandrin	$C_{24}H_{32}O_7$	Methanol	UPLC-MS/MS	Chen et al. (2022b)
155	Caffeic acid	$C_9H_8O_4$	Methanol and then n-hexane	HPLC	Qin et al. (2019)
156	trans-cinnamic acid	$C_9H_8O_2$	Methanol and then n-hexane	HPLC	Qin et al. (2019)
157	m-Coumaric acid	$C_9H_8O_3$	Ethanol	LC-ESI/MS	Cecarini et al. (2022)
158	5-caffeylquinic acid	$C_{16}H_{18}O_9$	H_2O	LC-ESI/MS	Al-Juhaimi et al. (2021)
Others					
159	Trehalose	$C_{12}H_{22}O_{11}$	Methanol	UPLC-MS/MS	Chen et al. (2022b)
160	Sucrose	$C_{12}H_{22}O_{11}$	Methanol	HPLC-Q-TOF MS	Zhou et al. (2021)
161	Berberine	$C_{20}H_{18}NO_4$	Methanol	UPLC-MS/MS	Chen et al. (2022b)
162	Tetrahydropalmatine	$C_{21}H_{25}NO_4$	Methanol	UPLC-MS/MS	Chen et al. (2022b)
163	Amygdalin amide	$C_{20}H_{29}NO_{12}$	Methanol	UPLC-MS/MS	Chen et al. (2022b)
164	Mandelamide	$C_8H_9NO_2$	H_2O	GC-MS	Shao et al. (2022)
165	N-Methoxy-N-methylbenzamide	$C_9H_{11}NO_2$	H_2O	GC-MS	Shao et al. (2022)
166	Nicotinamide	$C_6H_6N_2O$	Methanol	UPLC-MS/MS	Chen et al. (2022b)
167	Benzaldehyde	C_7H_6O	H2O	GC-MS	Geng et al. (2016), Li et al. (2016), Shao et al. (2022)
168	Nonanal	$C_9H_{18}O$	H2O	GC-MS	Li et al. (2016), Shao et al. (2022)
169	Benzyl alcohol	C_7H_8O	H2O	GC-MS	Geng et al. (2016), Li et al. (2016), Shao et al. (2022)
170	Benzyl cyanide	C_8H_7N	H2O	GC-MS	Geng et al. (2016)

amino acids. The protein content in ASA is more than 20%, and the content of important amino acids is reasonable and sufficient (Li et al., 2004). Currently, 18 amino acids **(51–68)** have been isolated and identified from ASA, among which leucine **(54)**, isoleucine **(55)**, phenylalanine **(56)**, tryptophan **(57)**, threonine **(58)**, methionine **(64)**, valine **(65)** and lysine **(66)** are essential amino acids, while histidine **(67)** is also an essential amino acid for infant growth. These amino acids are summarized in Table 1, and their chemical structures were drawn by ChemDraw 20.0 and presented in Figure 2.

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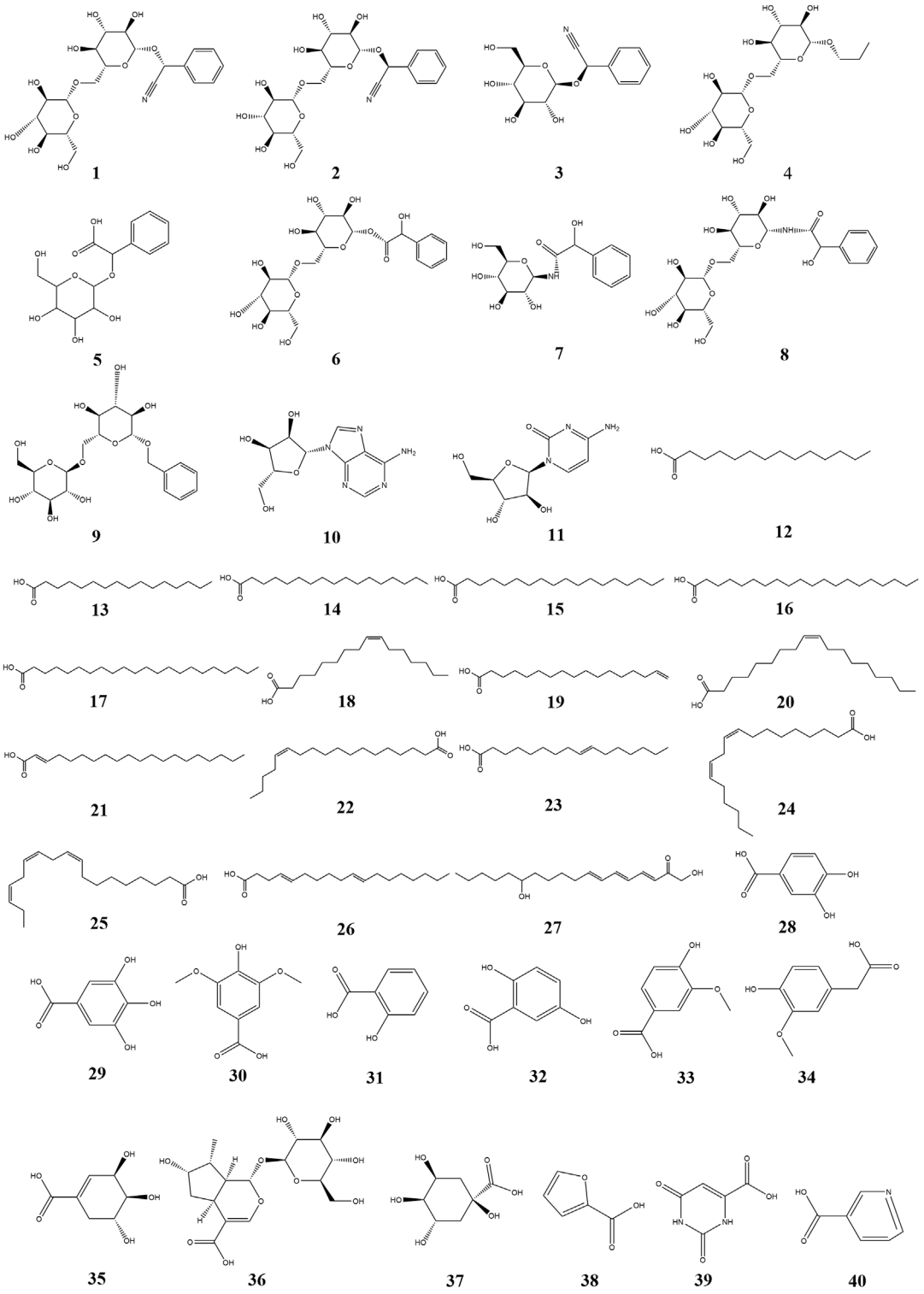

FIGURE 2
(Continued).

3.4 Flavonoids

Flavonoids have various physiological effects such as antioxidant, anti-inflammatory, and improvement of cardiovascular function (Feng et al., 2016; Shen et al., 2022). However, the content of flavonoids in ASA is 14.81 mg/100 g, less than 2‰ (Tanwar et al., 2018). Until now, 43 flavonoids **(69–112)** have been isolated and characterized from ASA, among which catechin **(69)**, epicatechin **(70)**, rutin trihydrate **(85)**, apigenin-7-glucoside **(86)**, luteolin 7-xyloside **(91)**, apigenin **(92)**, tricetin 3′-xyloside **(93)** are flavanols. Dimethoxyflavone **(71)**,

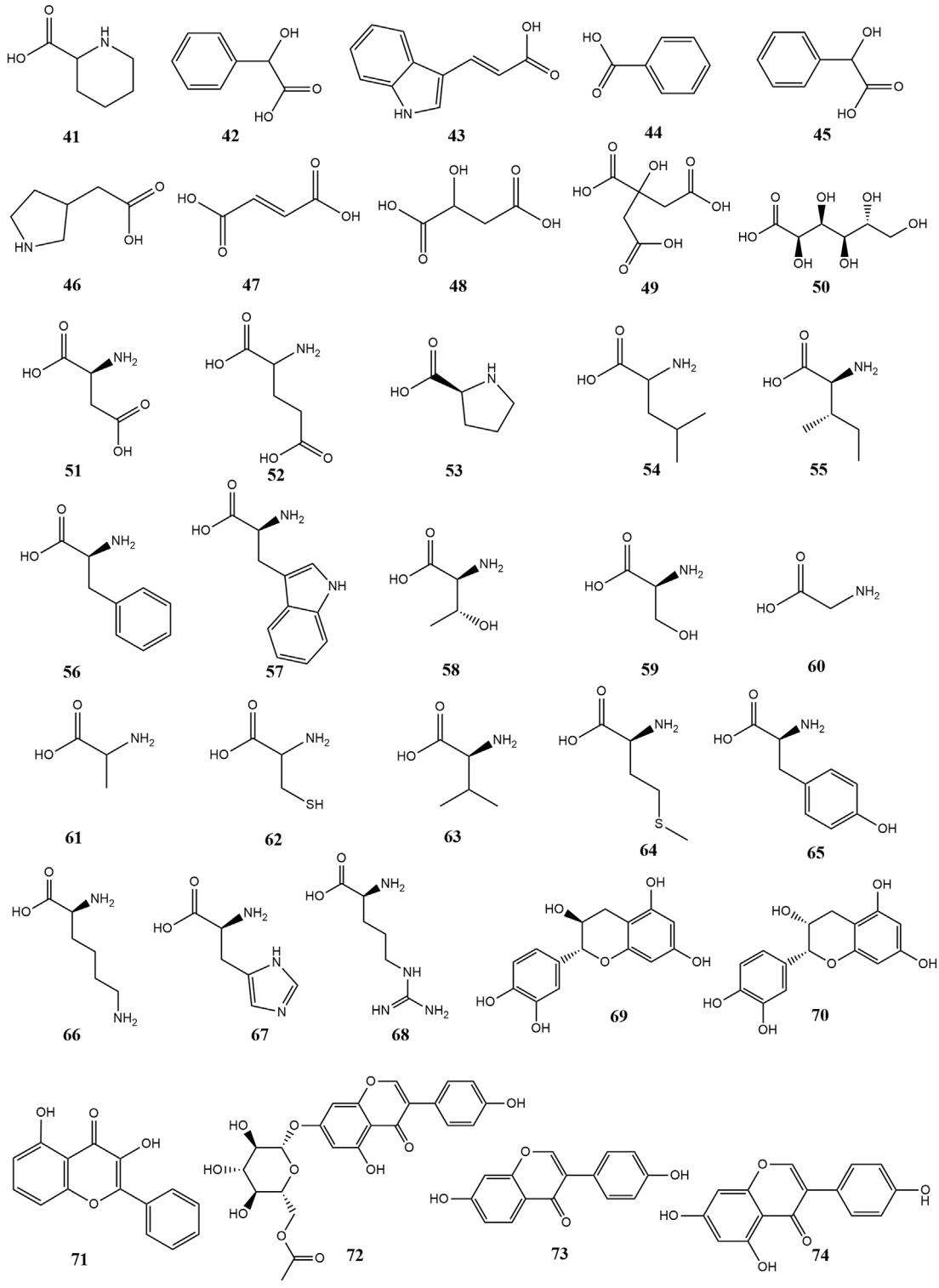

FIGURE 2
(Continued).

acetylgenistin **(72)**, daidzein **(73)**, genistein **(74)** and neobavaisoflavone **(75)** are isoflavones. Bavachinin **(76)**, naringenin hexoside **(77)**, procyanidin dimer **(78)** and isoliquiritigenin **(98)** are dihydroflavonoids. Phloridzin **(79)** and naringenin **(87)** are dihydrochalcones. Compounds **(80–84, 88–90, 94–97, 112)** are flavanols. Additionally, 12 anthocyanins **(99–111)** have been extracted from ASA skins, which belong to flavonoids as well (Qin et al., 2019; Cecarini et al., 2022). These flavonoids are summarized in Table 1, and their chemical structures were drawn using ChemDraw 20.0 and presented in Figure 2.

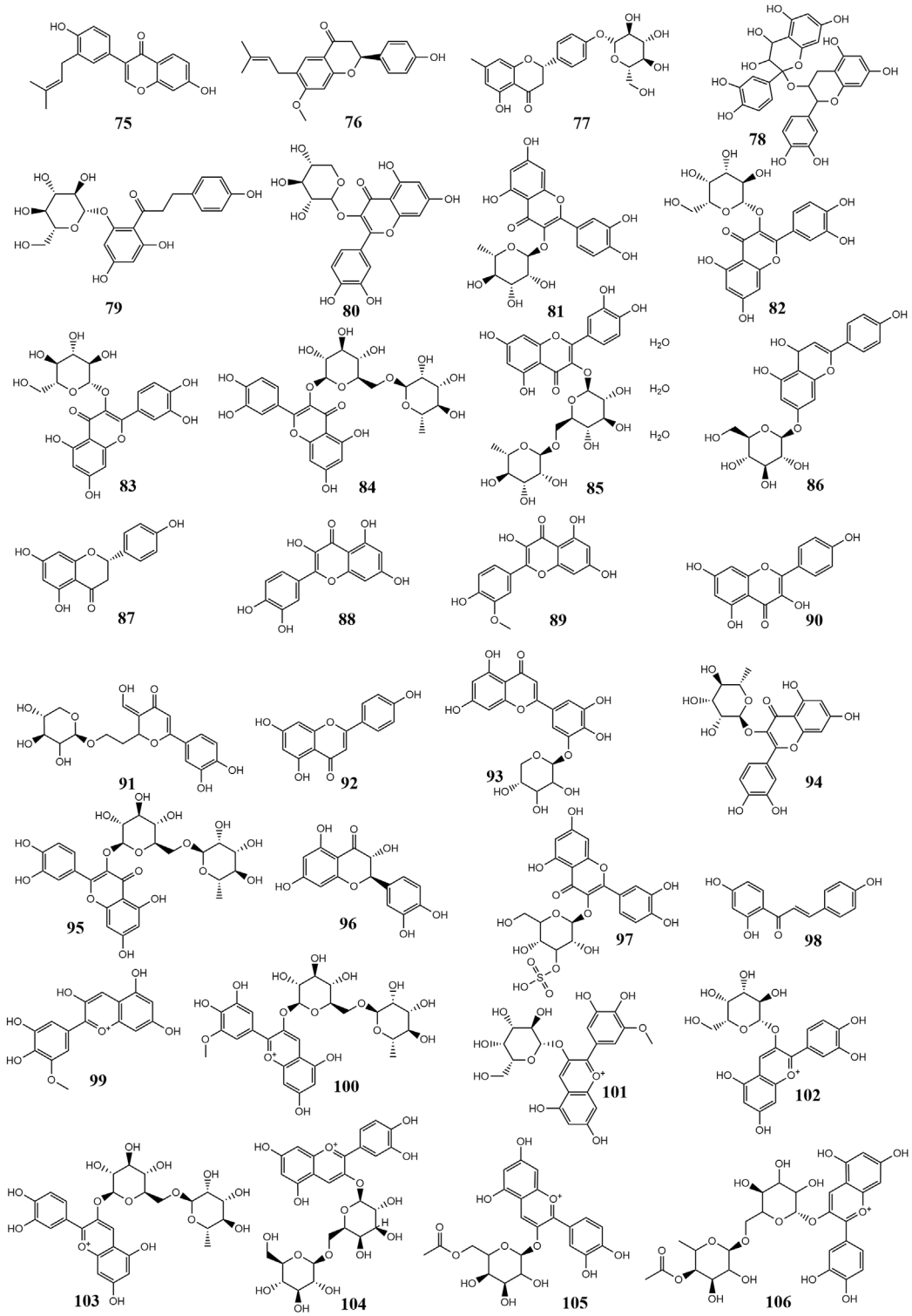

FIGURE 2
(Continued).

3.5 Terpenoids

Terpenoids, which consist of isoprene as the fundamental structural unit, are commonly found in Chinese herbal medicine and exhibit various pharmacological effects such as antioxidant, antimalarial, antibacterial, anti-inflammatory, and anti-cancer properties (Atriya et al., 2023). Currently, 20 terpenoids have been isolated and identified from ASA. These include 13 monoterpenoids **(113–125)**, 3 sesquiterpenoids **(126–128)**, two diterpenoids (trans-geranylgeraniol **(129)** and phytol **(130)**), and squalene **(131)**, which belongs to the

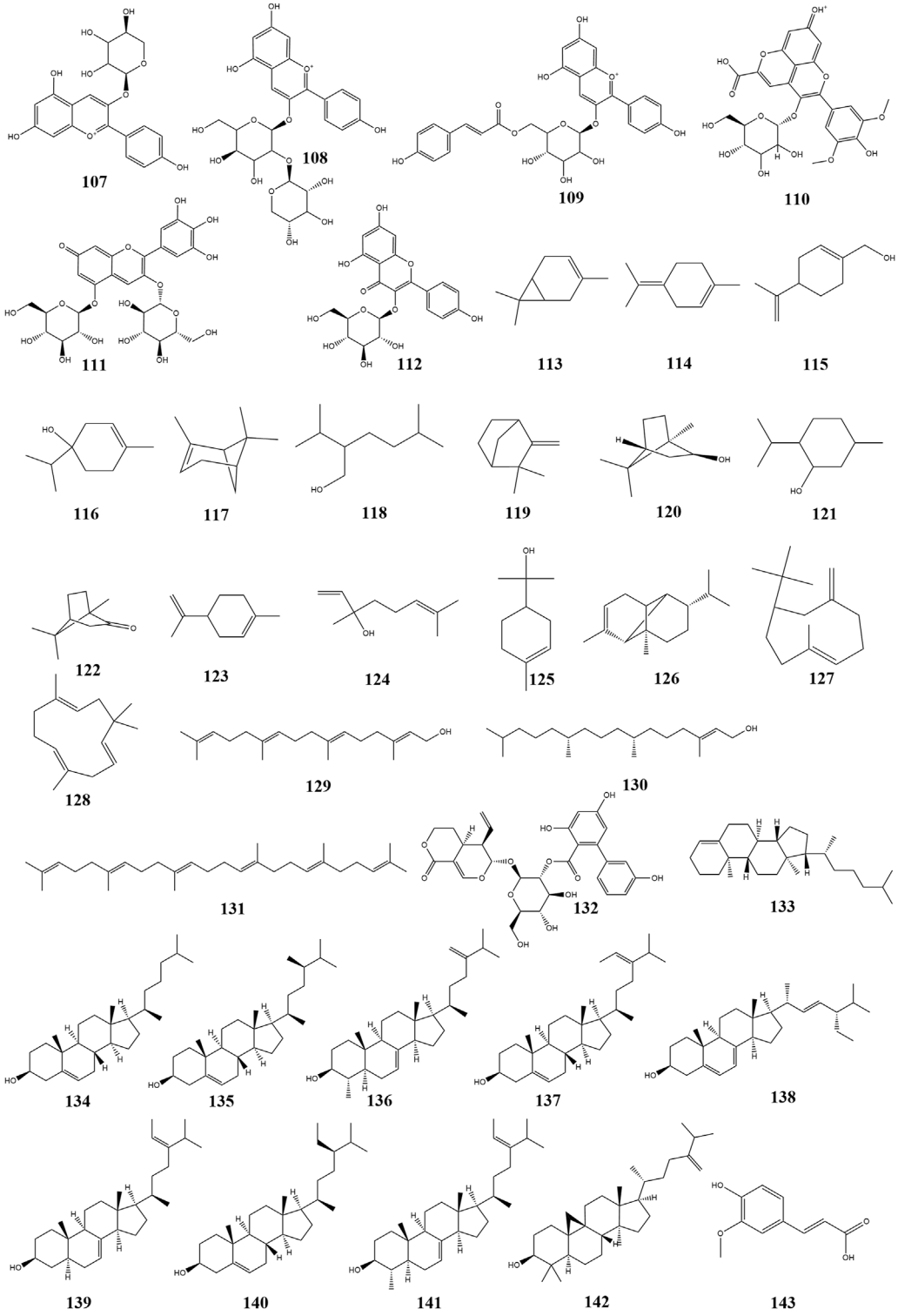

FIGURE 2
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triterpenoid group. Moreover, amarogentin **(132)**, a schizocyclic iridoterpenoid, has also been isolated from the aqueous extract of ASA. These terpenoids are summarized in Table 1, and their chemical structures were drawn by ChemDraw 20.0 and presented in Figure 2.

3.6 Physterols

The basic structure of sterols consists of cyclopentane polyhydrophenanthrene and a hydroxyl group. Phytosterols, a type of sterols, are commonly found in various parts of plants

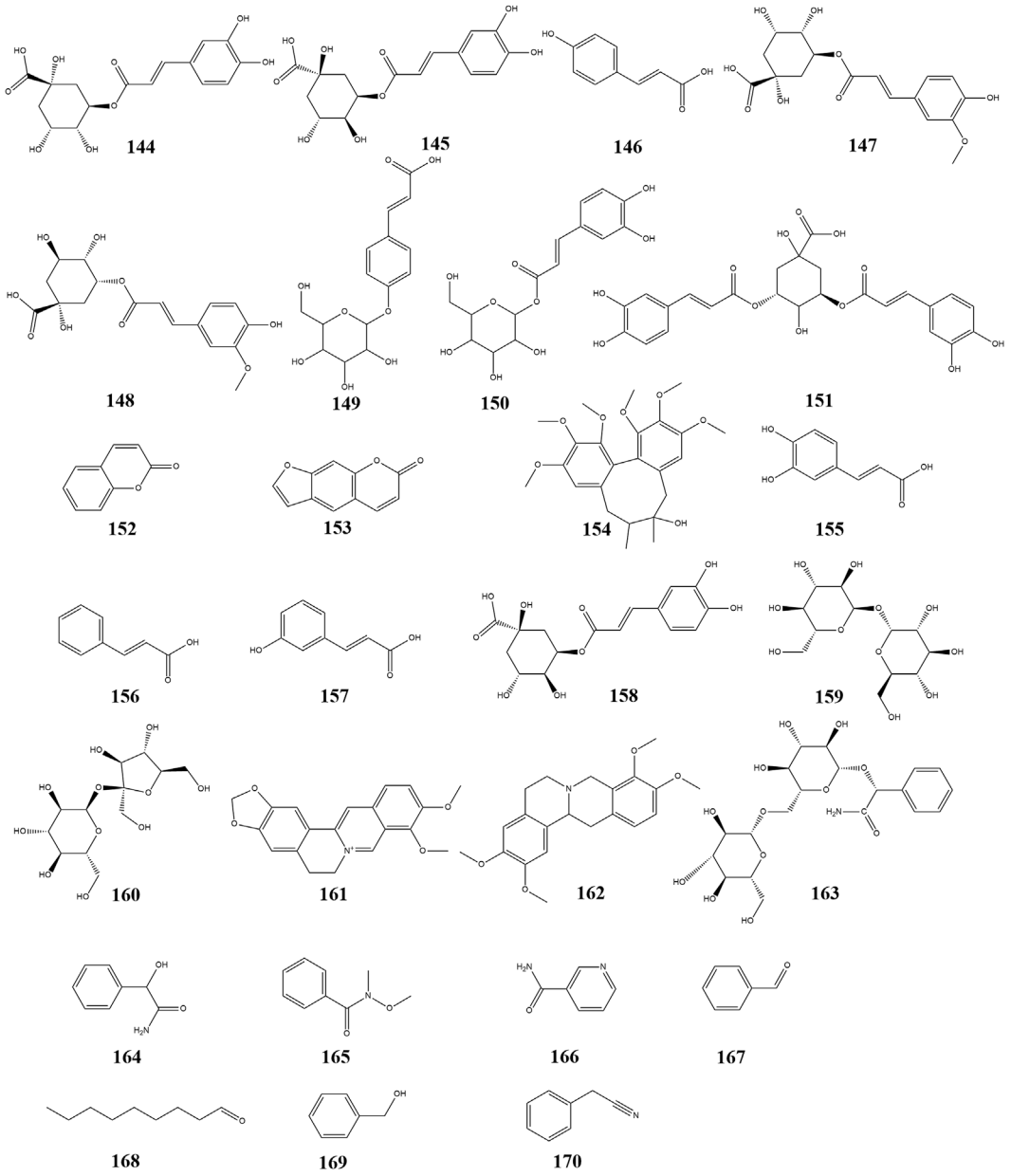

FIGURE 2
(Continued). Chemical structures of compounds isolated from *Armeniacae semen amarum*.

such as roots, stems, leaves, fruits, and seeds. Pharmacological studies have demonstrated the beneficial physiological effects of phytosterols, including their ability to prevent cardiovascular diseases, inhibit tumor growth, promote metabolism, and regulate hormone levels (Bakrim et al., 2022; Nattagh-Eshtivani et al., 2022). The total phytosterol content in different varieties of ASA ranges from 215.7 to 973.6 mg/100 g of bitter apricot kernel oil (Rudzińska et al., 2017). So far, researchers have isolated and identified 10 phytosterols **(133–142)** from ASA. In addition, Rudzińska Magdalena et al. analyzed the composition of ASA fat oil using TLC and capillary GLC methods, which revealed the presence of major phytosterols such as cholesterol **(134)**, campesterol **(135)**, gramisterol **(136)**, Δ5-avenasterol **(137)**, Δ7-stigmasterol **(138)**, Δ7-avenasterol **(139)**, β-sitosterol **(140)**, citrostadienol **(141)**, and 24-methylene-cycloartanol **(142)**. These physterols are summarized in Table 1. The corresponding chemical structures were drawn using ChemDraw 20.0 and presented in Figure 2.

3.7 Phenylpropanoids

The basic structural unit of phenylpropanoids consists of a benzene ring and three branched carbons (C6-C3). Until now, 16 phenylpropanoids have been successfully isolated and identified from ASA, among which **(143–151, 155–158)** are phenylpropanoic acids, coumarin **(152)** and psoralen **(153)** are coumarins, and schisandrin **(154)** is lignan. Besides, chlorogenic acid **(144)**, 5-feruloylquinic acid **(148)** and dicaffeoylquinic acid

(151) are polyphenols with significant anti-oxidant activity and free radical scavenging activity (Iwai et al., 2004; Cao et al., 2010; Park et al., 2015). These phenylpropanoids are summarized in Table 1, and their chemical structures were drawn by ChemDraw 20.0 and presented in Figure 2 as well.

3.8 Others

Besides the chemical constituents mentioned above, other components have also been investigated and summarized in Table 1, and the corresponding chemical structures are drawn by ChemDraw 20.0 in Figure 2. In brief, trehalose **(159)** and sucrose **(160)** are saccharides, berberine **(161)** and tetrahydropalmatine **(162)** are alkaloids, amygdalin amide **(163)**, mandelamide **(164)**, N-methoxy-N-methylbenzamide **(165)** and nicotinamide **(166)** are amide compounds. Furthermore, the compounds **(167–170)** are the main ingredients in ASA volatile oil.

4 Pharmacological activities

ASA exhibits a wide range of pharmacological activities and effects due to its abundance of chemical components and active substances. These include anticancer activity (breast carcinoma, prostatic cancer, hepatocellular carcinoma, lung cancer, renal cell carcinoma, bladder cancer and other cancers), anti-oxidant activity, antimicrobial activity, anti-inflammation activity, cardiovascular protection, neuroprotection, respiratory protection, digestive system protection, antidiabetic, liver and kidney protection, skin protection and other pharmacological activities (Figure 3). The following is a detailed introduction to the pharmacological effects of ASA.

4.1 Anticancer activity

In recent years, the overall incidence and mortality of cancer are still on the rise. Despite advances in various comprehensive therapies, the mortality rate of advanced malignant tumors remains high (Chen L. et al., 2021; Zhao et al., 2022a; Ming et al., 2022). ASA is rich in a variety of phytochemical ingredients, and amygdalin is one of its main active ingredients. Amygdalin is a phytochemical ingredient that has been extensively studied for its therapeutic effects on various types of cancers, including breast cancer, prostate cancer, hepatocellular carcinoma, renal cell carcinoma, lung cancer, bladder cancer, and others. Numerous studies have demonstrated the therapeutic potential of different ASA extracts and amygdalin. The therapeutic mechanism of ASA primarily involves inhibiting cancer cell adhesion, migration, and proliferation, as well as blocking the cell cycle, inducing cell oxidative damage and apoptosis, and regulating autophagy. However, it is important to note that the current research on the anticancer activity of ASA is mostly limited to *in vitro* cell studies, with fewer *in vivo* studies and a lack of clinical trials. Therefore, further investigation is needed to fully explore ASA as a potential alternative therapy for cancer. The effects of ASA and amygdalin on different types of cancer and their action mechanisms are summarized in Table 2 and Figure 4.

4.1.1 Breast carcinoma

Breast cancer is the most prevalent gynecological malignant tumor worldwide. The cure rate for patients diagnosed with early-stage breast cancer can reach 80%. However, treating patients in the advanced stages poses significant challenges (Zannetti, 2023). Conventional chemotherapy, radiotherapy, and targeted drug treatment are commonly used to treat breast cancer. Unfortunately, many patients develop drug resistance, experience cancer recurrence, and develop secondary diseases. *In vitro* studies, amygdalin, found in ASA, shows suppressive effects on various breast cancer cell lines including Hs578T, MCF-7, MDA-MB-231, SK-BR-3, and T47D cells, by inhibiting cancerous proliferation and migration, and inducing apoptosis, autophagy and oxidative stress.

Amygdalin impedes cell adhesion and migration by regulating integrin protein expression, which are cell adhesion molecules consisting of α and β subunits. Integrins facilitate the interaction between cancer cells and components of the extracellular matrix, thus influencing cell adhesion and eventually leading to cancer cell metastasis (Hoshino et al., 2015). In Hs578T breast cancer cells, amygdalin demonstrated a dose-dependent inhibition of cell adhesion, and it was observed that this inhibitory effect could potentially be attributed to the downregulation of integrin α5 protein expression (Lee and Moon, 2016). A decrease in mRNA levels of integrin αV/β3 and integrin α5 was observed in both MDA-MB-231 and MCF-7 cell lines, leading to the adhesion of cancer cells to fibronectin and collagen in the extracellular matrix. This decrease has an impact on the migration and metastasis of cancer cells. Notably, amygdalin shows a stronger inhibitory effect on integrin αV/β3 in MDA-MB-231 cells. Additionally, there were distinct variations in mRNA levels of integrin β1, β2, and β4 between the two cell lines. In MCF7 cells, integrin β1 and β4 levels increased, while integrin β2 levels decreased. Conversely, in MDA-MB-231 cells, the opposite trend was observed (Mosayyebi et al., 2021). The impact of amygdalin on cell adhesion and its effect on integrin protein expression have been extensively studied. However, the specific impact on different heterodimers is still not fully understood. A study conducted on MCF-7 cells showed that after 24 h and 48 h of amygdalin treatment, the IC50 values were determined to be 200.6 and 197 μg/mL, respectively. Additionally, Microarray Hybridization revealed that amygdalin can downregulate 19 out of 32 DNA replication-related genes, including MCM3, MCM6, MCM4, PCNA, and FEN1. This suggests that amygdalin may inhibit the proliferation of breast cancer cells by affecting DNA replication (Albogami and Alnefaie, 2021).

Apoptosis has long been recognized as a significant mechanism for preventing tumor development. The inhibitory effect of apoptosis is determined by the expression of Bcl-2 and Bax proteins (Czabotar et al., 2014). Studies have shown that amygdalin, at concentrations of 10 and 20 mg/mL, effectively suppresses the expression of Bcl-2 protein and enhances the expression of Bax in SK-BR-3 and MCF-7 cell lines (Moradipoodeh et al., 2020). This indicates that amygdalin can inhibit apoptosis in breast cancer cells. The human epidermal receptor 2 (HER2) is closely associated with breast cancer

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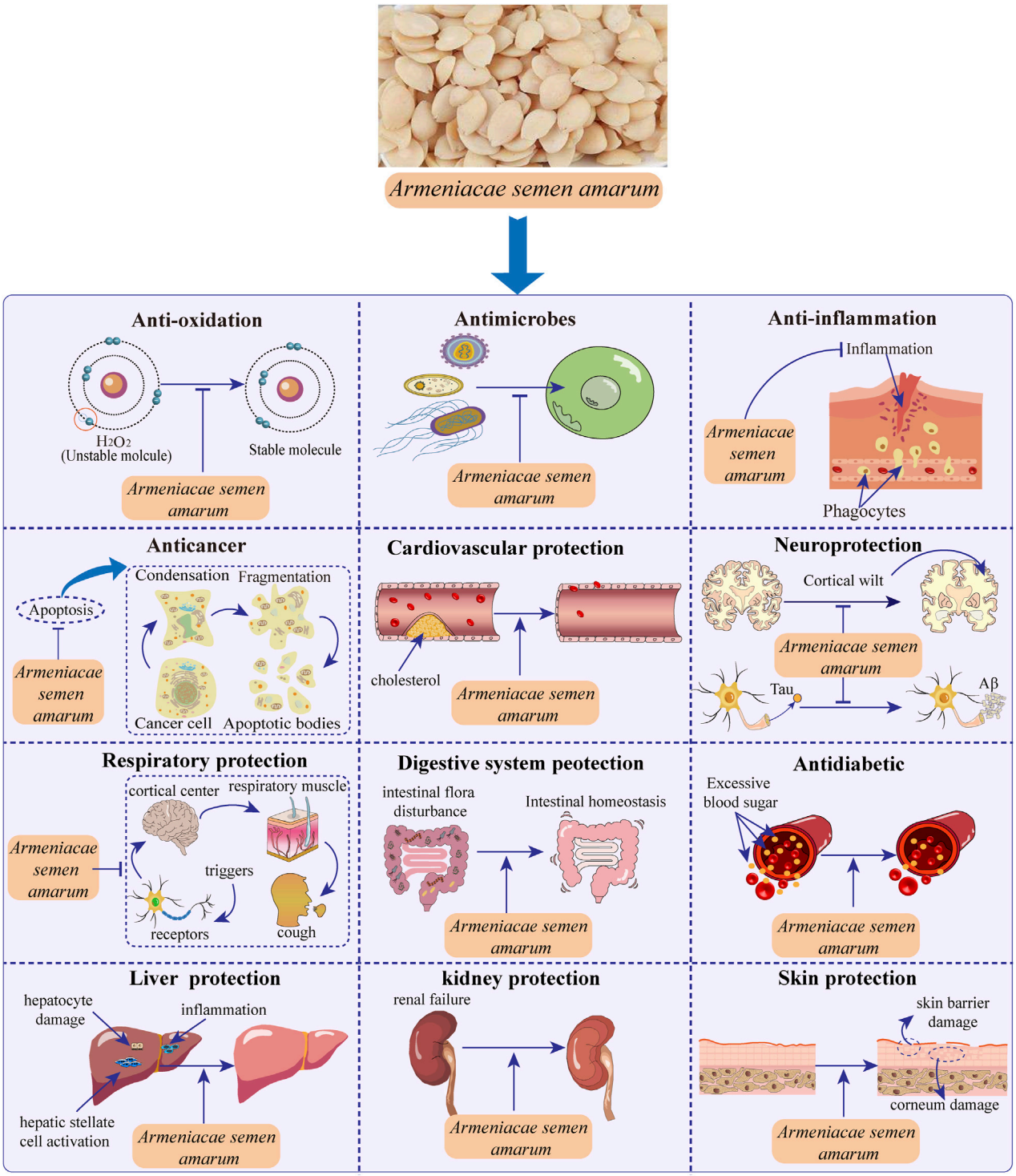

FIGURE 3
Pharmacological activities of *Armeniacae semen amarum*.

development and apoptosis (Shi et al., 2022). Molecular docking studies have revealed that amygdalin forms hydrogen bonds and hydrophobic interactions with Bcl-2 and the active site amino acids of HER2 in HER2-overexpressing SK-BR-3 cells. However, the binding ability of amygdalin to the active site amino acids of HER2 is weaker compared to lapatinib, a HER2 tyrosine kinase inhibitor. The metabolites of amygdalin, such as benzaldehyde, mandelonitrile, and cyanide, also bind to Bcl-2, although their binding affinity is weaker compared to amygdalin (Moradipoodeh et al., 2019). Another study found that amygdalin can diminish the apoptosis of Hs578T breast cancer cells by activating the p38 MAPK signaling pathway and regulating

TABLE 2 Anticancer activity of ASA.

Types of cancer	Cell line/ model	Compound	Minimal active concentration/dose	Mechanisms	Reference
Breast carcinoma	Hs578T cells	Amygdalin	10, 20, 40 mg/mL	Induction of apoptosis by intensifying the protein expressions of Bax and pp38 MAPK, while decreasing Bcl-2 and pro-caspase-3 protein expression; Decreasing adhesion via down-regulating integrin α5 protein expression	Lee and Moon (2016)
Breast carcinoma	MCF-7, MDA-MB-231 cells	Amygdalin	IC50 (MCF-7) = 34, 30 and 25 mg/mL for 24, 48 h and 72 h, respectively; IC50 (MDA-MB-231) = 28, 23.5 and 21 mg/mL for 24, 48 h and 72 h, respectively	Suppressing adhesion and migration, decreasing adhesion to fibronectin and collagen I, regulating mRNA levels of integrin α and β	Mosayyebi et al. (2021)
Breast carcinoma	MCF-7 cells	Amygdalin	IC50 = 200.6 and 197.9 μg/mL for 24 and 48 h, respectively	Inhibiting proliferation and blocking DNA replication	Albogami and Alnefaie (2021)
Breast carcinoma	MCF-7 and SK-BR-3 cells	Amygdalin	5, 10, 20 mg/mL	Inducing apoptosis, elevating Bax protein expression and descending Bcl-2 protein expression	Moradipoodeh et al. (2020)
Breast carcinoma	SK-BR-3 cells	Amygdalin	5, 10, 20 mg/mL	Inducing apoptosis, up-regulating Bax protein expression and down-regulating Bcl-2 protein expression	Moradipoodeh et al. (2019)
Breast carcinoma	MCF-7 cells	Amygdalin/ASA extracts	50 μM/1 mg/mL	Inhibition of autophagy cascade and migration due to downregulation of cathepsin B and L activities; Hampering the activities of proteasome 20S and 26S to induce apoptosis	Cecarini et al. (2022)
Breast carcinoma	MCF-7 and T47D cells	Amygdalin	65 mM	Induction of oxidative stress, Lowering MDA and GSSG levels, rising TGSH and GSH activities	Abboud et al. (2019)
Prostatic cancer	DU-145 cells	Amygdalin	10 mg/mL	Suppressing adhesion to HUVECs and immobilized collagen, repressing chemotaxis and migration; down-regulating integrin α6 protein expression while upregulating integrin α2 protein expression	Mani et al. (2020)
Prostatic cancer	LNCaP, DU-145 and PC3 cells	Amygdalin	10 mg/mL	Suppressing cell growth and promoting apoptosis, delaying cell cycle progression by repressing protein expression related to CDK1-cyclin B axis and AKT-mTOR pathway	Makarević et al. (2016)
Prostatic cancer	LNCaP and DU-145 cells	Amygdalin	0.1, 1 and 10 mg/mL	Promoting apoptosis by raising caspase-3 enzyme activity and Bax protein expression and lowering Bcl-2 protein expression	Chang et al. (2006)
Hepatocellular Carcinoma	HepG2 cells	Amygdalin	300 mg/mL	Arresting cell cycle at G2/M; Promoting cell apoptosis, heightening p53, Bax, cytochrome c and caspase-3 levels as well as diminishing Bcl-2 levels	El-Desouky et al. (2020)
Hepatocellular Carcinoma	HepG2 cells	Amygdalin	2.6 mg/mL	Delaying cell cycle at S and G2/M stages, inducing autophagy and apoptosis through inhibition of AMPK/mTOR and Bcl-2 pathway; Raising GSH levels and lessening MDA levels to alleviate cell necrosis caused by sorafenib	El-Sewedy et al. (2023)
Hepatocellular Carcinoma	DMBA-induced mice liver cancer	80% aqueous methanol of ASA; Amygdalin	ASA extract (400 mg/kg, oral administration) and amygdalin (1.85 mg/kg) once a day for 4 weeks	Up-regulating caspase-3 and downregulated Bcl-2 mRNA levels to inhibit apoptosis; Enhancing SOD, CAT, GSH, TAC levels and impeding MDA levels to exert antioxidant effects; Lowering beclin-1 mRNA level to	Hosny et al. (2021)

(Continued on following page)

TABLE 2 (*Continued*) Anticancer activity of ASA.

Types of cancer	Cell line/ model	Compound	Minimal active concentration/dose	Mechanisms	Reference
				regulate autophagy; Down-regulating the expressions of TNF-α, VEGF and PCNA to exert anti-inflammation, anti-angiogenesis and anti-proliferation effects, respectively	
Non-small cell lung cancer	H1299/M and PA/M cells	Amygdalin	2.5 and 5 mg/mL	Impeding proliferation, invasion and migration by hampering integrin β1, integrin β4, ILK, FAK, p-FAK, β-catenin, Akt and RICTOR protein expressions while up-regulating the expression of E-cadherin	Qian et al. (2015)
Lung Cancer	A549 and PC9 cells; Xenografted mice model	Amygdalin	*In vitro*: 10, 20 and 30 mg/mL; *In vivo*: 40 and 80 mg/kg; three times a week for 2 weeks	Activation of NFκB-1/NFκB signaling pathway and to prompt mitochondria-mediated apoptosis	Lin et al. (2022)
Renal cell carcinoma	Caki-1, KTC-26 and A498 cells	Amygdalin	10 mg/mL	Diminishing adhesion to HUVECs, immobilized collagen and fibronectin; Impeding chemotaxis and invasion ability through regulating the protein expressions of integrin α and β, and affecting the total content of integrin	Juengel et al. (2016a)
Renal cell carcinoma	Caki-1, KTC-26 and A498 cells	Amygdalin	10 mg/mL	Prompting cell cycle arrest and inhibition of growth, lessening CDK, CDK2, CDK4, cyclin A, cyclin B and cyclin D protein expressions; Altering cell differentiation, enhancing E-cadherin but hampering N-cadherin level	Juengel et al. (2016b)
Bladder cancer	UMUC-3, TCCSUP and RT112 cells	Amygdalin	10 mg/mL	Hampering the adhesion of UMUC-3, RT112 and TCCSUP cells to vascular endothelium and immobilized collagen; Repressing UMUC - 3, RT112 but boosting the cell migration capacity of TCCSUP cell	Makarević et al. (2014b)
Bladder cancer	UMUC-3, TCCSUP and RT112 cells	Amygdalin	10 mg/mL	Induction of apoptosis; Delaying cell cycle and 'arresting G0/G1 stage; Diminishing proliferation and growth by down-regulating the expression of CDK2 and cyclin A	Makarević et al. (2014a)
Cervical cancer	Hela cells; Xenografted nude mice	Amygdalin	*In vitro*:1.25, 2.5, 5, 10 and 20 mg/mL; *In vivo*: 300 mg/kg for 14 days	Hampering tumor growth; Induction of apoptosis by up-regulating Bax but down-regulating Bcl-2 protein expression, and intensifying caspase-3 enzyme activity	Chen et al. (2013)
Pancreatic cancer	PANC-1 cells	20% aqueous methanol of ASA; Amygdalin	ASA extracts:100–1,000 μg/mL; Amygdalin:5–40 mg/mL	Inhibition of cell growth, IC50 = 704 μg/mL at 72 h for ASA extracts and 35 mg/mL at 72 h for amygdalin; Activation of apoptotic through mitochondria-dependent pathway and enhancing mRNA level of caspase-3 and Bax/Bcl-2 mRNA expression ratio	Aamazadeh et al. (2020)
Acute leukemia	NALM-6 and KG-1 cells	Ethyl acetate extracts of ASA	IC50 = 0.388 mg/mL and 0.159 mg/mL for 48 h of NALM-6 and KG-1, respectively	Inducing apoptosis and up-regulating caspase-3 mRNA level	Mosadegh Manshadi et al. (2019)

the expression of Bcl-2 family and Caspase family proteins (Lee and Moon, 2016). Furthermore, when MCF-7 breast cancer cells and MCF-10A normal cells were treated with 50 μM amygdalin and 1 mg/mL ASA extract, it was observed that the activities of proteasomes 20S and 26S, Cathepsin B, and cathepsin L in MCF-7 cells were inhibited. Additionally, the expressions of p53, p27, and Bax were increased, indicating that amygdalin and ASA extract may promote apoptosis and regulate the autophagy cascade (Cecarini et al., 2022). Moreover, amygdalin can induce oxidative stress in breast cancer cells by increasing GSH activity and reducing MDA and oxidized glutathione levels, thereby exerting anti-cancer effects (Abboud et al., 2019).

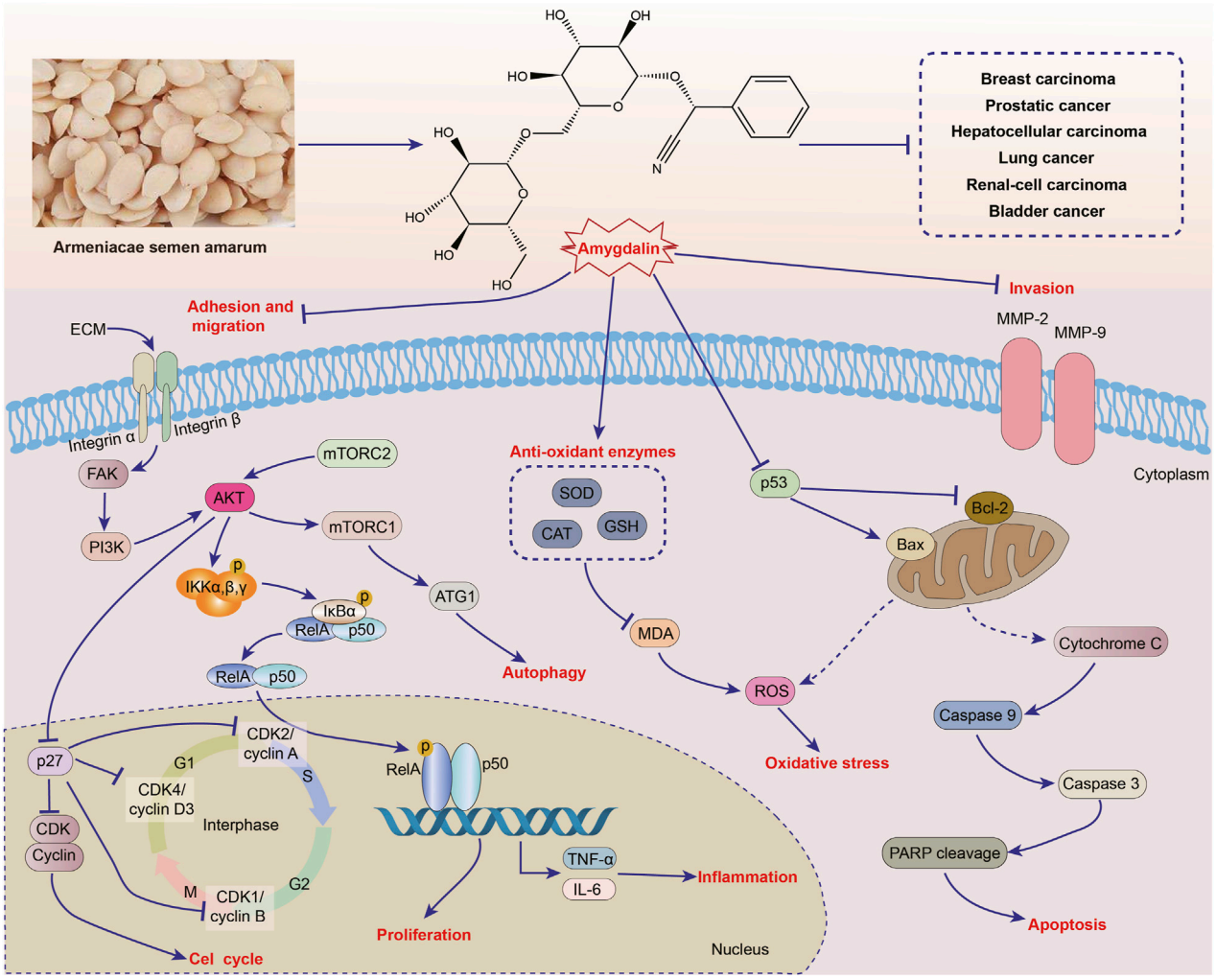

FIGURE 4
Anticancer activities of *Armeniacae semen amarum*.

4.1.2 Prostatic cancer

Prostatic cancer is the most common type of cancer in men, with approximately 40% of patients eventually developing other metastatic diseases. Therefore, it is crucial to investigate the potential of natural chemical components found in plants as alternative therapies for prostate cancer treatment (Martínez-Piñeiro et al., 2003). Amygdalin has demonstrated anti-prostate cancer activity in LNCaP, DU-145, and PC3 cells. Its primary mechanisms involve inhibiting cell adhesion, migration and metastasis, and inducing apoptosis and cell cycle arrest, attributed to its downregulation of integrin α6 and Bcl-2, while upregulation of integrin α2, Bax and caspase-3, as well as inhibition of CDK1-cyclin B axis and the AKT-mTOR pathway.

A study demonstrated that treating DU-145 prostate cancer cells with 10 mg/mL amygdalin for 24 h inhibited their adhesion, chemotaxis, and migration. This inhibition was attributed to the downregulation of integrin α2 and the upregulation of α6. Integrin α2 plays a critical role in cell adhesion, which in turn regulates cell invasion and metastasis. However, a decrease in adhesion of PC3 cells was observed only after 2 weeks of amygdalin treatment, with no impact on their chemotaxis and migration abilities. Further experiments involving the knockout of integrins α2, α6, and β1 revealed distinct changes in the adhesion, chemotaxis, and migration abilities of DU-145 and PC3 cells (Mani et al., 2020). In conclusion, the effects of amygdalin on cell adhesion, migration, and metastasis are influenced by the epigenetics of tumor cells, and each cell line may have a specific set of receptors. Amygdalin has shown potential anticancer activities by influencing the cell cycle. In a 2-week study, amygdalin administration resulted in the prolongation of the G0/G1 phase and the shortening of the S phase and G2/M phase in LNCaP, DU-145, and PC3 cells. Additionally, it inhibited the expression of cell cycle regulatory proteins, including CDK1, CDK2, CDK4, cyclin A, cyclin B and cyclin D3, as well as the AKT-mTOR signaling cascade (Makarević et al., 2016). Furthermore, amygdalin has been found to enhance cell apoptosis by increasing caspase-3 enzyme activity and Bax protein expression, while decreasing Bcl-2 protein expression (Chang et al., 2006).

4.1.3 Hepatocellular carcinoma

Hepatocellular carcinoma is a prevalent type of cancer. A study involving 148 hepatocellular carcinoma patients found that 75 of them died within 22 months. Cirrhosis developed in 77% of the patients, and the 1-year and 3-year survival rates were 70.8% and 47.6% respectively (Wongjarupong et al., 2021). After administering ASA treatment, there was a significant increase in the proportion of early apoptosis, late apoptosis, and necrosis cells in HepG2 hepatocellular carcinoma. This effect was positively correlated with the upregulation of p53, Caspase-3, and Bcl-2 activities, as well as the downregulation of Bax. It is worth noting that the pro-apoptotic effect of amygdalin is enhanced with the addition of zinc (El-Desouky et al., 2020). Sorafenib, a commonly used targeted drug for liver cancer treatment, often leads to severe side effects and drug resistance in patients (Zheng et al., 2014). Experiments have demonstrated that 2.6 mg/mL amygdalin alone or in combination with sorafenib can induce cell cycle arrest in HepG2 cells and trigger autophagy and apoptosis. These results align with the upregulation of AMPK, HMGB1, beclin-1, and ATG5 mRNA levels, as well as the downregulation of mTOR and Bcl-2 levels. Unlike sorafenib, amygdalin can increase GSH level, reduce MDA level, and exhibit strong DPPH free radical scavenging ability (El-Sewedy et al., 2023). These findings suggest that amygdalin holds significant potential for the treatment of hepatocellular carcinoma.

The therapeutic effects of ASA extract on liver cancer have been demonstrated *in vivo*. When liver cancer is induced by 2,2′-Bis (hydroxymethyl)butyric (DMBA), ASA methanol-water extract and amygdalin have been shown to significantly increase the levels of SOD, CAT, GSH, and TAC, while inhibiting MDA levels. These effects contribute to the anti-oxidant properties of ASA, which are crucial in protecting the liver from oxidative damage. Additionally, ASA has been found to downregulate the mRNA levels of Bcl-2 and beclin-1, reduce TNF-α and VEGF contents, and downregulate PCNA protein expression in mouse liver tissues (Hosny et al., 2021). These findings indicate that ASA can inhibit inflammation through apoptosis, autophagy, angiogenesis, and proliferation pathways, thereby exerting anti-cancer effects.

4.1.4 Lung cancer

Lung cancer is a prevalent and deadly malignant tumor that often metastasizes to various organs including the brain, bone, liver, and kidney. Current treatments primarily focus on primary lung cancer, leading to a poor prognosis for metastatic patients (Yin et al., 2021). However, in highly metastatic non-small cell lung cancer cell lines H1299/M and PA/M, amygdalin at concentrations of 2.5 and 5 mg/mL significantly inhibits cell proliferation, migration, and invasion. The inhibition rates of cell proliferation decreased by 15.6% and 25.1% respectively under these concentrations. Amygdalin achieves its function by reducing the levels of integrin β1 and β4, while upregulating the level of E-cadherin (Qian et al., 2015). This not only affects tumor cell adhesion but also activates FAK, β-catenin, and the downstream AKT-mTOR signaling pathway to mediate cell proliferation, adhesion, and metastasis. Additionally, amygdalin effectively promotes cancer cell apoptosis in A549 and PC9 cancer cells *in vitro*, as well as in A549 cell xenograft mice. This is achieved by inhibiting the NF-κB signaling pathway through increased protein expression of NF-κB-1 and further altering the expression of apoptosis-related proteins Bax, Bcl-2, cytochrome C, caspase 9, caspase 3, and PARP (Lin et al., 2022). In conclusion, amygdalin shows promising potential for treating lung cancer and may serve as a potential NF-κB-1 agonist.

4.1.5 Renal cell carcinoma

Renal cell carcinoma, which accounts for 80% of all kidney cancers, is a common type of urinary tract tumor. In the United States, there are approximately 64,000 new cases and 14,000 deaths associated with renal cell carcinoma each year (Singh, 2021). Amygdalin has demonstrated anti-renal cell carcinoma activity *in vitro*, specifically in Caki-1, KTC-26, and A498 cells. This activity is attributed to the regulation of integrin α and β protein expressions, leading to the inhibition of adhesion and migration. Additionally, amygdalin inhibits CDK/cyclin complexes, thereby arresting the cell cycle.

Amygdalin at a concentration of 10 mg/mL has been found to inhibit the adhesion, chemotaxis and migration of Caki-1, KTC-26 and A498 cells, due to the downregulation of integrins α5 and α6 levels. Furthermore, the expression changes of other integrin subtypes in these cells vary, suggesting that the integrin profile may be specific to each cell line (Juengel et al., 2016a). Additionally, amygdalin induces cell cycle arrest by increasing the number of cells in the G0/G1 phase of Caki-1 and A498 cells, and in the S phase of KTC-26 cells, which may be attributed to the diminishment of CDK, CDK2, CDK4, cyclin A, cyclin B and cyclin D protein expressions (Juengel et al., 2016b). Notably, amygdalin may impact cancer cell differentiation by regulating N-cadherin and E-cadherin, potentially influencing the prognosis of the cancer. However, further research is necessary to investigate the specific impact of cadherin on cell differentiation in renal cell carcinoma.

4.1.6 Bladder cancer

Bladder cancer is a prevalent form of cancer that affects the urinary system, leading to significant morbidity and mortality. A key symptom of bladder cancer is painless hematuria. As the disease progresses, patients may experience urinary retention, poor urination, and urinary tract obstruction (Xiang et al., 2021). In recent studies, amygdalin has shown promise in inhibiting the adhesion of bladder cancer cells (UMUC-3, TCCSUP, and RT112) by potentially affecting integrin expression. However, the specific integrin profile in different cell lines appears to play a more significant role. Furthermore, amygdalin has been observed to impede the migration of UMUC-3 and RT112 cells, while paradoxically increasing the migration of TCCSUP cells (Makarević et al., 2014b). It is important to note that although amygdalin can inhibit cancer cell adhesion, prolonged exposure to certain cancer cells may promote the migration of non-adherent cells. Additionally, amygdalin has demonstrated inhibitory effects on the growth and proliferation of UMUC-3, TCCSUP, and RT112 cancer cells. This is primarily achieved by causing cell cycle delay and arresting cells in the G0/G1 phase, possibly through the downregulation of CDK2 and cyclin A protein expression (Makarević et al., 2014a).

4.1.7 Other cancers

In addition to its therapeutic potential for the above cancer types, ASA has also shown suppression of cervical cancer, pancreatic

cancer and blood cancer, mainly based on the effects of amygdalin. Both *in vivo* and *in vitro* studies have demonstrated that amygdalin has positive therapeutic effects on cervical cancer. The main mechanism of amygdalin's therapeutic effect is inhibition of cell growth and promotion of apoptosis (Chen et al., 2013). Furthermore, research has shown that the methanol aqueous extract of ASA and amygdalin can promote apoptosis in PANC-1 pancreatic cancer cells (Aamazadeh et al., 2020). Additionally, a separate study found that the ethyl acetate extract of ASA has an inhibitory effect on NALM-6 acute B lymphoid leukemia cells and KG-1 myeloid leukemia cells (Mosadegh Manshadi et al., 2019).

4.2 Anti-oxidation

The anti-oxidant activity of ASA primarily involves the elimination of lipid peroxidation, reduction of reactive oxygen species (ROS) accumulation, and enhancement of anti-oxidant enzyme activity, and the main functional substances are polyphenols (Table 3). Malondialdehyde (MDA) is a crucial marker for LPO resulting from the oxidation of polyunsaturated fatty acids (Liu et al., 2018). A study with the ethanol-induced rat liver injury and oxidative stress model has demonstrated that consumption of ASA significantly decreases LDH content in serum, MDA level in red blood cells, brain, kidney and heart of rats while increasing the content of anti-oxidant enzymes such as superoxide dismutase (SOD) and glutathione S-transferase (GST) in the liver (Yurt and Celik, 2011). This indicates that ASA can prevent liver injury by increasing the activity of anti-oxidant enzymes and inhibiting lipid peroxides to resist oxidative stress. *Mahboub, H.H. et al.* have also reported that ASA consumption significantly enhances the overall anti-oxidant capacity within *cyprinus carpio*, which may be attributed to the upregulation of anti-oxidant enzymes. When 10 g/kg ASA was added to the basic diet for continuous feeding over a period of 60 days, the total anti-oxidant capacity (TAC), glutathione (GSH), and SOD contents in liver tissue were increased from 16.66 ng/mg to 58.33 ng/mg, 30.33 mmol/g to 66.33 mmol/g, and 14 to 48 U/mg respectively, meanwhile, SOD, GPX, and GSS mRNA levels in spleen were also intensified (Mahboub et al., 2022).

In addition, the anti-oxidant capacity of ASA is positively correlated with the total phenolic content in the extract. Phenolic compounds have the ability to scavenge free radicals and participate in redox reactions to protect cells from oxidative damage (Desmarchelier et al., 2005). Qin, F. et al. extracted ASA with 50% ethanol and found that the extract had a total phenolic content of 874.49 ± 6.75 mg GAE (gallic acid equivalent)/100 g fresh weight. This extract demonstrated excellent free radical scavenging ability in free radical scavenging assays. The extract showed significantly stronger total reducing activity, 2′-Azinobis-(3-ethylbenzthiazoline-6-sulphonate) (ABTS) free radical scavenging activity, and H_2O_2 scavenging activity compared to ascorbic acid. However, its 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging ability, hydroxide ion, and peroxy ion were comparable to that of ascorbic acid (Qin et al., 2019). However, Yiğit, D. et al. extracted ASA with methanol and water, the total phenolic content was 0.4 and 0.5 μg GAE/mL, respectively, while the DPPH free radical scavenging activity was poor at the concentration of 100–300 μg/mL, indicating that the total phenolic content of ASA is a key factor affecting its anti-oxidant capacity (Yiğit et al., 2009). Furthermore, the variety and origin of ASA also play important roles in determining the total phenolic content. Among the five varieties of ASA in Poland, the "Somo" variety had the highest total phenolic content of 1.22 mM GAE/L, and this variety showed the best anti-oxidant activity according to the ferric reducing anti-oxidant power (FRAP) test. Similarly, ASA from five different regions of Pakistan exhibited significant differences in anti-oxidant activity after extraction with n-hexane. The ASA from Badoghur had a total phenol content of 5,005 mg GAE/100 g dry weight, which was significantly higher than that of other origins. Additionally, ASA from Badoghur showed the smallest half maximal inhibitory concentration (IC50) value in total anti-oxidant capability, hydrogen peroxide scavenging, DPPH, and FRAP experiments, indicating the strongest anti-oxidant activity (Tareen et al., 2021).

Moreover, recent studies have revealed that ASA contains other components, besides phenols, that have anti-oxidant capacity. One such component is a neutral polysaccharide called AP-1, which was extracted and isolated from ASA. AP-1 exhibited a maximum inhibition rate of 87.74% for DPPH radical scavenging activity at a concentration of 10 mg/mL, which is slightly lower than that of vitamin C. However, ABTS assay revealed that AP-1 has comparable free radical scavenging ability and hydroxyl radicals to vitamin C (Peng et al., 2023). Furthermore, amygdalin also demonstrated anti-oxidant capacity by inhibiting ROS accumulation and activating anti-oxidant enzyme activities such as catalase (CAT) and SOD in RAW264.7 cells (Trang et al., 2022).

4.3 Antimicrobial activity

A growing number of experimental studies have demonstrated the broad spectrum of antibacterial activity exhibited by ASA. Different extracts of ASA have varying degrees of antibacterial activity, as outlined in Table 4. Among these extracts, ASA volatile oil stands out for its extensive antibacterial activity, which is likely attributed to its main component, benzaldehyde. This component has been widely utilized in cosmetics due to its antibacterial, antiseptic, and stabilizing effects (Rodrigues and de Carvalho, 2022). ASA volatile oil exhibits excellent antibacterial activity against Gram-positive bacteria such as *Staphylococcus aureus*, *Staphylococcus epidermidis* and *methicillin-resistant S. aureus* as well as Gram-negative bacteria including *Escherichia coli*, *Pseudomonas aeruginosa*, *P. aeruginosa D24*, *Salmonella typhimurium* and *Shigella sonnei*. Complete growth inhibition was observed with a minimum inhibitory concentration (MIC) ranging from 250 to 500 μg/mL. Furthermore, the ASA essential oil also displayed certain antibacterial activity against several other clinical pathogenic bacteria (Lee et al., 2014). Additionally, ASA volatile oil exhibited a significant inhibitory effect on *Listeria monocytogenes* in solid medium, micro-atmospheric medium, liquid medium and beef slices (Wang et al., 2020). *Listeria monocytogenes* is an intracellular parasite, primarily transmitted through food, and severe poisoning can result in blood and brain infections (Stevens et al., 2006). Studies have indicated that polyphenols can bind to bacterial cell membrane, disrupt bacterial cell membrane proteins, induce bacterial metabolic

TABLE 3 Anti-oxidant activity of ASA.

Extract/ compound	Mechanism	Minimal active concentration/dose	*In vitro*/ *In vivo*	Reference
ASA ethanol extract	Decreasing LDH content in serum and MDA accumulation in erythrocyte, brain, kidney, and heart, while heightening SOD and GST content in liver in ethanol-induced rats liver injury and oxidative stress model	15% ASA +20% alcohol-water	*In vivo*	Yurt and Celik (2011)
ASA	Raising TAC, SOD, and GSH content in liver tissue of *Cyprinus carpio*, while up-regulating SOD, GPX, and GSS mRNA levels in spleen	2.5, 5, and 10 g/kg	*In vivo*	Mahboub et al. (2022)
ASA polyphenols	Reducing ferric, Scavenging ABTS radicals, hydrogen peroxide radicals, DPPH radicals, hydroxy radicals, and peroxy radicals	IC50 = 3.05, 0.24, 18.71, 13.77, 37.64, and 32.46 mg/mL, respectively	*In vitro*	Qin et al. (2019)
ASA polyphenols	Scavenging DPPH radicals	100 and 300 μg/mL, respectively	*In vitro*	Yiğit et al. (2009)
ASA oil	Reducing ferric	IC50 = 1.07–1.38 mM $Fe2^{+}$/L in 5 different ASA varieties	*In vitro*	Stryjecka et al. (2019)
ASA n-hexane extract	Reducing ferric, intensifying TAC, scavenging DPPH radicals, and hydrogen peroxide radicals	IC50 = 163.35, 110.80, 98.61, and 516.63 μg/mL, respectively	*In vitro*	Tareen et al. (2021)
A neutral polysaccharide (AP-1)	Scavenging DPPH radicals, ABTS radicals, and hydroxyl radicals	IC50 = 2.95, 0.522, and 0.053 mg/mL, respectively	*In vitro*	Peng et al. (2023)
Amygdalin	Inhibiting the production of ROS in RAW264.7 cells, while elevating the content of CAT and SOD	10 and 40 μM	*In vitro*	Trang et al. (2022)

disorders, and ultimately inhibit bacterial growth or kill bacteria (Messaoudene et al., 2022). ASA is rich in polyphenols, which exhibit significant antibacterial activity against both Gram-negative bacteria (*E. coli* and *Acetobacter aceti*) and Gram-positive bacteria (*S. aureus*, *Bacillus subtilis* and *Bacillus cereus*). The inhibitory zone ranges from 13.0 to 18.6 mm and MIC between 31.25 and 250 μg/mL (Qin et al., 2019). However, ASA demonstrates a stronger antibacterial effect against Gram-positive bacteria. This could be attributed to the outer membrane permeability barrier of Gram-negative bacteria cell wall, which limits the interaction between antibacterial agents and their targets within bacterial cells. Moreover, both aqueous and alcoholic extracts of ASA display significant antibacterial activity against *E. coli* and *S. aureus* with inhibitory diameters ranging from 13 to 15 mm and MIC values of 0.312–0.625 mg/mL (Yiğit et al., 2009). However, the ASA base oil exhibits poor antibacterial activity, consistent with previous findings that the fatty acids in ASA lack antibacterial properties (Moola et al., 2022).

Millions of people worldwide are affected by superficial fungal infections, the most common skin disease caused by dermatophytes that parasitize on the surface layer of the stratum corneum. *Microsporum canis* and *Microsporum* are often implicated in these infections. The clinical symptoms of dermatophytosis are generally mild, and active lesions typically heal within 6–8 weeks. ASA volatile oil has demonstrated significant antibacterial activity against keratinophilic fungi, completely inhibiting their growth at a concentration of 100 μg/mL (Ibrahim and Abd El-Salam, 2015). In addition, among various ASA extracts, volatile oil exhibited notable inhibitory effects on *Malassezia furfur* and *Candida albicans*, with MIC of 250 and 1,000 μg/mL (Lee et al., 2014), respectively. However, ASA polyphenols only showed moderate inhibition against *candida*, while base oil displayed poor inhibitory activity (Yiğit et al., 2009; Moola et al., 2022). Furthermore, ASA volatile oil exhibited inhibitory effect on 19 plant pathogenic fungi, suggesting its potential as a plant and agricultural fungicide (Geng et al., 2016).

ASA is known to contain antibacterial substances such as volatile oil and polyphenols, which contribute to its excellent antibacterial potential. While there have been numerous studies on the antibacterial activity of ASA, few have explored its underlying mechanism. Mahboub, H.H. et al. suggested that the antibacterial effect of ASA might be attributed to immune enhancement (Mahboub et al., 2022), while Mikoshiba, S. et al. proposed that metabolism could play a vital role (Mikoshiba et al., 2006). However, these studies are still limited, and further research is necessary to fully understand the antibacterial mechanism of ASA.

4.4 Anti-inflammation

The main substance exerting anti-inflammation effect in ASA may be amygdalin, which can inhibit the abnormal activation of TGF-β1/Smad signaling pathway and TLR4/NF-κB signaling pathway (Figure 5, Table 5). It was found that intraperitoneal injection of 4 mg/kg amygdalin significantly alleviate bleomycin-induced neutrophil inflammatory infiltration in mouse lung tissues and reduced the number of macrophages and neutrophils in BALF, which are precursors of immune defense. The underlying mechanism may be the inhibition of TGF-β1/Smad signaling pathway (Jiao et al., 2023). In addition, amygdalin can directly hamper the expression of cytokines to exert anti-inflammatory effect. In the model of intraplantar injection of formalin, 1 mg/kg amygdalin significantly inhibited TNF-α and IL-1β mRNA levels in rat paw skins, which was comparable to that of indomethacin (Hwang et al., 2008). Besides, amygdalin can regulate the expression of inflammation-related enzymes and play an indirect anti-inflammatory role. Cyclooxygenase-2 (COX-2) and inducible

TABLE 4 Antimicrobial activity of ASA.

Pathogenic microorganism	Extract/ compound	*In vitro*/ *In vivo*	Minimal active concentration/dose	Mechanisms	Reference
Bacillus cereus	Volatile oil	*In vitro*	MIC = 2,000 μg/mL	Moderate inhibition of growth	Lee et al. (2014)
Enterococcus faecalis	Volatile oil	*In vitro*	MIC = 4,000 μg/mL	Weak inhibition of growth	Lee et al. (2014)
Methicillin-resistant	Volatile oil	*In vitro*	MIC = 500 μg/mL	Complete inhibition of growth	Lee et al. (2014)
S.aureus (MRSA) P15					
Staphylococcus aureus	Volatile oil	*In vitro*	MIC = 500 μg/mL	Complete inhibition of growth	Lee et al. (2014)
Staphylococcus epidermidis	Volatile oil	*In vitro*	MIC = 250 μg/mL	Complete inhibition of growth	Lee et al. (2014)
Citrobacter freundii	Volatile oil	*In vitro*	MIC = 2,000 μg/mL	Moderate inhibition of growth	Lee et al. (2014)
Enterobacter aerogenes	Volatile oil	*In vitro*	MIC = 2000 μg/mL	Moderate inhibition of growth	Lee et al. (2014)
Enterobacter cloacae	Volatile oil	*In vitro*	MIC = 1,000 μg/mL	Moderate inhibition of growth	Lee et al. (2014)
Escherichia coli	Volatile oil	*In vitro*	MIC = 500 μg/mL	Complete inhibition of growth	Lee et al. (2014)
Klebsiella pneumoniae	Volatile oil	*In vitro*	MIC = 2,000 μg/mL	Moderate inhibition of growth	Lee et al. (2014)
Proteus mirabilis	Volatile oil	*In vitro*	MIC = 2,000 μg/mL	Moderate inhibition of growth	Lee et al. (2014)
Pseudomonas aeruginosa	Volatile oil	*In vitro*	MIC = 500 μg/mL	Complete inhibition of growth	Lee et al. (2014)
P.aeruginosa D24	Volatile oil	*In vitro*	MIC = 500 μg/mL	Complete inhibition of growth	Lee et al. (2014)
Salmonella typhimurium	Volatile oil	*In vitro*	MIC = 500 μg/mL	Complete inhibition of growth	Lee et al. (2014)
Serratia marcescens	Volatile oil	*In vitro*	MIC = 1,000 μg/mL	Moderate inhibition of growth	Lee et al. (2014)
Shigella sonnei	Volatile oil	*In vitro*	MIC = 500 μg/mL	Complete inhibition of growth	Lee et al. (2014)
Candida albicans	Volatile oil	*In vitro*	MIC = 1,000 μg/mL	Complete inhibition of growth	Lee et al. (2014)
Malassezia furfur	Volatile oil	*In vitro*	MIC = 250 μg/mL	Complete inhibition of growth	Lee et al. (2014)
Listeria monocytogenes	Volatile oil	*In vitro*	0.5% and 1%	Displaying antimicrobial effects in solid medium, micro-atmosphere, liquid media and sliced beef	Wang et al. (2020)
Escherichia coli	Polyphenols	*In vitro*	MIC = 250 μg/mL	Great antimicrobial potency	Qin et al. (2019)
Staphylococcus aureus	Polyphenols	*In vitro*	MIC = 125 μg/mL	Great antimicrobial potency	Qin et al. (2019)
Bacillus subtilis	Polyphenols	*In vitro*	MIC = 31.25 μg/mL	Great antimicrobial potency	Qin et al. (2019)
Bacillus cereus	Polyphenols	*In vitro*	MIC = 250 μg/mL	Great antimicrobial potency	Qin et al. (2019)
Aspergillus niger	Polyphenols	*In vitro*	--	No antimicrobial potency	Qin et al. (2019)
Acetobacter aceti	Polyphenols	*In vitro*	MIC = 62.5 μg/mL	Great antimicrobial potency	Qin et al. (2019)
Escherichia coli	Methanol extract and water extract	*In vitro*	MIC = 0.312, 0.625 mg/mL, respectively	Significant antibacterial activity	Yiğit et al. (2009)
Proteus mirabilis	Methanol extract and water extract	*In vitro*	MIC = 0.625 mg/mL for water extract	Significant antibacterial activity	Yiğit et al. (2009)
Staphylococcus aureus	Methanol extract and water extract	*In vitro*	MIC = 0.312 mg/mL	Significant antibacterial activity	Yiğit et al. (2009)
Candida albicans	Methanol extract and water extract	*In vitro*	MIC = 0.625, 2.5 mg/mL, respectively	Moderate antibacterial activity	Yiğit et al. (2009)
Candida glabrata	Methanol extract and water extract	*In vitro*	MIC = 1.25 mg/mL for methanol extract	Moderate antibacterial activity	Yiğit et al. (2009)
Candida parapisilosis	Methanol extract and water extract	*In vitro*	MIC = 2.5 mg/mL	Moderate antibacterial activity	Yiğit et al. (2009)
Enterococcus faecium	Carrier oil	*In vitro*	MIC = 4 mg/mL	Poor antimicrobial activity	Moola et al. (2022)

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TABLE 4 (*Continued*) Antimicrobial activity of ASA.

Pathogenic microorganism	Extract/ compound	*In vitro*/ *In vivo*	Minimal active concentration/dose	Mechanisms	Reference
Staphylococcus aureus	Carrier oil	*In vitro*	MIC = 3 mg/mL	Poor antimicrobial activity	Moola et al. (2022)
Klebsiella pneumoniae	Carrier oil	*In vitro*	MIC = 2 mg/mL	Poor antimicrobial activity	Moola et al. (2022)
Acinetobacter baumannii	Carrier oil	*In vitro*	MIC = 4 mg/mL	Poor antimicrobial activity	Moola et al. (2022)
Pseudomonas aeruginosa	Carrier oil	*In vitro*	MIC = 3.33 mg/mL	Poor antimicrobial activity	Moola et al. (2022)
Escherichia coli	Carrier oil	*In vitro*	MIC = 3.5 mg/mL	Poor antimicrobial activity	Moola et al. (2022)
Candida albicans	Carrier oil	*In vitro*	MIC = 1 mg/mL	Moderate antimicrobial activity	Moola et al. (2022)
Aeromonas veronii	ASA powder	*In vivo*	2.5, 5 and 10 g/kg	Dose-dependently lowering mortality rate	Mahboub et al. (2022)
Corynebacterium xerosis	Ethanol extract	*In vitro*	62.5, 125 ppm	Did not exhibit a bactericidal effect	Mikoshiba et al. (2006)
Microsporum canis	Volatile oil	*In vitro*	0.5–4 μL/mL	Completely mycelial growth inhibition	Ibrahim and Abd El-Salam (2015)
Epidermophyton floccosum	Volatile oil	*In vitro*	0.5–4 μL/mL	Completely mycelial growth inhibition	Ibrahim and Abd El-Salam (2015)
Trichophyton rubrum	Volatile oil	*In vitro*	0.5–4 μL/mL	Completely mycelial growth inhibition	Ibrahim and Abd El-Salam (2015)
Trichophyton mentagrophytes	Volatile oil	*In vitro*	0.5–4 μL/mL	Completely mycelial growth inhibition	Ibrahim and Abd El-Salam (2015)
Fusarium oxysporum sp. *cucumebrium* Owen	Volatile oil	*In vitro*	EC50 = 511.7 μg/mL	Antifungal Activity	Geng et al. (2016)
Valsa mali Miyabe et Yamade	Volatile oil	*In vitro*	EC50 = 610.8 μg/mL	Antifungal Activity	Geng et al. (2016)
Pyricularia oryzae cavgra	Volatile oil	*In vitro*	EC50 = 429.3 μg/mL	Antifungal Activity	Geng et al. (2016)
Fusarium graminearum	Volatile oil	*In vitro*	EC50 = 627.9 μg/mL	Antifungal Activity	Geng et al. (2016)
Alternaria alternata (Fr) Keissler	Volatile oil	*In vitro*	EC50 = 642.0 μg/mL	Antifungal Activity	Geng et al. (2016)
Alternaria solani	Volatile oil	*In vitro*	EC50 = 103.2 μg/mL	Antifungal Activity	Geng et al. (2016)
Phytophthora capsici Leonian	Volatile oil	*In vitro*	EC50 = 600.5 μg/mL	Antifungal Activity	Geng et al. (2016)
Gloeosporium fructigenum	Volatile oil	*In vitro*	EC50 = 225.9 μg/mL	Antifungal Activity	Geng et al. (2016)
Fusarium oxysporum f. sp. *lycopersici* Synder et Hansen	Volatile oil	*In vitro*	EC50 = 295.1 μg/mL	Antifungal Activity	Geng et al. (2016)
Gloeosporium orbiculare	Volatile oil	*In vitro*	EC50 = 273.7 μg/mL	Antifungal Activity	Geng et al. (2016)
Verticillium dahliae Kleb	Volatile oil	*In vitro*	EC50 = 325.2 μg/mL	Antifungal Activity	Geng et al. (2016)
Gaeumannomyces graminis var. *tritici*	Volatile oil	*In vitro*	EC50 = 192.0 μg/mL	Antifungal Activity	Geng et al. (2016)
Botrytis cinerea	Volatile oil	*In vitro*	EC50 = 217.0 μg/mL	Antifungal Activity	Geng et al. (2016)
Fusarium oxysporum f. sp. *vasinfectum*	Volatile oil	*In vitro*	EC50 = 526.7 μg/mL	Antifungal Activity	Geng et al. (2016)
Curvularia lunata	Volatile oil	*In vitro*	EC50 = 509.5 μg/mL	Antifungal Activity	Geng et al. (2016)
Fusarium oxysporum (Schlecht.)	Volatile oil	*In vitro*	EC50 = 423.8 μg/mL	Antifungal Activity	Geng et al. (2016)
Colletotrichum gloeosporioides (Penz.) et Sacc	Volatile oil	*In vitro*	EC50 = 381.8 μg/mL	Antifungal Activity	Geng et al. (2016)
Fusarium oxysporum f. sp. *niveum*	Volatile oil	*In vitro*	EC50 = 569.3 μg/mL	Antifungal Activity	Geng et al. (2016)
Alternaria brassicae	Volatile oil	*In vitro*	EC50 = 50.2 μg/mL	Antifungal Activity	Geng et al. (2016)

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TABLE 4 (*Continued*) Antimicrobial activity of ASA.

Pathogenic microorganism	Extract/ compound	*In vitro*/ *In vivo*	Minimal active concentration/dose	Mechanisms	Reference
Gloeosporium orbiculare	Volatile oil	*In vivo*	4–12 mg/mL	High protective and therapeutic effects	Geng et al. (2016)
Blumeria graminis	Volatile oil	*In vivo*	4–12 mg/mL	Medium protective effect and weak therapeutic effect	Geng et al. (2016)

nitric oxide synthase (iNOS) are involved in the inflammatory response and induce the production of inflammatory mediators prostaglandin E2 (PGE2) and NO, respectively (Chang et al., 2005). In LPS-stimulated BV2 cell model, treatment with 10 or 100 μg/mL amygdalin and 0.1 or 1 mg/mL ASA aqueous extract can significantly downregulate COX-2 and iNOS mRNA levels, and the contents of PGE2 and NO (Chang et al., 2005; Yang et al., 2007). Furthermore, in a model of HUVEC injury induced by PM2.5, amygdalin at concentrations of 2.5, 5, and 10 μg/mL has been shown to diminish the levels of COX-2, IL-6, TNF-α, and IL-1β, while promoting apoptosis of damaged cells via impeding aberrant activation of TLR4/NF-κB signaling pathway (Wang et al., 2022). Moreover, it has been discovered that oral administration of 15 mg/kg amygdalin can restore Th1/Th2 immune imbalance to alleviate airway inflammation in an ovalbumin-induced asthma mice model (Cui et al., 2023). However, further studies are needed to determine whether other components of ASA have anti-oxidant effects.

4.5 Cardiovascular protection

The latest evidence indicates that cardiovascular disease is responsible for 31% of global deaths. It has been established that adopting a healthy diet is crucial in reducing the risk of cardiovascular diseases (Dikariyanto et al., 2021). Cardiovascular diseases encompass various heart and vascular conditions such as coronary heart disease, hypertension, heart failure, peripheral vascular disease, cerebrovascular disease, vascular disease, and rheumatic heart disease. ASA, which is rich in unsaturated fatty acids, has been proven to effectively lower biochemical and arterial markers associated with cardiovascular risk (de Oliveira et al., 2017). Moreover, ASA is abundant in anthocyanins, flavonoids, and phenolic acids, with concentrations of up to 118.17 mg/100 g, 113.66 mg/L, and 91.42 mg/100 mL, respectively (Qin et al., 2019). These substances have also demonstrated positive effects on cardiovascular diseases (Perez-Vizcaino and Duarte, 2010; Blesso, 2019; Potì et al., 2019; Mattioli et al., 2020). Therefore, ASA exhibits significant potential and advantages in the treatment of cardiovascular system diseases, mainly due to the functions of unsaturated fatty acids, polyphenols, flavonoids, and amygdalin.

Currently, ASA and its active ingredients have been shown to contribute to cardiovascular health in both *in vivo* experiments and clinical studies (Table 5). In a rat myocardial ischemia-reperfusion injury model, it was observed that continuous treatment with 2, 6, and 10 mL/kg of ASA oil for 2 weeks resulted in a significant reduction in the myocardial infarction area of rats. Additionally, the activities of serum creatine kinase and aspartate aminotransferase increased, leading to an increased production of ATP. This increase in ATP production provides sufficient energy for the physiological needs of the heart. Moreover, supplementation with ASA oil also demonstrated a significant increase in the activity of antioxidant enzymes such as myocardial CAT, SOD, and glutathione peroxidase. This increase in anti-oxidant enzyme activity enhances the anti-oxidant defense system while reducing the content of MDA and inhibiting lipid peroxidation. Ultimately, these effects provide a protective effect against myocardial ischemia-reperfusion injury in cardiomyocytes (Zhang et al., 2011). In recent years, there has been increasing attention on amygdalin, the main component of ASA. It has been demonstrated *in vitro* that amygdalin can effectively inhibit Ang II-induced cardiomyocyte hypertrophy, reduce inflammatory response, and exhibit anti-oxidant activity when treating H9C2 cells induced by Ang II at concentrations of 80, 160, and 320 μM. These effects of amygdalin are primarily achieved through the reduction of atrial natriuretic peptide, B-type natriuretic peptide, and β-MHC, which are related to cardiac hypertrophy. Additionally, amygdalin inhibits the expression of inflammatory markers such as TNF-α, iNOS, COX-2, and phospho-NF-κB protein. Furthermore, amygdalin increases the expression of Nrf2, CAT, SOD-2, and GPX-4, which are proteins related to oxidative stress (Kung et al., 2021). Both *in vitro* and *in vivo* studies have also indicated that amygdalin can alleviate atherosclerosis. This effect may be attributed to its inhibition of the inflammatory response, enhancement of immune regulatory function in regulatory T cells, or inhibition of the TLR4/NF-κB and Bcl-2/Bax signaling pathways (Jiagang et al., 2011; Wang et al., 2022).

The benefits of ASA for cardiovascular disease have been extensively studied due to its various components and proven efficacy. ASA has been shown to exert cardiovascular protective effects by reducing cholesterol levels, particularly low-density lipoprotein cholesterol (LDL-C) (Kopčeková et al., 2021). Clinical research reports have demonstrated that after 6 consecutive weeks of taking 60 mg/kg ASA, volunteers experienced a significant decrease in serum LDL-C levels. It is important to note that elevated levels of LDL-C can contribute to the development of cardiovascular atherosclerosis and the blockage of blood vessels by causing excessive fat absorption in extrahepatic cell tissues (Siri-Tarino et al., 2010). In another clinical study, it was observed that after 12 weeks of taking 60 mg/kg ASA, total cholesterol levels decreased by 8.64% and LDL-C levels decreased by 21.2%. Additionally, there was a slight increase in high-density lipoprotein cholesterol (HDL-C) levels, along with an increase in C-reactive protein and serum creatine kinase levels (Kopčeková et al., 2018). Importantly, studies have shown that for every 1% reduction in LDL-C, the risk of coronary heart disease is reduced by up to 3% (Brown and Goldstein, 2006). This indicates that consuming ASA can significantly reduce the risk of cardiovascular disease. Further investigation revealed that

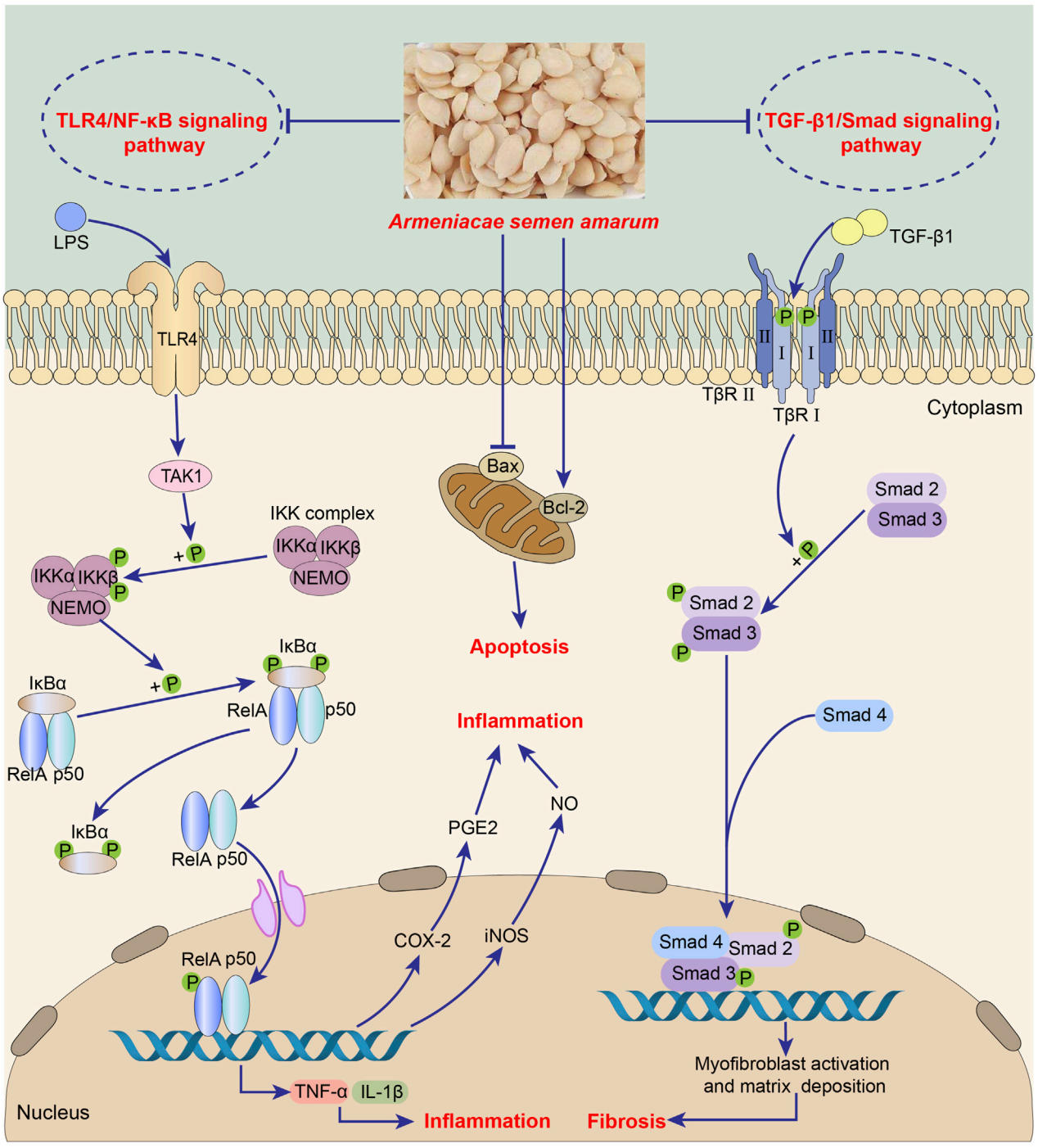

FIGURE 5
Anti-inflammation of *Armeniacae semen amarum*.

after a 6-week administration of ASA to 21 individuals with normal cholesterol levels and 13 patients with high cholesterol levels, there was no significant change observed in the total cholesterol content and average LDL-C levels of the normal individuals. Similarly, the average total cholesterol content and average LDL-C levels of the patients with HDL-C levels also did not exhibit a significant change. However, a reduction in density cholesterol levels was observed, and the LDL_{3-7} subfractions were only detected in one individual (Kopčeková et al., 2022). It is important to note that the LDL_{3-7} subfractions, which are part of very low-density lipoproteins, have smaller particle sizes compared to LDL_1 and LDL_2, and are associated with a higher risk of atherosclerosis (Qiao et al., 2022). In simpler terms, the intake of ASA can modify the lipoprotein profile of individuals with hypercholesterolemia by primarily reducing low-density lipoprotein levels, without negatively affecting lipid metabolism in healthy individuals.

In summary, ASA exerts cardiovascular protection mainly by reducing LDL levels, inhibiting oxidative stress and regulating

immunity, which strongly supports the use of ASA in the management of cardiovascular diseases.

4.6 Neuroprotection

Alzheimer's disease and Parkinson's disease are two common neurodegenerative diseases characterized by neuronal damage and behavioral dysfunction. The pathological processes involved in these diseases include immune inflammation, oxidative stress, and mitochondrial dysfunction (Chen W. et al., 2022). Phytochemicals with anti-oxidant properties are known to have the potential to provide neuroprotection (Chakraborty et al., 2022). ASA, abundant in flavonoids, polyphenols, and other anti-oxidative compounds, shows promising potential for treating neurodegenerative diseases by suppressing inflammation, oxidative stress and acetylcholinesterase (AchE) activity (Table 5).

Microglia, immune effector cells in the central nervous system, play a role in releasing inflammatory mediators that contribute to neurotoxicity and the development of neurodegenerative diseases (Simpson and Oliver, 2020). Studies have demonstrated that ASA extract can inhibit COX-2 and iNOS mRNA levels in BV2 cells stimulated by LPS. This inhibition leads to a reduction in the synthesis of PGE2 and the production of NO, thereby suppressing immune and inflammatory responses and exerting a neuroprotective effect (Chang et al., 2005; Yang et al., 2007). AchE, present in neurons, serves as an indicator of neuronal damage (Olasehinde and Olaniran, 2022). *In vitro* studies, ASA water extract exhibits significant anticholinesterase activity with an IC50 of 134.93 μg/mL. Additionally, treatment with 100 μg/mL ASA water extract demonstrates a favorable neuroprotective effect against H_2O_2-induced damage to PC12 neuron cells, resulting in a cell survival rate of 70.71%. In comparison, PC12 cells treated with 400 μM hydrogen peroxide exhibit a survival rate of less than 40% (Vahedi-Mazdabadi et al., 2020).

It has been demonstrated *in vivo* studies that the methanol extract of ASA at concentrations of 100, 300, and 800 mg/kg has a protective effect on haloperidol-induced Parkinson's disease model. Behavioral analysis has shown that ASA treatment improves motor activity, motor coordination, and exploratory activities in rats. It also reduces depression, anxiety, and convulsive seizures, accompanied by a decrease in dopamine, 5-hydroxytryptamine, and norepinephrine neurotransmitter levels. Additionally, there is a significant increase and decrease in AchE levels. Furthermore, behavioral improvement and brain function recovery are positively correlated with increased anti-oxidant enzyme activity in the body (Saleem et al., 2022). Moreover, amygdalin also shows potential neuroprotective effects, possibly due to its induction of calreticulin protein expression, which plays a vital role in the survival, differentiation, and regulation of neurons (Cheng et al., 2015).

4.7 Respiratory protection

Respiratory system diseases are diverse and common, affecting the trachea, bronchi, and lungs. Some prevalent conditions in this category include asthma, COVID-19, acute lung injury, and chronic obstructive pneumonia (Tavares et al., 2020). ASA, an important Chinese herbal medicine, is used to treat cough and has various functions such as enhancing lung function, relieving constipation, and promoting intestinal peristalsis. According to traditional Chinese medicine, bitter purgation helps disperse and move lung Qi, thereby eliminating phlegm (Gao et al., 2011). Pharmacological studies have shown that amygdalin, an effective component of ASA, is hydrolyzed to hydrocyanic acid and benzaldehyde in the body after oral administration, thereby relieving cough, asthma and other respiratory system diseases (Figure 6).

The COVID-19 pandemic, caused by the 2019 novel coronavirus, is spreading globally. It is characterized by symptoms such as fever, dry cough, and fatigue, which can lead to severe respiratory failure and even death. Additionally, patients may experience muscle aches and diarrhea, and in severe cases, they may develop acute respiratory distress syndrome, septic shock, or succumb to the disease (Du et al., 2021). Through network pharmacology and molecular docking, it was found that stigmasterol, sitosterol, sholesterol, (6Z,10E,14E,18E)-2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene, oestrone, diisooctyl succinate, 11,14-eicosadienoic acid, and amygdalin are suggested to be the nine key active ingredients for the treatment of COVID-19. Moreover, IL6, SRC, MAPK1, MAPK3, VEGFA, EGFR, HRAS, and CASP3 are identified as potential core targets for ASA treatment. It has been demonstrated that a therapeutic potential of amygdalin *in vivo* experiments. Moreover, The administration of 0.5–2 mg/kg of amygdalin has been shown to regulate the PI3K-AKT signaling pathway, VEGF signaling pathway, and MAPK signaling pathway, resulting in significant inhibition of EGFR, phospho-AKT, phospho-SRC, VEGFA, MAPK1, IL-6, IL-1β, and TNF-α protein expressions (Wang et al., 2021). However, more research is required to support the use of ASA in the treatment of COVID-19.

Allergic asthma, which is the most common type of asthma, is characterized by chronic airway inflammation involving T lymphocytes, mast cells, eosinophils, and other cells (Possa et al., 2013). Several studies have demonstrated that ASA aqueous extract shows promising therapeutic effects in both an ovalbumin-induced allergic airway inflammation model *in vivo* and lymph node primary cells *in vitro*. This therapeutic effect of ASA is attributed to a reduction in IL-4 and IL-5 levels (Do et al., 2006). IL-4 is responsible for the transformation of regulatory T cells into helper T cells, while IL-5 regulates the growth, differentiation, and activation of eosinophils (Jin et al., 2019). However, further research is necessary to determine whether ASA exhibits similar therapeutic effects on other types of asthma and to investigate the underlying molecular mechanisms involved.

Acute lung injury (ALI) is a severe medical condition associated with significant morbidity and mortality. It is characterized by damage to the alveolar epithelial cells and pulmonary capillary endothelial cells, resulting from non-cardiogenic factors (Tang et al., 2023). The clinical manifestations of ALI include dyspnea and intractable hypoxemia, which can progress to severe respiratory disorders. ALI is characterized by the infiltration of a large number of neutrophils into lung tissue, leading to the release of inflammatory cytokines and damage to pulmonary endothelial and epithelial cells. LPS, also known as endotoxin, is a major component of the outer membrane of Gram-negative microorganisms and is highly

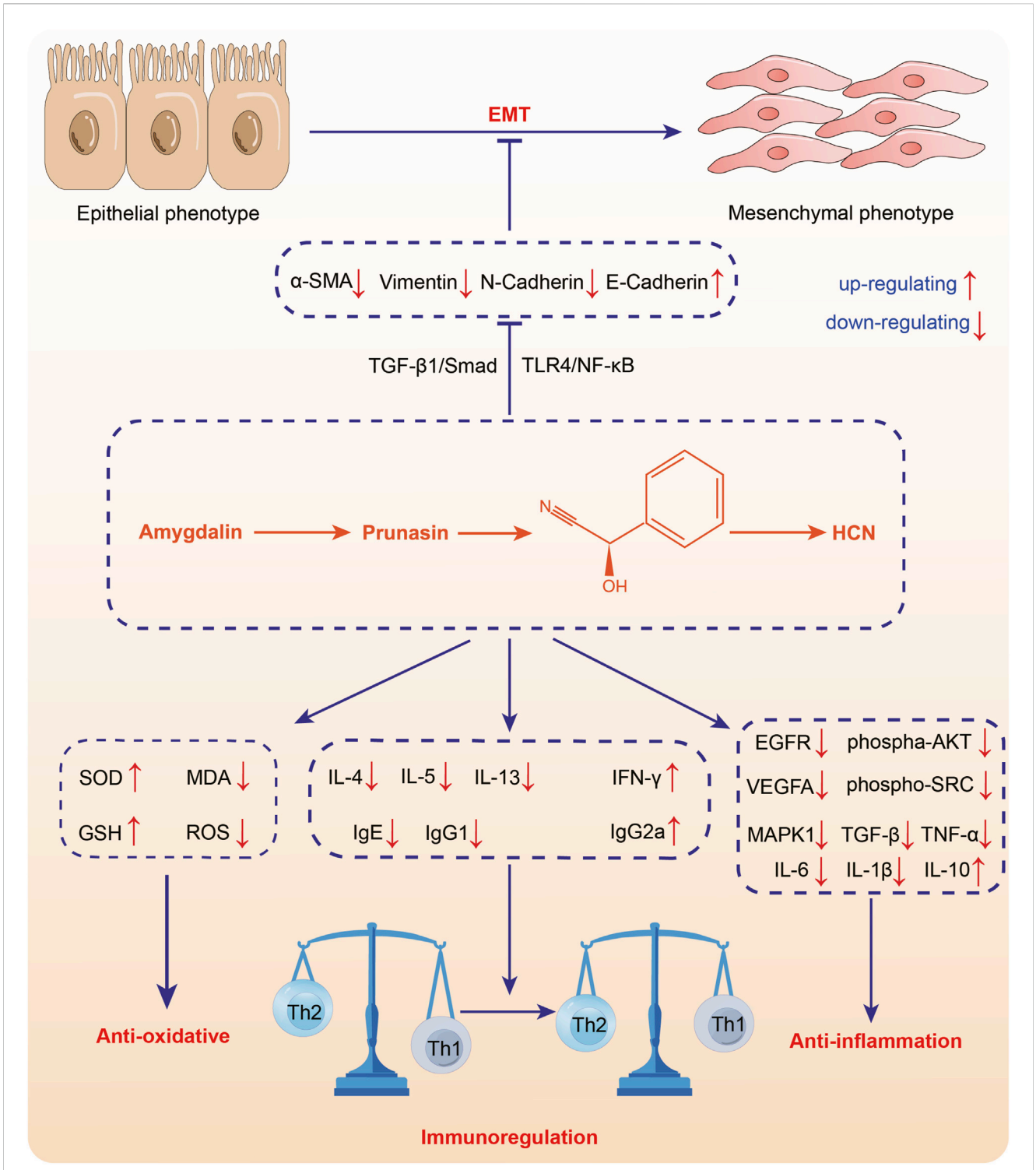

FIGURE 6
Respiratory protection of *Armeniacae semen amarum*.

pathogenic (Liu et al., 2020). The ASA carbon nano-material has demonstrated its ability to inhibit the release of IL-6, IL-1β, and TNF-α inflammatory mediators in rat serum. Moreover, it has been shown to reduce the increase of neutrophils in the blood. Additionally, it exhibits a decrease in the chemotaxis of neutrophils to inflammatory sites and inhibits the injury and aggravation of LPS to lung tissue. These findings suggest that ASA carbon nano-material shows promising potential as a candidate treatment for ALI (Zhao Y. et al., 2022).

In addition, amygdalin may also have therapeutic effects on chronic obstructive pulmonary disease (COPD) (Sun et al., 2020). COPD is characterized by airway remodeling, which involves

epithelial-mesenchymal transition (EMT). Recent studies have shown that amygdalin, administered at doses of 5, 10, and 20 mg/kg, has a protective effect on the EMT process in COPD mice induced by cigarette smoke. These findings are consistent with the observed inhibition of TGF-β1 protein expression and Smad2/3 phosphorylation by amygdalin, indicating its potential role in suppressing the TGF-β/smad pathway. Moreover, amygdalin also demonstrates inhibitory effects on the EMT process in BEAS-2B cells stimulated by cigarette smoke *in vitro*, suggesting its potential use in COPD treatment (Wang et al., 2019). Furthermore, the mechanism by which amygdalin exerts its therapeutic effect may also be related to the inhibition of LPS-induced EMT and TLR4/NF-κB signaling cascade (Si and Zhang, 2021).

Numerous formulas containing ASA have been extensively studied and utilized in the research and treatment of various respiratory diseases such as colds, asthma, COVID-19, and pulmonary fibrosis (Li et al., 2010; Lin et al., 2016; Sun et al., 2018; Bai et al., 2022; Li et al., 2022). This further demonstrates the potential respiratory protection activity of ASA (Table 5).

4.8 Digestive system protection

Limited reports exist on the protective effects of ASA on the digestive system. This section provides a summary of the protective effects of ASA on the digestive tract and digestive glands (Table 5). Studies have shown that 400 mg/kg ASA can enhance the damage caused by gamma-radiation of 5 Gy to the salivary glands of *Rattus Norvegicus*, specifically affecting the acinar cells. This effect is primarily attributed to the downregulation of EGF protein expression and the upregulation of TGF-β protein expression, indicating that ASA mitigates oxidative damage and inflammatory responses, thereby protecting against salivary gland damage (Abdaulmoneam et al., 2023). In addition, ASA oil has been found to possess gastroprotective effects. In an ethanol-induced rat gastric ulcer model, ASA oil reduces the release of cytokines such as IL-6, increases levels of oxidative stress markers like SOD and CAT, decreases lipid oxidation, and inhibits mucosal cell apoptosis, demonstrating its gastroprotective properties. Recent research also suggests that amygdalin may have potential pancreatic protective effects (Karaboğa et al., 2018). Intravenous injection of 10 mg/kg amygdalin improves pancreatic fibrosis in rats with chronic pancreatitis induced by dibutyldichlorotin, as evidenced by reduced production of profibrotic growth factors and inhibition of pancreatic stellate cell activation. The mechanism may involve improved microcirculation through reduced endothelin-1 expression and upregulated expression of calcitonin gene-related peptide (Zhang et al., 2018). Similarly, ASA ethanol extract can induce apoptosis of pancreatic cancer cells *in vitro* (Aamazadeh et al., 2020).

In summary, ASA has been found to have a protective effect on parotid glands, pancreas and stomach. Its mechanism of action is believed to involve the inhibition of inflammatory response and oxidative stress, along with the induction of cell apoptosis. However, the specific substances responsible for the therapeutic effects of ASA are still unidentified and the protective effects on other digestive organs and digestive glands have not been defined, thus the protective effects of ASA on the digestive system need to be further investigated.

4.9 Antidiabetic effect

Diabetes mellitus (DM) is a group of metabolic disorders that poses a significant global health burden, affecting approximately 6% of the population. The majority of diabetic patients (90%–95%) have type II diabetes, while the remaining have type I diabetes. Currently, the options for DM treatment are limited, and long-term use of available drugs may result in severe side effects (Das and Chakrabarti, 2005). ASA has shown specific effects on DM and offers a promising alternative treatment option due to its cost-effectiveness and easy accessibility. Both *in vivo* and *in vitro* studies have demonstrated that the antidiabetic activity of ASA is primarily associated with its ability to enhance insulin secretion, leading to reduced blood pressure and mitigation of oxidative stress (Table 5).

In an alloxan-induced rat DM, ASA demonstrated a dose-dependent reduction in blood glucose levels, an increase in body weight, a decrease in lipid peroxidation levels, and an increase in serum CAT levels. ASA significantly increased insulin levels after 8 weeks, and exhibited an inhibitory effect on α-glucosidase, suggesting that its anti-diabetic properties may be attributed to the reduction of oxidative stress caused by glucose, inhibition of α-glucosidase, and significant mediation by elevated insulin (Raafat et al., 2018). Interestingly, ASA also showed a significant reduction in glycosylated hemoglobin levels, indicating its potential to prevent complications associated with DM. Higher levels of Hemoglobin A1C (HbA1c) in diabetic patients are indicative of poorer regulation of blood glucose and an increased risk of diabetes-related complications (Klonoff, 2020). Furthermore, amygdalin was found to alleviate diabetic retinopathy, a complication of DM. In high glucose-stimulated HRECs cells, 40 μM amygdalin demonstrated a significant inhibition on oxidative stress and ferroptosis, evidenced by increased GSH/GSSG ratio, SOD, CAT, GPX4 activity and reduced MDA and ROS levels, as well as significant downregulation of ferroptosis marker proteins including RAS, TFR1, and ACSL4. Notably, the antidiabetic retinopathy effects of amygdalin were found to be associated with the activation of the NRF2/ARE pathway, leading to the activation of NRF2 and HO-1 and an increase in NQO1 protein expression (Li et al., 2023).

Recently, the antihypertensive effects of natural chemical constituents of ASA have attracted great attention from researchers. A polypeptide, Arg-Pro-Pro-Ser-Glu-Asp-Glu-Asp-Gln-Glu, has been identified in ASA albumin lately. This polypeptide acts as a non-competitive inhibitor of angiotensin-converting enzyme (ACE) with an IC50 value of 205.50 μM. Additionally, it has exhibited positive antihypertensive effects on spontaneously hypertensive rats at concentrations of 100 and 150 mg/mL. Although not as effective as 10 mg/kg captopril, this polypeptide has led to a significant decrease in systolic and diastolic blood pressure (Qin et al., 2023). These findings suggest that the polypeptide holds the potential for anti-DM effects and could be utilized in the development of anti-DM drugs. Furthermore, a neutral polysaccharide (AP-1), which has a triple helix structure, has recently been extracted from ASA. AP-1 primarily consists of glucose, arabinose, galactose, and mannose. It has strong inhibition of α-glucosidase enzyme and the ability to scavenge DPPH, ABTS, and Hydroxyl free radicals *in vitro* (Peng et al., 2023). These findings

indicate that AP-1 may serve as a natural anti-oxidant and hypoglycemic agent in the treatment of DM.

4.10 Liver protection

Oxidative stress is widely recognized as the underlying cause of both acute and chronic liver diseases (Cui et al., 2021). ASA, a natural source of plant antioxidants, shows promising potential for the treatment of liver diseases. Recent studies have revealed that amygdalin not only alleviates symptoms of Ehrlich ascites cancer but also, helps prevent liver cancer and mitigate associated liver damage when combined with sorafenib. These hepatoprotective effects are attributed to the direct reduction of liver function indicators such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transferase (GGT), as well as the significant antioxidant activity of amygdalin (Attia et al., 2022). In addition, another study also suggests that the key role of ASA in liver protection may be related to oxidative stress (Yurt and Celik, 2011).

ASA has demonstrated hepatoprotective effects at various stages of liver disease development. In the early stages, ASA exhibits anti-inflammatory properties, effectively inhibiting disease progression. The main component of ASA, amygdalin, not only inhibits excessive oxidative stress and reduces the levels of liver injury-related enzymes, but also suppresses the production of TNF-α, IL-6 and IL-1β as well as the expressions of inflammation-related proteins such as iNOS and COX-2, thereby mitigating inflammatory response and providing resistance against acute liver injury (Tang et al., 2019). Hepatic fibrosis, a compensatory pathophysiological process, occurs when the liver is damaged by chronic inflammation, leading to tissue degeneration, inflammatory infiltration, necrosis, and constant repair of liver collagen and extracellular matrix (Tsuchida et al., 2018). Amygdalin, the active ingredient of ASA, has been found to inhibit the activation of hepatic stellate cells induced by transforming growth factors. It also reduces the secretion of cytokines and the levels of ALT and AST, exerting anti-inflammatory effects and protecting the liver from fibrosis (Zhang et al., 2022b). Moreover, amygdalin has a protective effect on advanced liver failure. In the case of acetaminophen-induced acute liver failure, intraperitoneal injection of 2.5 or 5 mg/kg amygdalin has been found to reduce the area of necrosis in liver tissue, lower the levels of liver function-related indicators ALT and AST, and decrease neutrophil and macrophage counts. These effects are associated with the inhibition of oxidative damage, increased protein expression of Nrf2/NQO1/HO1, phospho-AKT, and inhibition of the JNK/RIP3/MLKL signaling pathway (Zhang et al., 2022a).

Overall, ASA and amygdalin have promising liver protection effects both *in vivo* and *in vitro* experiments due to their potent anti-oxidant activities (Table 5). However, further research is needed to explore the potential of ASA as a therapeutic drug for different stages of liver disease development (Figure 7).

4.11 Kidney protection

ASA has therapeutic effects on both renal cell carcinoma and chronic kidney disease, such as renal fibrosis (Table 5). The main component of ASA, amygdalin, inhibits the proliferation and production of transforming growth factors in renal interstitial fibroblasts, which plays a crucial role in the development of renal interstitial fibrosis (Bai et al., 2020). In a rat model of unilateral ureteral obstruction, treatment with amygdalin at concentrations of 3 and 5 mg/kg resulted in reduced renal damage and delayed progression of renal interstitial fibrosis (Guo et al., 2013). However, the accumulation of hydrocyanic acid, a metabolite of amygdalin in ASA, can lead to nervous system depression, limiting its application. Nevertheless, a study found that oral administration of 2 g/kg ASA water extract to rats did not exhibit nephrotoxicity but increased antioxidant activity, manifesting as increased levels of renal function indicators such as urea, creatinine and urea nitrogen as well as increased activities of anti-oxidant enzymes such as SOD and GSH (Zehra and Naz, 2021). In short, although the metabolism of ASA can lead to the accumulation of toxic substances, its rich natural chemical components have shown promising effects in the research of various diseases. Further research is needed to fully understand the impact of ASA on kidney diseases.

4.12 Other pharmacological activities

In addition to its pharmacological effects described above, ASA oil also exhibits skin protective effects. It can inhibit the growth of human keratinocytes and enhance their programmed cell death, making it a potential treatment option for psoriasis (Li et al., 2016). Furthermore, preliminary clinical studies have shown that massage with ASA oil during early pregnancy can effectively reduce the formation of stretch marks (Timur Taşhan and Kafkasli, 2012). Additionally, ASA extract has demonstrated positive effects in relieving symptoms of dry eye syndrome and dry keratitis (Kim et al., 2016; Hyun et al., 2019). Moreover, ASA has also been found to promote fracture healing (Ying et al., 2020; Trang et al., 2022) and regulate the immune system (Tian et al., 2016) (Table 5).

5 Clinical applications

There is mounting evidence supporting the use of ASA in the treatment of cough, lung, and other respiratory-related diseases. Studies have shown that ASA liquids can reduce the sensitivity of the trachea to ammonia stimulation, thereby relieving cough and promoting intestinal peristalsis (Gao et al., 2012). In a research study investigating the effectiveness of traditional Chinese medicine compounds for treating COVID-19, a total of 166 compounds containing 179 traditional Chinese medicines were collected. Among the candidate prescriptions for COVID-19 treatment selected through complex system entropy and unsupervised hierarchical clustering, ASA ranked third in terms of frequency of use and was included in the first formula (Luo et al., 2020). Furthermore, a data mining analysis examining traditional Chinese medicine prescriptions for respiratory diseases analyzed 562 prescriptions specifically targeting the respiratory system. The results revealed that ASA was utilized in 36.7% of the prescriptions, ranking second after *Glycyrrgizae radix et rhizoma*—roots and rhizomes of *Glycyrrhiza glabra* L. (Fabaceae), which was used in 47.2% of the prescriptions (Fu et al., 2013). These

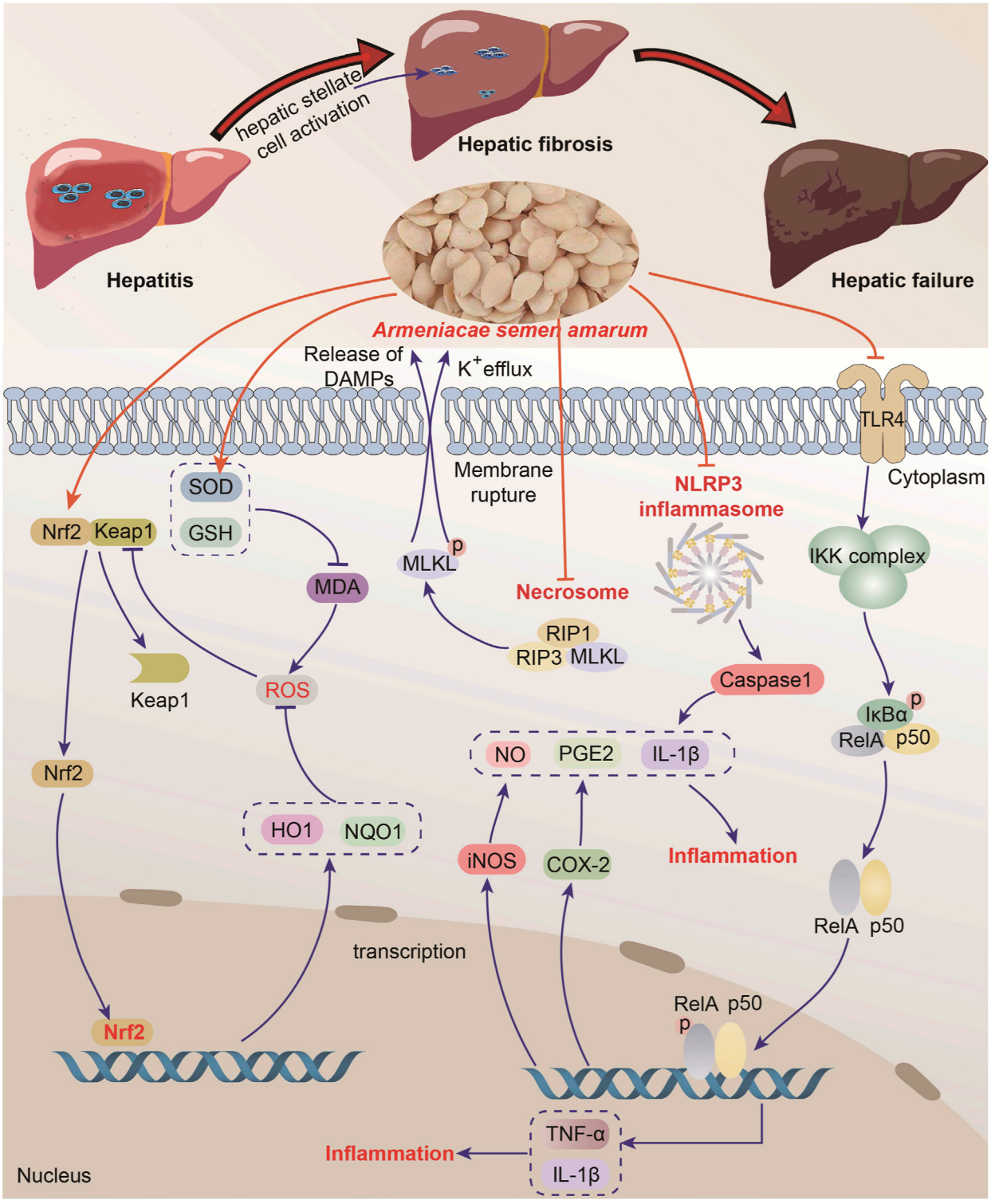

FIGURE 7
Liver protection of *Armeniacae semen amarum*.

findings suggest that ASA holds promise as an effective treatment for respiratory diseases.

The plant kingdom contains many substances that may have the potential to prevent or treat human disease (Zhao et al., 2022b; Sun et al., 2023; Zhao et al., 2023), but these bioactive components (such as vitamins and alkaloids) usually show low bioavailability or biological instability. Recently, various techniques for improving drug delivery have been developed to solve the problems of bioavailability and stability. Nanoparticle is the most promising drug carrier, which can effectively deliver bioactive compounds and

improve bioavailability. Currently, a protein belonging to the 11S globulin family was isolated from ASA water extract. This protein is composed of three polypeptides connected by disulfide bonds. Upon heat treatment, these bonds rearrange, resulting in the formation of a spherical-shaped dimer. The unique structure of this protein makes it a potential candidate for use as a nanocarrier. It efficiently encapsulates paclitaxel with a maximum encapsulation efficiency of 92.6% and a maximum release of paclitaxel of 57.4% (Lin et al., 2020). Additionally, a recent study developed liposomes loaded with amygdalin using a molar ratio of Tween 60: cholesterol: dihexadecyl phosphate as 1: 2: 0.1. These liposome-loaded amygdalin formulations demonstrated significant effects in reducing tumor volume, decreasing epidermal hyperplasia, and eliminating edema in a rat tumor model induced by 7,12-dimethylphenanthrene. Surprisingly, the anti-tumor activity of these liposomes surpassed that of tamoxifen, a well-known anti-tumor drug (El-Ela et al., 2022). Moreover, a polypeptide extracted from ASA water extract has displayed the ability to form a complex with zinc ions, exhibiting remarkablely lowering blood pressure effect. This polypeptide shows promise for further development as an antihypertensive drug (Qin et al., 2023).

Numerous studies have demonstrated that formula preparations containing ASA exhibit powerful therapeutic effects in the treatment of lung disease, liver disease, eye disease, and other diseases, especially respiratory diseases. The ASA-containing formulas may significantly relieve symptoms such as fever, cough and runny nose. Table 6 provides a summary of ASA-containing formulations and their clinical applications as outlined in the Chinese Pharmacopoeia 2020 edition.

6 Toxicological effects including adverse reactions

The main toxic substance in ASA is hydrocyanic acid, which is produced when amygdalin is metabolized. Amygdalin is broken down by β-D-glucosidase into mandelonitrile, which further breaks down into benzaldehyde and hydrocyanic acid. HCN is eventually absorbed into the bloodstream, leading to cyanide poisoning. It is important to note that the toxic doses of amygdalin vary greatly depending on the method of administration. The lethal dose of amygdalin through intravenous injection in humans is 5 g, while oral consumption is 0.5–3.5 mg/kg body weight (Song et al., 2016). When injected intravenously, amygdalin can bypass enzymatic hydrolysis in the gastrointestinal tract, resulting in high blood concentration and detectable amygdalin in the plasma. Additionally, 80% of the injected amygdalin is absorbed by the body within 24 h and eliminated through urine (He et al., 2020). Ingesting 50 ASA consecutively can cause poisoning symptoms in adults, whereas babies can be poisoned by consuming only 5–10 (Chaouali et al., 2013). Cyanide poisoning can lead to rapid hemodynamic and neurological impairment. Studies have shown that hydrocyanic acid can inhibit the activity of cytochrome oxidase in cell mitochondria, causing respiratory inhibition in tissue cells and cell death due to hypoxia. The clinical manifestations of cyanide poisoning depend on the route, duration, dose, and source of exposure. Common symptoms include nausea, vomiting, diarrhea, respiratory failure, hypotension, arrhythmia, cardiac arrest, the odor of bitter almonds, and cherry red skin (Jaszczak-Wilke et al., 2021).

Modern pharmacological research has revealed significant variations in the toxicity of different extracted components of ASA (Table 7). One study found that the median lethal dose (LD50) of lyophilized ASA aqueous extract on Kunming mice was 29.9 g/kg (Song et al., 2016), while another study reported an LD50 of approximately 22.5 g/kg for raw ASA aqueous extract on Kunming mice (Chen and Jia, 2012). However, a separate study administered ASA oil at a dosage of 10 mg/day to Wistar rats for 13 weeks, and no adverse reactions or fatalities were observed (Gandhi et al., 1997). In contrast, when amygdalin was directly administered to Wistar rats, the rats exhibited quadriplegia, muscle-twitching, difficulty in breathing, apnea, and subsequent death, with an LD50 of 880 mg/kg (Adewusi and Oke, 1985). These findings indicate that ASA oil does not exhibit obvious toxicity, whereas ASA water or alcohol extract demonstrates strong toxicity. Furthermore, the toxicity of amygdalin alone is more significant than that of ASA water or alcohol extract.

β-D-glucosidase plays a crucial role in the hydrolysis process of amygdalin. When amygdalin was administered alone, the IC50 of HepG-2 was 458.10 mg/mL. However, co-administration of amygdalin with β-D-glucosidase resulted in a more than 100-fold decrease in IC50 to 3.2 mg/mL, highlighting the critical role of β-D-glucosidase in the pathway of amygdalin poisoning (Zhou et al., 2012). Similarly, there was a notable difference in the IC50 values of PC12 and MDCK cells when amygdalin was administered alone or in combination with β-D-glucosidase. The IC50 of PC12 cells decreased from 35.83 to 5.97 μM, and the IC50 of MDCK cells decreased from 63.97 to 3.93 μM (Song et al., 2016). Although amygdalin itself is stable, it becomes highly toxic after hydrolysis by β-D-glucosidase. Unfortunately, β-D-glucosidase is widely present in humans, animals, plant seeds, and microorganisms. Therefore, it is crucial to explore methods for attenuating amygdalin poisoning and implementing preventive measures.

Traditional Chinese medicine suggests that ASA should undergo processing before use to inhibit the activity of amygdalin and preserve its properties. The 2020 edition of the Chinese Pharmacopoeia states that the main methods for processing and detoxifying ASA include the Clear fried method and the Chan method (Wei et al., 2023). It has been discovered that the combined use of *ephedare herba*—herbaceous stems of *Ephedra sinica* Stapf (Ephedraceae) with ASA effectively reduces the toxicity of ASA without impacting the amygdalin content. When mice were orally administered ASA alone, the LD50 was found to be 29.9 g/kg. However, when different ratios of *ephedare herba* and ASA (MX (4:1), MX (2:1), MX (1:1), MX (1:2), and MX (1:4)) were orally administered, the LD50 of mice was 87.9, 81.6, 81.4, 64.6, and 59.3 g/kg respectively, indicating the detoxification effect of *Ephedra sinica Stapf* on ASA. Furthermore, the HPLC method was used to measure the difference in amygdalin content among the mentioned groups above. The content of amygdalin in the ASA water extract was found to be 11.77 mg/g. However, co-extraction with ephedra did not result in significant differences in the amygdalin content (Song et al., 2016).

Another detoxification method for ASA has recently been reported. The method involves soaking ASA powder in a 25% sodium chloride solution for 12 h, followed by rinsing with tap

TABLE 5 Anti-inflammation, cardiovascular protection, neuroprotection, respiratory and digestive system protection, antidiabetic, liver and kidney protection and other pharmacological activities of ASA.

Extract/ compound	Cell line/model	*In vitro*/ *In vivo*	Dose	Mechanisms	Reference
Anti-inflammation					
Amygdalin	BLM-induced mice lung fibrosis model	*In vivo*	4 mg/kg, i.p.	Decreasing lung index, diminishing macrophages, neutrophils, and IL-2 levels in BALF, hampering TGF-β1, Smad2, Smad3, phospho-Smad2, and phospho-Smad3 protein expressions	Jiao et al. (2023)
Amygdalin	Formalin-induced mice model	*In vivo*	0.1, 0.5, 1, and 10 mg/kg, i.v.	Down-regulating TNF-α and IL-1β mRNA levels	Hwang et al. (2008)
ASA aqueous extract	LPS stimulated BV-2 cell inflammation	*In vitro*	0.01, 0.1, and 1 mg/mL	Inhibiting COX-1, COX-2, iNOS mRNA levels and protein expressions to impede the production of PGE2 and NO	Chang et al. (2005)
Amygdalin	LPS-induced BV-2 cell inflammation	*In vitro*	10 and 100 μg/mL	Reducing COX-1, COX-2 and iNOS mRNA levels and protein expressions to hamper the accumulation of PGE2 and NO	Yang et al. (2007)
Amygdalin	PM2.5-induced HUVEC injury	*In vitro*	2.5, 5, and 10 μg/mL	Decreasing the productions and mRNA levels of IL-6, TNF-α, and IL-1β, Lessening COX-2 mRNA level and protein expression, Diminishing TLR4, phospho-NF-κB p65, NF-κB p50, phospho-IκBα, and Bax protein expressions, while strengthening Bcl-2 protein expression	Wang et al. (2022)
Amygdalin	Ovalbumin-induced asthma mice model	*In vivo*	15 mg/kg, i.g	Alleviating airway inflammation, reducing macrophages, eosinophils, neutrophils, lymphocytes, and total cells in BALF, depressing IL-4, IL-5, and IL-13 contents in BALF to restore Th1/Th2 immune imbalance	Cui et al. (2023)
Cardiovascular protection					
ASA oil	Rats myocardial ischemia-reperfusion injury model	*In vivo*	2, 6, and 10 mL/kg	Shrinking myocardial infarction size, lowering serum and myocardial CK and AST activities, enhancing myocardial CAT, SOD, and GSH-Px levels, while diminishing MDA content, inhibiting iNOS but activating cNOS and heightening NO content	Zhang et al. (2011)
Amygdalin	Left anterior descending coronary artery induced mice myocardial infarction model	*In vivo*	3 mg/kg, i.p	Improving cardiac function and shrinking myocardial infarction size, alleviating myocardial injury and fibrosis by hampering IL-1β, IL-6, and TNF-α mRNA levels, CD68 and COX-2 protein expressions, and TGF-β/Smad signaling pathway activation	Guo et al. (2023)
Amygdalin	Angiotensin II-induced H9C2 cell hypertrophy	*In vitro*	80, 160, and 320 μM	Reducing protein expressions of ANP, BNP, β-MHC, calcineurin, and phospho-GATA-4, intensifying phospho-Nrf2, SOD-2 and CAT protein expressions, impeding phospho-NF-κB p65, COX-2, iNOS, and TNF-α protein expressions	Kung et al. (2021)

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TABLE 5 (*Continued*) Anti-inflammation, cardiovascular protection, neuroprotection, respiratory and digestive system protection, antidiabetic, liver and kidney protection and other pharmacological activities of ASA.

Extract/ compound	Cell line/model	*In vitro*/ *In vivo*	Dose	Mechanisms	Reference
Amygdalin	High-fat diet-induced mice atherosclerosis model	*In vivo*	1 mg/kg	Lowering blood triglyceride, total cholesterol, and LDL content, enhancing IL-10 and TGF-β level, up-regulating $CD4^+CD25^+Foxp3^+$ Treg cells level, Foxp3 mRNA level and protein expression, inducing cell apoptosis	Jiagang et al. (2011)
ASA	Healthy Slovak women in their reproductive age (41.60 ± 11.28 years)	*In vivo*	60 mg/kg for 42 days	Lessening total cholesterol and LDL-C content, while slightly elevating HDL-C content, intensifying follicle stimulating hormone, luteinizing hormone, and prolactin content in plasma, reducing progesterone and 17-β-estradiol content, while heightening the content of testosterone and androstenedione	Kopčeková et al. (2021)
ASA	Healthy adults in Slovak (5 females and 7 males)	*In vivo*	60 mg/kg for 84 days	Diminishing total cholesterol and LDL-C contents in plasma, slightly enhancing HDL-C level, reducing hs-CRP and AST levels and increasing CK and GGT levels	Kopčeková et al. (2018)
ASA	Adults with elevated total cholesterol levels	*In vivo*	60 mg/kg for 42 days	Decreasing total cholesterol and LDL-C content, reducing LDL_1, LDL_2, and atherogenic LDL_{3-7} subfractions, increasing mean LDL particle size	Kopčeková et al. (2022)
Neuroprotection					
Amygdalin	LPS-induced BV-2 cells	*In vitro*	0.01, 0.1, and 1 mg/mL	Suppressing COX-1, COX-2, iNOS mRNA levels and protein expressions to impede the production of PGE2 and NO	Chang et al. (2005)
Amygdalin	LPS-induced BV-2 cells	*In vitro*	10 and 100 μg/mL	Hampering the accumulation of PGE2 and NO by inhibiting COX-1, COX-2 and iNOS mRNA levels and protein expressions	Yang et al. (2007)
ASA aqueous extract	H_2O_2-induced PC12 cells	*In vitro*	1, 10, and 100 μg/mL	Anti-AchE activity with the IC50 value of 134.93 μg/mL	Vahedi-Mazdabadi et al. (2020)
ASA methanol extract	Haloperidol-induced rats Parkinsonism model	*In vivo*	100, 300, and 800 mg/kg	Improving motor function deficits and behavioral disturbances, alleviating brain tissue injury, strengthening dopamine, noradrenaline, and serotonin levels, while depressing AchE activity in brain homogenates, elevating SOD, CAT, and GSH levels, while reducing MDA and nitrite levels	Saleem et al. (2022)
Amygdalin	PC12 cells	*In vitro*	2.5, 5, 10, and 20 μM	Enhancing NGF-induced neurite outgrowth, and protecting PC12 cells from 6-OHDA-induced injury by up-regulating calreticulin protein expressing and intracellular calcium concentration	Cheng et al. (2015)
Respiratory protection					
Amygdalin	LPS-induced mice lung inflammation model	*In vivo*	0.5, 1, and 2 mg/kg, i.p.	Preventing LPS-induced lung inflammation, reducing W/D ratio of lung tissues and ROS content, suppressing EGFR, phospho-AKT, phospho-SRC, VEGFA, MAPK1, IL-6, TNF-α, IL-1β, and TGF-β1 protein expressions	Wang et al. (2021)

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TABLE 5 (*Continued*) Anti-inflammation, cardiovascular protection, neuroprotection, respiratory and digestive system protection, antidiabetic, liver and kidney protection and other pharmacological activities of ASA.

Extract/ compound	Cell line/model	*In vitro*/ *In vivo*	Dose	Mechanisms	Reference
ASA aqueous extract	OVA-induced allergic airway inflammation, and peribronchial lymph node cells	*In vivo* and *in vitro*	1 and 10 mg/mL	Reducing airway hyperreactivity, and numbers of eosinophils neutrophils and lymphocytes in BALF, lowering IL-4 level in BALF, OVA-specific IgE level in serum and BALF, and IgG1 level in serum, while increasing IgG2a level in serum, inhibiting Th2 response by diminishing IL-4, IL-5, and IL-13 production in lymph node cells	Do et al. (2006)
ASA carbonisata-derived carbon dots	LPS-induced acute lung injury	*In vivo*	0.94, 1.88, and 3.75 mg/kg, i.p	Ameliorating LPS-induced acute lung injury by reducing IL-6, IL-1β, and TNF-α levels while intensifying IL-10 content, as well as elevating SOD and GSH content and diminishing MPO and MDA accumulation	Zhao et al. (2022c)
Amygdalin	Cigarette smoke combined with LPS-induced mice COPD model and BEAS-2B cells	*In vivo* and *in vitro*	*In vivo*: 5, 10, and 20 mg/kg; *in vitro*: 100, 200, and 400 μg/mL	Inhibiting EMT process by inhibition of TGF-β/Smad pathway, suppressing vimentin, TGF-β1, phospho-Smad3, and phospho-Smad2 mRNA levels and protein expressions while up-regulating E-cadherin mRNA level and protein expression	Wang et al. (2019)
Amygdalin	LPS-treated BEAS-2B cells	*In vitro*	200, 400 μg/mL	Counteracting LPS-induced apoptosis and inflammatory responses by decreasing apoptosis rate and content of TNF-α, IL-6, IL-8, and MUC5AC. Suppressing LPS-induced EMT and activation of TLR4/NF-κB signaling by inhibiting N-Cadherin, α-SMA, vimentin, TLR4, phospho-p65, phospho-IκBα while intensifying E-Cadherin and IκBα protein expressions	Si and Zhang (2021)
Digestive system protection					
ASA	Irradiation-induced rats parotid glands degenerative model	*In vivo*	400 mg/kg	Suppressing EGF and TGF-β2 levels to alleviate rat parotid gland injury	Abdaulmoneam et al. (2023)
ASA oil	Ethanol-induced rat gastric mucosal injury	*In vivo*	1 mL/rat, i.g.	Relieving gastric mucosa injury by hampering iNOS protein expression, IL-6 and MDA levels while heightening IL-10, CAT and SOD levels	Karaboğa et al. (2018)
Amygdalin	Dibutyltin dichloride-induced rats chronic pancreatitis model	*In vivo*	10 mg/kg	Lessening α-SMA, PDGF-BB, TGF-β1, and ET-1 levels while enhancing CGRP level to alleviate microcirculatory disturbance, attenuates PSCs activation and relieves inflammation	Zhang et al. (2018)
ASA ethanolic extract and amygdalin	PANC-1 cells	*In vitro*	704 μg/mL and 35 mg/mL, respectively	Inducing cell apoptosis by regulating Bax, Bcl-2, and caspase-3 mRNA expression	Aamazadeh et al. (2020)
Antidiabetic					
ASA	Alloxan-induced rats diabetes model	*In vivo*	2, 3, and 4 mg/kg, i.p	Lowering blood glucose, HbA1c, LPO, and α-glucosidase levels and increasing serum insulin and CAT levels	Raafat et al. (2018)

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TABLE 5 (*Continued*) Anti-inflammation, cardiovascular protection, neuroprotection, respiratory and digestive system protection, antidiabetic, liver and kidney protection and other pharmacological activities of ASA.

Extract/ compound	Cell line/model	*In vitro*/ *In vivo*	Dose	Mechanisms	Reference
Amygdalin	Streptozotocin-induced rats diabetic retinopathy model, and high-glucose-stimulated HREC cells	*In vivo* and *in vitro*	*In vivo*: 10 mg/kg; *In vitro*: 10, 20, 40, and 80 μM	Relieving diabetic retinopathy progression, intensifying NRF2, HO-1, and NQO1 protein expressions, and CAT, SOD levels, while suppressing LDH, MDA, ROS levels and protein expressions of RAS, TFR1, and ACSL4, decreasing HbA1c, blood glucose levels and increasing body weight	Li et al. (2023)
ASA peptides	Spontaneously hypertensive rats	*In vitro*	50, 100, and 150 mg/kg	Reducing systolic blood pressure and diastolic blood pressure	Qin et al. (2023)
A neutral polysaccharide (AP-1)	Not mentioned	*In vitro*	0.5–10 mg/mL	Scavenging DPPH radicals, ABTS radicals, and hydroxyl radicals, and inhibiting α-glucosidase activity	Peng et al. (2023)
Amygdalin	High glucose-induced rats diabetic nephropathy model and HBZY-1 cells	*In vivo* and *in vitro*	1, 3, and 10 mg/kg	Suppressing ROS, fasting blood glucose, IL-12, IFN-γ, MDA, 24 h-urine proteins, Scr and BUN levels by inhibiting Smad/TGF-β pathway and ECM accumulation as well as transformation	Chen et al. (2021a)
Liver protection					
Amygdalin	Ehrlich ascites carcinoma-induced liver damage mice model	*In vivo*	300 mg/kg	Decreasing tumor volume and number of viable tumor cells, reducing hepatic MDA content, MMP9 and VEGF mRNA levels, while elevating GSH, SOD content and Nrf2 mRNA level	Attia et al. (2022)
ASA	Ethanol-induced rat liver injury	*In vivo*	15% or 30% ASA +20% alcohol-water	Reducing LDH content in serum and MDA production in erythrocyte, brain, kidney, and heart, while heightening SOD and GST content	Yurt and Celik (2011)
Amygdalin	D-galactosamine and LPS-induced mice acute liver injury	*In vivo*	4 and 8 mg/kg	Lowering serum ALT and AST, liver MDA, levels of MPO, TNF-α, IL-6, IL-1β, iNOS and COX-2 by inhibition of NLRP3 inflammasome and NF-κB signaling cascade, and activation of Nrf2/NQO1 signaling pathway	Tang et al. (2019)
Amygdalin and prunasin	CCl4-induced rats liver injury and fibrosis, TGF-β1 stimulated JS1 cells, and LPS-stimulated RAW264.7 cells	*In vivo* and *in vitro*	2.5, 5, and 10 μM	Inhibiting α-SMA, Col1A1, NO, serum AST, serum ALT levels to impede macrophage inflammation and hepatic stellate cell activation	Zhang et al. (2022b)
Amygdalin	Acetaminophen-induced mice acute liver failure model	*In vivo*	2.5 and 5 mg/kg	Reducing ALT, AST, necrosis area, TNF-α, IL-6, IL-1β, MDA, phospho-JUK, phospho-MLKL, and phospho-RIP3 levels, while elevating SOD, Nrf2, NQO1, HO1, and phospho-AKT levels by activation of AKT/JNK/ Nrf2 signalling pathway	Zhang et al. (2022a)
kidney protection					
Amygdalin	Unilateral ureteral obstruction induced rats renal fibrosis, and primary kidney fibroblast cells	*In vivo* and *in vitro*	3 and 5 mg/kg	Hampering kidney fibroblast proliferation, TGF-β1 secretion, and renal interstitial fibrosis	Guo et al. (2013)
ASA aqueous extract	Not mentioned	*In vivo*	1,000, 1,500, and 2,000 mg/kg	Diminishing ALT, AST, ALP, BIL, and MDA levels, while increasing creatinine, urea, BUN, CAT, SOD, and GSH levels	Zehra and Naz (2021)

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TABLE 5 (*Continued*) Anti-inflammation, cardiovascular protection, neuroprotection, respiratory and digestive system protection, antidiabetic, liver and kidney protection and other pharmacological activities of ASA.

Extract/ compound	Cell line/model	*In vitro*/ *In vivo*	Dose	Mechanisms	Reference
Other pharmacological activities					
ASA volatile oil	HaCaT cells	*In vitro*	1, 2.5, and 5 g/mL	Inducing G0/G1 cell cycle arrest, increasing early and late apoptotic cells, decreasing caspase3, caspase8, caspase9, PARP, Bax, TNF-α and NF-κB p65 protein expressions while intensifying Bcl-2 and IκBα protein expressions	Li et al. (2016)
ASA oil	Primiparous women	*In vivo*	15 min massage per day	Reducing the development of striae gravidarum during pregnancy	Timur Taşhan and Kafkasli (2012)
Dry eye syndrome					
ASA aqueous extract and Amygdalin	Urban particulate matter-induced rats keratoconjunctivitis sicca	*In vivo*	1, 10, and 100 μg/kg for ASA aqueous extract, and 0.1, 1, and 10 μg/mL	Inhibiting MMP activity and down-regulating MMP-9 mRNA level, reducing TNF-α and IL-6 content and mRNA level	Hyun et al. (2019)
ASA methanol aqueous extract	Exorbital lacrimal gland excision-induced mice model	*In vivo*	0.5 and 1 mg/mL	Increasing aqueous tear secretion, alleviating corneal epithelial damage and corneal irregularity, inhibiting Muc4 and TNF-α protein expressions	Kim et al. (2016)
Fracture Healing					
Amygdalin	RANKL-induced RAW264.7 cells	*In vitro*	5, 10, 20, and 40 μM	Impeding osteoclast differentiation and formation, endoplasmic reticulum stress and oxidative stress in by suppressing BIP, phospho-eIFα, ROS, NFATc1, c-fos, dcstamp, acp5, ATP6v0d2, ctsk, phospho-ERK, phospho-P38, and phospho-JUK levels while enhancing CAT and SOD levels	Trang et al. (2022)
Amygdalin	Mice tibial fracture model, and TGF-β1 stimulated C3H10 T1/2 cells	*In vivo* and *in vitro*	10 μM	Promoting the migration and differentiation of MSCs to accelerate the fracture healing process by regulating TGF-β/Smad signaling	Ying et al. (2020)
Immunoregulation					
ASA oil	Cyclophosphamide-induced rats immunosuppression model	*In vivo*	0.5 mL/100 g	Increasing organ indexes of spleen and thymus, white blood cell counts, platelet counts, bone marrow karyocyte counts, IgA, IgM, IgG, IL-2, IL-12, TNF-α. SOD, and GSH-Px levels while decreasing MDA production	Tian et al. (2016)

water until the liquid becomes clear. This process is repeated once, and then the ASA powder is soaked again in the 25% sodium chloride solution for another 12 h. After rinsing until the liquid is clear, the ASA powder is dried at 45° for 36 h, resulting in the detoxified ASA. This method effectively eliminates the toxic component HCN and significantly reduces the levels of antinutrient factors such as phytates, phytate phosphorus, and oxalate by 71.83%, 23.92%, and 38% respectively compared to raw ASA. The fat content and crude fiber content do not show significant changes. However, there is a reduction in the contents of Vitamin C, β-carotene, minerals, and protein to varying degrees (Tanwar et al., 2018). Overall, this method can be employed in ASA oil and functional food production. Nevertheless, further research is needed to fully explore the medicinal potential of ASA and investigate the effects of different processing methods on ASA.

7 Pharmacokinetic profile

Studies on the pharmacokinetics of ASA primarily focus on amygdalin and its metabolite prunasin (Table 8). When ASA water extract is administered orally, amygdalin and prunasin can be detected in the plasma of rats, exhibiting significantly different pharmacokinetic parameters, particularly in terms of the

TABLE 6 The clinical uses of ASA.

No.	Formula name	Main compositions	Traditional and clinical uses	Reference
1	Juhong Capsules	**ASA**; Citri Grandis Exocarpium; Citri Reticulatae Pericarpium; Pinelliae Rhizoma Praeparatum; Poria; Glycyrrhizae Radix et Rhizoma; Platycodonis Radix; Perillae Fructus (stir-fried); Asteris Radix et Rhizoma; Farfarae Flos; Trlchosanthis Pericarpium; Fritillariae Thunbergii Bulbus; Rehmanniae Radix; Ophiopogonis Radix; Gypsum Fibrosum	Phlegm-heat cough with profuse yellow thick greasy sputum, oppression in the chest, and dry mouth	Chinese Pharmacopoeia Commission (2020)
2	Juhong Huatan Pills	ASA; Citri Grandis Exocarpium; Physalis Calyx seu Fructus; Fritillariae Cirrhosae Bulbus; Papaveris Pericarpium; Schisandrae Chinensis Fructus; Alumen; Glycyrrhizae Radix et Rhizoma	Pattern of internal phlegm-turbidity obstruction due to lung Qi deficiency, manifested by cough with sputum, wheezing, panting, fullness and oppression in the chest and the diaphragm	
3	Juhong Tanke Mixture	**ASA**; Citri Grandis Exocarpium; Stemonae Radix (stir-baked with honey); Poria; Pinelliae Rhizoma (processed); Cynanchi Stauntonii Rhizoma et Radix; Glycyrrhizae Rhizoma et Radix; Schisandrae Chinensis Fructus	Pattern of phlegm turbidity obstructing the lung, manifested as cough, wheezing, and profuse sputum. Common cold, bronchitis, and laryngopharyngitis with the symptoms described above	
4	Zhike Juhong Mixture	ASA (peeled and stir-baked); Citri Grandis Exocarpium; Citri Reticulatae Pericarpium; Pinelliae Rhizoma Praeparatum; Peria; Farfarae Flos; Glycirrhizae Radix et Rhizoma; Trichosanthis Pericarpium; Asteris Radix et Rhizoma; Ophiopogonis Radix; Anemarrhenae Rhizoma; Platycodonis Radix; Rehmanniae Radix; Gypsum Fibrosum; Perillae Fructus (stir-baked)	Cough with profuse sputum, oppression in the chest, shortness of breath, dry and itching throat due to phlegm-heat obstructing the lung	
5	Shema Mixture	**ASA**; Ephedrae Herba; Arisaema Cum Bile; Gypsum Fibrosum; Mori Cortex (processed with honey); Belamcandae Rhizoma; Raphani Semen (stir-baked); Cynanchi Stauntonii Rhizoma et Radix; Scutellariae Radix; Schisandrae Chinensis Fructus (processed with vinegar)	Cough with profuse and sticky sputum, oppression in the chest, panting, rattling sound in the throat, fever in some patients, yellow or yellow and white tongue coating, or red tongue, and wiry and slippery or slippery and rapid pulse	
6	Kechuanning Mixture	**ASA**; Ephedrae Herba; Gypsum Fibrosum; Platycodonis Radix; Stemonae Radix; Papaveris Pericarpium; Glycyrrhizae Radix et Rhizoma	Frequent cough, expectoration of yellow sputum, wheezing and panting, and oppression in the chest due to phlegm-heat obstructing the lung	
7	Yifei Qinghua Concentrated Decoction	**ASA**; Astragali Radix; Codonpsis Radix; Glehniae Radix; Ophiopogonis Radix; Agrimoniae Herba; Bistortae Rhizoma; Patriniae Herba; Oldenlandiae Diffusae Herba; Paeoniae Radix Alba; Asteris Radix et Rhizoma; Platycodonis Radix; Glycyrrhizae Radix er Rhizoma	Shortness of breath, lack of strength, cough, hemoptysis chest pain due to dual deficiency of Qi and Yin; Adjuvant therapy against advanced lung cancer with the symptoms described above	
8	Kugan Granules	**ASA**; Ephedrae Herba; Menthae Haplocalycis Herba; Cicadae Periostracum; Lonicerae Japonicae Flos; Scutellariae Radix; Platycodonis Radix; Fritillariae Thunbergii Bulbus; Glycyrrhizae Radix et Rhiwma	Aversion to cold, fever, headache, sore throat, cough, expectoration and panting due to wind-heat cold and lung heat caused by wind-heat. Upper respiratory tract infection, influenza and acute tracheitis and bronchitis with the symptoms described above	
9	Ermu Ansou Pills	**ASA**; Anemarrhenae Rhizoma; Scrophulariae Radix; Papaveris Pericarpium; Ophiopogonis Radix; Farfarae Flos; Asteris Radix et Rhizoma; Lilii Bulbus; Fritillariae Thunbergii Bulbus	Persistent cough in consumptive diseases, manifested as cough with phlegm or wheezing, bone-steaming tidal fever, hoarse voice, dry mouth and tongue, profuse sputum and drooling	
10	Jiusheng Powder	**ASA**; Atractylodis Rhizoma; Phellodendri Chinensis Cortex; Perillae Folium; Menthae Haplocalycis Herba; Olibanum; Myrrha; Calomelas; Hydrargyri Oxydum Rubrum	Damp toxin obstructing the skin, leading to eczema, chronic ulcer in the leg, and impetiginous sores, manifested by wet, oozing and ulcerative skin infections with pus discharge	
11	Ertong Qingfei Pills	**ASA (stir-baked)**; Ephedrae Herba; Gypsum Fibrosum; Glycyrrh_izae Radix et Rhizoma; Mori Cortex (stir-baked with honey); Trichosanthis Pericarpium; Scutellariae Radix; Isatidis Radix; Citri Exocarpium Rubrum; Pinelliae Rhizoma Praeparatum; Perillae Fructus (stir-baked); Descurainiae Semen Lepidii Semen; Fritillariae Thunbergii Bulbus; Perillae Folium; Asari Radix et Rhizoma; Menthae Haplocalycis Herba; Eriobotryae Folium (stir-baked with honey); Cynanchi Stauntonii	Wind cold fettering the exterior with phlegm-heat in the lung meridian in pediatric patients, manifested as fever with reddened complexion, cough and wheezing, profuse, thick arid greasy sputum, sore throat arid hoarse voice	

(Continued on following page)

TABLE 6 (*Continued*) The clinical uses of ASA.

No.	Formula name	Main compositions	Traditional and clinical uses	Reference
		Rhizoma et Radix; Peucedani Radix; Acori Tatarinowii Rhizoma; Trichosanthis Radix; Chloriti Lapis (calcined)		
12	Ergan Tuirening Mixture	**ASA**; Artemisiae Annuae Herba; Isatidis Radix; Chrysanthemi Flos; Platycodonis Radix; Forsythiae Fructus; Menthae Haplocalycis Herba; Glycyrrhizae Radix et Rhizoma	Externally contracted wind heat and internal constraint heat transforming into fire in pediatric patients, manifested as headache with fever, cough, and swollen sore throat	
13	Zhisou Huatan Pills	**ASA**; Papaveris Pericarpium; Platycodonis Radix; Anemarrhenae Rhizoma; Peucedani Radix; Citri Reticulatae Pericarpium; Rhei Radix et Rhizorna (processed); Glycirrhizae Radix et Rhizoma praeparata cum Melle; Fritillariae Cirrhosae Bulbus; Gypsum Fibrosum; Perillae Folium; Descurainiae Semen Lepidii semen; Farfarae Flos (processed); Stemonae Radix (processed); Scrophulariae Radix; Ophiopogonis Radix; Buddlejae Flos; Asparagi Radix; Schisandrae Chinensis Fructus (processed); Aurantii Fructus (stir-baked); Trichosanthis Semen; Pinelliae Rhizoma (processed with ginger juice); Aucklandiae Radix; Aristolochiae Fructus (processed); Mori Folium	Pattern of phlegm-heat obstructing the lung, manifested as persistent cough, hemoptysis, sputum, wheezing and Qi counterflow, inability to sleep because of coughing and dyspnea	
14	Zhisou Dingchuan Mixture	**ASA**; Ephedrae Herba; Glycirrhizae Radix et Rhizoma; Gypsum Fibrosum	Pattern of exterior cold with internal heat, manifested as body fever with thirst, cough with profuse expectoration, wheezing and panting, fullness and oppression in the chest and the diaphragm; Acute bronchitis with the symptoms described above	
15	Fenghan Kesou Granules	**ASA;** Citri Reticulatae Periearpiilln; Zlrigiberis Rhizoma Rcess; Pillelliae Rhizoma Praeparatun; Citri Reticulatae Pericapium Viride; Ephedrae Herba; Perinae Folium; Schisandrae Chinensis Fructus; Mori Cortex; Glycyrrhizae Radix et Rhizoma Praeparata cum Melle	Cough and panting due to externally contracted wind-cold and lung Qi failing to diffuse, manifested as headache, stuffy nose, profuse sputum, cough, oppression in the chest and wheezing	
16	Ruyi Dingchuan Tablets	**ASA**; Gecko; Bufonis Venenum (processed); Astragali Radix; Pheretima; Ephedrae Herba; Codonopsis Radix; Ginkgo Semen; Aurantii Fructus Immaturus; Asparagui Radix; Schisandrae Sphenantherae Fructus (steamed with wine); Ophiopogonis Radix; Asteris Radix et Rhizoma; Stemonae Radix; Lycii Fructus; Rehmanniae Radix Praeparata; Polygalae Radix; Lepidii Semen; Daturae Flos; Gypsum Fibrosum; Glycyrrhizae Radix et Rhizoma Praeparata cum Melle	Chronic cough and panting, weak constitution and profuse sputum due to dual deficiency of Qi and Yin; bronchial asthma, pulmonary emphysema, and pulmonary heart disease with the symptoms described above	
17	Kechuanshun Pills	**ASA**; Perillae Fructus; Trichosanthis Semen; Poria; Houttuyniae Herba; Pinelliae Rhizoma (processed); Farfarae Fies; Mori Cortex; Peucedani Radix; Asteris Radix et Rhizoma; Citri Reticulatae Pericarpium; Glycyrrhizae Radix et Rhizoma	Pattern of phlegm turbidity obstructing the lung and lung Qi failing to diffuse, manifested as cough, wheezing, profuse sputum, oppression in the chest; chronic bronchitis, bronchial asthma, and pulmonary emphysema with the symptoms described above	
18	Yangshen Baofei Pill	**ASA**; Papaveris Pericarpium; Schisandrae Chinesis Fructus (stir-baked with vinegar); Fritillariae Cirrhosae Bulbus; Citri Reticulatae Pericarpium; Amomi Fructus; Aurantii Immaturus Fructus; Ephedrae Herba; Gypsum Fibrosum; Glycyrrhizae Radix et Rhizoma; Scrophulariae Radix; Panacis Quinquefolii Radix	Pattern of Yin deficiency and lung heat, manifested as cough with phlegm, panting, oppression in the chest, shortness of breath, dry mouth and throat, and restlessness at night	
19	Runfei Zhisou Pills	**ASA (stir-baked)**; Asparagi Radix; Rehmannlae Radix; Trichosanthis Radix; Trichosanthis Semen (stir-baked with honey); Mori Cortex (stir-baked with honey); Perillae Fructus (stir-baked); Asteris Radix et Rhizom; Fritillariae Thunbergii Bulbus; Farfarae Flos; Platycodonis Radix; Schisandrae Chinesis Fnictus (processed with vinegar); Peucedani Radix; Citri Reticulatae Pericarpium Viride (processed with vinegar); Citri Reticulatae Pericaipium; Astragali Radix Praeparata cum Melle; Ziziphi Spinosae Semen	Cough, wheezing, panting, excessive sputum and drooling, and hoarseness due to lung Qi deficiency	

(Continued on following page)

TABLE 6 (*Continued*) The clinical uses of ASA.

No.	Formula name	Main compositions	Traditional and clinical uses	Reference
		(stir-baked); Scutellariae Radix; Anemarrhenae Rhizoma; Loophatheri Herba; Glycyrrhizae Radix et Rhizoma Praeparata cum Melle		
20	Sangjiang Ganrnao Tablets	**ASA**; Mori Folium; Chrysanthemi Flos; Perillae Folium; Forsythiae Fructus; Zingiberis Rhizoma	Common cold due to externally contracted wind-heat, and phlegm turbidity obstructing the lung, manifested as fever, headache, swollen sore throat, and cough with white sputum	
21	Sangju Ganrnao Mixture	**ASA**; Mori Folium; Chrysanthemi Flos; Forsythiae Fructus; Menthae Haplocalycis Herba; Platycodonis Radix; Glycyqhizae Radix et Rhizoma; Phragmitis Rhizoma	Early onset of common cold due to wind-heat, manifested as headache, cough, dry mouth, and sore throat	
22	Maren Pills	**ASA**; Cannabis Semen; Rhei Radix et Rhizoma; Aurantii Fructus Immaturus (stir-baked); Magnoliae Officinalis Cortex (processed with ginger); Paeoniae Radix Alba (stir-baked)	Constipation due to intestinal dryness and body fluid deficiency, manifested as dry feces, and abdominal distension and discomfort; habitual constipation with the symptoms described above	
23	Maren Runchang Pills	**ASA (peeled and stir-baked)**; Cannabis Semen; Rhei Radix et Rhizoma; Aucklandiae Radix; Citri Reticulatae Pericarpium; Paeoniae Radix Alba	Heat in the stomach and intestines with chest and abdominal distension, and constipation	
24	Maren Zipi Pills	**ASA (peeled and stir-baked)**; Rhei Radix et Rhizoma (processed); Cannabis Semen; Angenlia Senesis Radix; Magnolia Officinalis Cortex (processed with ginger); Aurantii Fructus Imrnaturus (stir-baked with bran); Pruni Semen; Paeoniae Radix Alba	Constipation, chest and abdominal distension, loss of appetite, irritability, red tongue with fluid deficiency due to enterogastric heat, intestinal dryness and body fluid deficiency	
25	Qingfei Huatan Pills	**ASA**; Scutellariae Radix (processed with wine); Trichosanthis Semen; Fritillariae Cirrhosae Bulbus; Arisaema cum Bile (stir-baked with sand); Pinelliae Rhizoma Praeparatum (stir-baked with sand); Citri Reticulatae Pericarpium; Poria. Aurantii Fructus (stir-baked with bran); Ephedrae Herba (processed with honey); Platycodonis Radix; Perillae Typicae Fructus; Raphani Semen (stir-baked); Farfarae Flos (processed with honey); Glycyrrhizae Radix et Rhizoma	Lung-heat cough and inhibited lung Qi, manifested as profuse sputum and even wheezing, and difficulty in breathing	
26	Qingqi Huatan Pills	**ASA**; Scutellariae Radix (stir-baked with wine); Thchosanthis Semen Pulveratum; Pinelliae Rhizoma (processed); Arisaema Cum Bile; Critri Reticulatae Pericarpium; Aurantii Fructus lrnmaturus; Poria	Profuse sputum, yellow thick greasy sputum, cough, fullness and oppression in the chest and the abdomen due to phlegm-heat obstructing the lung	
27	Qingfei Xiaoyan Pills	**ASA (stir-baked)**; Ephedrae Herba; Gypsum Fibrosum; Pheretima; Arctii Fructus; Lepidii Semen or Descurainiae Semen; Bovis Calculus Artifactus; Saigae Tataricae Comu	Pattern of phlegm-heat obstructing the lung, manifested as coughing and wheezing, distending pain in the hypochondrium, and yellow thick greasy sputum; Upper respiratory tract infection, acute bronchitis, acute episode of chronic bronchitis, lung infections with the symptoms described above	
28	Lusika Pills	**ASA**; Ephedrae Herba; Gypsum Fibrosum; Glycyrrhizae Radix et Rhizoma; Asari Radix et Rhizoma; Perillae Fructus (stir-baked); Sinapis Semen (stir-baked); Arctii Fructus (stir-baked); Trichosanthis Pericarpium; Belamcandae Rhizoma; Indigo Naturalis; Meretricis Concha or Cyclinae Concha; Trichosanthis Radix; Gardeniae Fructus (stir-baked with ginger); Bovis Calculus Artifactus	Whooping cough, cough due to phlegm turbidity obstructing the lung, manifested as paroxysmal cough, rattling sound in the throat, wheezing, dry throat, and hoarse voice; pertussis with the symptoms described above	
29	Lianhuaqingwen Capsules	**ASA (stir-baked)**; Forsythiae Fructus; Lonicerae Japonicae Flos; Ephedrae Herba (processed with honey); Gypsum Fibrosum; Isatidis Radix; Dryopteridis Crassirhizoma Rhizoma; Houttuyniae Herba; Pogostemonis Herba; Rhei Radix et Rhizorna; Rbodiolae Crenulatae Radixet Rhizorna; Menthol; Glycyrrhizae Radix et Rhizorna	Patterns of heat toxin assailing the lung in influenza, manifested as fever, aversion to cold, muscle soreness, stuffy and runny nose, cough, headache, dry and sore throat, reddish tongue, and yellow or yellow and greasy tongue coating	
30	Qingxuan Zhike Granules	**ASA (stir-baked)**; Mori Folium; Menthae Haplocalycis Herba; Platycodonis Radix; Paeoniae Radix Alba; Aurantii Fructus; Citri Reticulatae Pericarpium; Asteris Radix et Rhizoma; Glycyrrhizae Radix et Rhizoma	Cough due to externally contracted wind-heat in children, manifested as cough, expectoration of sputum, fever or nasal congestion, runny nose, slight aversion to wind-cold, red or sore throat, and thin and yellow tongue coating	

(Continued on following page)

TABLE 6 (*Continued*) The clinical uses of ASA.

No.	Formula name	Main compositions	Traditional and clinical uses	Reference
31	Yinhuang Qingfei Capsules	**ASA**; Descurainiae Semen Lepidii semen; Ephedrae Herba (processed with honey); Fritillariae Thunbergii Bulbus; Eriobotryae Folium; Isatidis Folium; Acori Tatarinowii Rhizoma; Dioscoreae Nipponicae Rhizoma; Arternisiae Rupestris Herba; Ginkgo Folium; Schisandrae Chinensis Fructus; Aurantii Fructus Imrnaturus; Gypsum; Glycyrrhizae Radix et Rhizoma	Acute attack of chronic bronchitis with the pattern of phlegm-heat obstructing the lung, manifested as cough with yellow and sticky phlegm, oppression in the chest, wheezing, fever, thirst, dry stools, yellow urine, red tongue and yellow, greasy coating	
32	Fengliaoxing Fengshi Dieda Wine	**ASA**; Erycibes Caulis; Cinnamomi Ramulus; Ephedrae Herba; Notopterygii Rhizoma et Radix; Anglicae Sinensis Radix; Chuanxiong Rhiroma; Angelicae Dahuricae Radix; Psoraleae Fructus; Olibanum; Gleditsiae Fructus Abnormalis; Citri Reticulatae Pericarpium; Atractylodis Rhizoma; Magnoliae Officinalis Cortex; Cyperi Rhizoma; Aucklandiae Radix; Aurantii Fructus; Atractylodis Macrocephalae Rhizoma; Dioscoreae Rhizoma; Polygonati Rhizoma; Cuscutae Semen; Foeniculi Fructus; Alismatis Rhizoma; Trogopterori Faeces; Bombycis Feculae; Moutan Cortex; Myrrha	*Bi* disorders due to wind, cold and dampness, numbness of the extremities, soreness and weakness in the lower back and knees; Traumatic injuries and swelling pain due to stasis	
33	San' ao Tablets	**ASA**; Ephedrae Herba; Glycyrrhizae Radix et Rhiroma; Zingiberis Rhizoma Recens	Pattern of wind-cold assailing the lung, manifested as cough, deep hoarse voice, profuse white clear sputum; Acute bronchitis with the symptoms described above	
34	Keke Tablets	**ASA**; Ephedrae Herba; Papaveris Pericarpium; Glycyrrhizae Radix et Rhizoma; Raphani Semen; Platycodonis Radix; Gypsum Fibrosum	Cough, wheezing and shortness of breath	
35	Lingyang Qingfei Granules	ASA (stir-baked); Fritillariae Thunbergii Bulbus; cortex Mori (processed with honey); Peucedani Radix; Ophiopogonis Radix; Asparagi Radix; Trichosanthis Radix; Rehmanniae Radix; Scrophulariae Radix; Dendrobii Herba; Platycodonis Radix; Eriobotryae Folium (processed with honey); Tinosporae Radix; Lonicerae Japonicae Flos; Isatidis Folium; Garedeniae Frucrus; Scutellariae Radix; Isatidis Radix; Moutan Cortex; Menthae Haplocalycis Herba; Glycyrrhizae Radix et Rhiwrna; Rhei Radix et Rhizoma Praeparata; Citri eticulatae Pericarpium; Saigae Tataricae Comu Pulvis	Considerable heat in lung and stomach, with infection of seasonal pathogenic factors; manifested as fever, dizziness, heavy aching limbs, cough, abundant expectoration, swollen sore throat, nosebleed, hemoptysis, dry mouth and tongue	
36	Zhichuanling Injection	**ASA**; Ephedrae Herba; Daturae Flos; Forsythiae Fructus	Wheezing, cough, oppression in the chest, and profuse sputum due to phlegm turbidity obstructing the lung and lung failing to diffuse and downbear; Bronchial asthma and asthmatic bronchitis with the symptoms described above	
37	Niuhuang Qingxin Pills	**ASA (stir-baked)**; Bovis Calculus; Angelicae Sinensis Radix; Chuanxiong Rhizoma; Glycyrrhizae Radix et Rhizoma; Dioscoreae Rhizoma; Scutellariae Radix; Sojae Semen Germinatum; Jujubae Fructus; Atractylodis Macrocephalae Rhizoma (stir-baked); Poria; Platycodi Radix; Saposhnikoviae Radix; Bupleuri Radix; Asini Corii Colla; Zingiberis Rhizoma; Paeoniae Radix Alba; Ginseng Radix et Rhizorna; Massa Medicata Fermentata (stir-baked); Cinnamomi Cortex; Ophiopogonis Radix; Ampelopsis Radix; Typhae Pollen (stir-baked); Moschus or Moschus Artifactus; Bomeolum Syntheticum; Powerdered Buffalo Horn Extract; Saigae Tataricae Cornu; Cinnabaris; Realgar	Pattern of heat entering the pericardium and exuberant heat stirring up wind, manifested as vexation and restlessness in high fever; loss of consciousness and delirious speech; seizures in children due to high fever	
38	Qihuang Tongmi Soft Capsules	**ASA (stir-baked)**; Astragali Radix; Polygoni Multiflori Radix; Angelicae Sinensis Radix; Cistanches Herba; Sesami Semen Nigrum; Juglandis Semen; Rhei Radix et Rhizoma (prepared); Cassiae Semen; Aurantii Fructus Immaturus; Persicae Semen	Functional constipation due to deficiency	

(Continued on following page)

TABLE 6 (*Continued*) The clinical uses of ASA.

No.	Formula name	Main compositions	Traditional and clinical uses	Reference
39	Shenyan Jiere Tablets	**ASA (stir-baked)**; Imperatae Rhizorna; Forsythiae Fructus; Schizonepetae Herba; Citri Retiuculatae Pericarpium; Arecae Pericarpium; Alismatis Rhizoma (stir-baked with salt water); Poria; Cinnamomi amulus; Plantaginis Semen (stir-baked); Vignae Semen; Gypsum Fibrosum; Taraxaci Herba; Cicadae Periostracum	Edema caused by wind-heat invasion of the lung, manifested as fever, cold, swelling of the head and face, sore throat, aching limbs, short red urine, thin yellow tongue coating, pulse floating number, and acute nephritis with the symptoms described above	
40	Jinlian Qingre Granules	**ASA (stir-baked)**; Trollii Chinensis Flos; Isatidis Folium; Gypsum Fibrosum; Anemarrhenae Rhizoma; Rehmanniae Radix; Scrophulariae Radix	Pattern of exuberant heat toxin in common cold, manifested as high fever, thirst, dry throat, cough, thick phlegm; influenza and upper respiratory tract infection with the symptoms described above	
41	Jinsang Kaiyin Granules	**ASA (rinsed with boiling water)**; Lonicerae Japonicae Flos; Forsythiae Fructus; Scrophulariae Radix; Isatidis Radix; Paeoniae Radix Rubra; Scutellariae Radix; Mori Folium; Chrysanthemi Flos; Peucedani Radix; Arctii Fructus; Alismatis Rhizoma; Sterculiae Lychnophorae Semen; Bombyx Batryticatus (stir-baked); Cicadae Periostracum; Oroxyli Semen	Swelling and sore of the throat, hoarseness; acute pharyngitis, sub-acute pharyngitis, and laryngitis with the symptoms described above	
42	Fufang Haqing Tablets	ASA; Bufonis Venenum; Astragali Radix; Ginkgo Semen; Asteris Radix et Rhizoma; Peucedani Radix; Aconiti Lateralis Radix Praeparata; Schisandrae Sphenantherae Fructus; Piperis Nigrum Fructus	Pattern of lung deficiency, manifested as coughing and wheezing with profuse sputum; chronic tracheitis, pulmonary emphysema, and asthmatic bronchitis with the symptoms described above	
43	Biaoshi Ganmao Granules	**ASA (stir-baked)**; Perillae Folium; Puerariae Lobatae Radix; Angelicae Dahuricae Radix; Ephedrae Herba; Saposhnikoviae Radix; Platycodonis Radix; Cinnamomi Ramulus; Glycyrrhizae Radix et Rhizoma; Citri Pericarpium Reticulatae; Zingiberis Rhizoma Recens	Common cold of exterior excess wind-cold pattern, manifested as severe chills with mild fever. Absence of sweating, headache, painful stiff nape, clear, runny nose, and cough with white and watery phlegm	
44	Biaoxu Ganmao Granules	**ASA (stir-baked)**; Cinnamomi Ramulus; Puerariae Lobatae Radix; Paeoniae Radix Alba; Zingiberis Rhizoma Recens; Jujubae Fructus	Common cold due to exterior deficiency wind-cold pattern, manifested as fever, chills, sweating, headache, painful stiff nape, cough with white phlegm, stuffy nose and dry retching, thin white coating, and floating and moderate pulse	
45	Ganmao Qingre Chewable Tablets	**ASA**; Schizonepetae Spica; Menthae Haplocalycis Herba; Saposhnikoviae Radix; Radix Bupleuri; Perillae Folium; Puerariae lobatae Radix; Platycodonis Radix; Angelicae Dahuricae Radix; Corydalis bungeanae Herba; Phragmitis Rhizoma	Wind-cold common cold, manifested as headache, fever, chills, general body aches, clear runny nose, cough and dry throat	
46	Ganmao Zhike Syrup	**ASA**; Bupleuri Radix; Lonicerae Flos; Puerariae Lobatae Radix; Artemisiae Annuae Herba; Forsythiae Fructus; Scutellariae Radix; Platycodonis Radix; Menthol	Common cold due to externally contracted wind-heat, manifested as fever, aversion to wind, headache, stuffy nose, swollen sore throat, cough and general malaise	
47	Baikejing Syrup	**ASA (stir-baked)**; Citri Reticulatae Pericarpium; Ophiopogonis Radix; Peucedani Radix; Pinelliae Rhizoma Praeparatum cum Alumine; Scutellariae Radix; Stemonae Radix (processed with honey); Phellodendri Chinensis Cortex; Mori Cortex; Glycyrrhizae Radix et Rhizoma; Ephedrae Herba (processed with honey); Descurainiae Semen Lepidii Semen (stir-baked); Perillae Fructus (stir-baked); Arisaematis Rhizoma (stir-baked); Platycodonis Radix; Trichosanthis Semen (stir-baked)	Cough, expectoration of sputum due to externally contracted wind-heat; Common cold, acute and chronic bronchitis, pertussis with the symptoms. Described above	
48	Baokening Granules	**ASA (stir-baked)**; Perillae Folium; Mori Folium; Peucedani Radix; Fritillariae Thunbergii Bulbus; Ephedrae Herba; Platycodonis Radix; Rhizoma Arisaematis Rhizoma (processed); Citri Reticulntae Pcricarpium; Scutellariae Radix; Indigo Naturalis; Trichosanthis Radix; Aurantii Fructus (stir-baked with bran); Crataegi Fructus (stir-baked); Glycyrrhizae Radix et Rhizoma; Bovis Calculus Artifactus	Pattern of externally contracted wind-cold and internal heat due to food retention in children, manifested as headache with fever, coughing with copious sputum, panting and even wheezing, swollen sore throat, vexation and restlessness	

(Continued on following page)

TABLE 6 (*Continued*) The clinical uses of ASA.

No.	Formula name	Main compositions	Traditional and clinical uses	Reference
49	Gejie Dingchuan Capsules	**ASA (stir-baked)**; Gecko; Perillae Fructus (stir-baked); Trichosanthis Semen; Ephedrae Herba: Gypsum Fibrosum; Glycyrrhizae Radix et Rhizoma; Asteris Radix et Rhizoma; Trionycis Carapax (processed with vinegar); Seutellariae Radix; Ophiopogonis Radix; Coptidis Rhizoma; Lilii Bulbus; Gypsum Fibrosum	Persistent cough in consumptive diseases and wheezing in the elderly due to lung heat with Yin deficiency, manifested as shortness of breath, heat vexation, fullness and oppression in the chest, spontaneous sweating, and night sweating	
50	Jieji Ningsou Pills	**ASA;** Perillae Folium; Peucedani Radix; Puerariae Lobatae Radix; Platycodonis Radix; Pinelliae Rhizoma (processed); Citri Reticulatae Pericarpium; Fritillariae Thunbergii Bulbus; Trichosanthis Radix; Scrophulariae Radix; Glycyrrhizae Radix et Rhizoma	Common cold with fever, cough, and profuse sputum in children due to external contraction of wind-cold, and phlegm turbidity obstructing the lung	
51	Chaiyin Mixture	**ASA**; Bupleuri Radix; Lonicerae Japonicae Flos; Scutellariae Radix; Pueraiae Lobatae Radix; Schizonepetae Herba; Artemisiac Annuae Herba; Forsythiae Fructus; Platycodonis Radix; Menthae Haplocalyc is; Houttuyniae Herba	Upper respiratory tract infection due to externally contracted wind-heat, manifested as fever, aversion to wind, headache, sore throat, sweating, stuffy and runny nose, cough, reddened tongue tip and margins with thin yellow coating	
52	Dahuang Zhechong Pills	**ASA (stir-baked)**; Rhei Radix et Rhizoma (processed); Eupolyphaga Steleophaga (stir-baked); Hirudo (processed); Tabanus (removed from wings and feet,stir-baked); Holotrichia Diomphalia (stir-baked); Toxicodendri Resina (calcined); Persicae Semen; Scutellariae Radix; Rehmanniae Radix; Paconiae Alba Radix; Glycyrrhizae Radix et Rhizoma	Abdominal masses and amenorrhea due to internal static blood retention, manifested as abdominal masses, scaly dry skin, dark complexion, tidal fever, emaciation, and amenorrhea	
53	Jinbei Tankeqing Granules	**ASA (stir-baked)**; Fritillariae Thunbcrgii Bulbus; Lonicerae Japonicae Flos; Peucedani Radix; Moil Cortex; Platycodonis Radix; Belamcandae Rhizoma; Ephedrae Herba; Chuanxiong Rhizoma; Glycyrrhizae Radix et Rhizoma	Cough, yellow thick greasy phlegm, wheezing caused by phlegm-heat obstructing the lung; Acute episode of chronic bronchitis with the symptoms described above	
54	Fufang Yigan Pills	**ASA**; Artemisiae Scopariae Herba; lsatidis Radix; Gentianae Radix; Chrysanthemi Indici Flos; Taraxaci Herba; Sophorae Tonkinensis Radix et Rhizoma; Sedi Herba; Cicadae Periostracum; Bovis Calculus Artifactus; Spica Prunellae; Plantaginis Semen; Smilacis Glabrae Rhizoma; Picrorhizae Rhizoma; Moutan Cortex; Salviae Miltiorrhizae Radix et Rhizoma; Carthami Flos; Rhei Radix et Rhizoma; Cyperi Rhizoma; Citri Reticulatae Viride Pericarpium; Aurantii Fructus; Arecae Semen; Gigeriae Galli Endothelium Cornrum; Ginseng Radix et Rhizoma; Cinnamomi Ramulus; Schisaindrae Chinensis Fructus; Bupleuri Radix; Glycyrrhizae Radix et Rhizoma Praeparata Cum Melle	Panern of retained dampness-heat at toxin, manifested as distending pain in the hypochondria, jaundice, dry mouth, bitter taste in the mouth, yellow tongue coating and string-like pulse; Acute and chronic hepatitis with the symptoms described above	

maximum concentration (C_{max}). After oral administration of ASA water extract, amygdalin is rapidly absorbed with a T_{max} at 0.5 h and a C_{max} at 223.6 ng/mL. Subsequently, a substantial amount of amygdalin is hydrolyzed to prunasin within a short time, with a T_{max} of 0.58 h and a C_{max} of 5,212.8 ng/mL (Song et al., 2015). The volume of distribution/bioavailability (Vz/F) of amygdalin is 196.8 L/kg, while the Vz/F of prunasin is 15.9 L/kg, indicating that amygdalin exhibits high tissue distribution specificity and may be concentrated in certain organs compared to prunasin (Helmy et al., 2013). Recent research revealed that the concentration of amygdalin in lung tissue (309.335 ± 13.662 ng/g) was significantly higher than in plasma (44.774 ± 7.397), heart (23.693 ± 6.097), liver (43.391 ± 5.963), spleen (53.745 ± 6.584), and kidney (55.373 ± 4.467) (Yang et al., 2021), suggesting that amygdalin may be concentrated in lung tissue. The elimination half-life ($t_{1/2}$) of amygdalin and prunasin are 1.15 ± 0.26 h and 2.21 ± 0.52 h, respectively. Similarly, the mean residence time (MRT) for amygdalin and prunasin are 1.33 ± 0.23 h and 1.57 ± 0.22 h, respectively (Song et al., 2015). This observation can be attributed to the hydrolysis of β-D-glucosidase. Additionally, the clearance/bioavailability (CLz/F) of amygdalin is significantly higher at 121.1 ± 31.4 L/kg·h compared to prunasin, which has a CLz/F of only 5.1 ± 0.9 L/kg·h. This difference may be linked to the higher blood concentrations of prunasin. It is worth noting that amygdalin exists in two isomers, D and L, with the latter being stable only at temperatures higher than 40°C (Wahab et al., 2015). After administration of ASA water extract, the plasma concentrations of the two isomers are almost the same, with values of 147.8 ± 34.9 and 138.7 ± 32.4 ng/mL, respectively. However, their metabolites, D-Prunasin and L-Prunasin, exhibit significant differences in concentration, with values of 2,101.4 ± 453.0 and 3,561.2 ± 619.8 ng/mL, respectively. Importantly, the content of L-Prunasin is considerably higher than that of D-Prunasin, indicating stereoselective metabolism of amygdalin. Besides, the bioavailability of amygdalin was found to be only 0.19% ± 0.08% when orally administered to rats,

TABLE 7 Toxicological effects including adverse reactions of ASA.

Extract/ Compound	Animal/cell line/subject	Minimal toxic concentration/Dose	Toxic and side effects	Reference
Lyophilized ASA aqueous extracts	Male and female Kunming mice	LD50 = 29.9 g/kg	Death	Song et al. (2016)
Raw ASA aqueous extracts	Kunming mice	LD50 = 22.4874 g/kg	Death	Chen and Jia (2012)
ASA Methanol water extracts	Marine bacterium *V. logei* (wild strain)	IC50 = 1.61–2.03 mg/mL ranges from different varieties	Inhibiting bacterial emission	Tareen et al. (2021)
Wild Apricot Oil	Haffkine Wistar strain rats	10 mg per day for 13 weeks	Survival with on clinical signs of any abnormality	Gandhi et al. (1997)
Bitter apricot essential oil	HaCaT (human skin keratinocyte cells)	IC50 = 142.45 μg/mL at 48 h	Suppressing the proliferation	Li et al. (2016)
Amygdalin	Wistar strain rats	LD50 = 880 mg/kg	Quadriplegia, muscle-twitching, difficulty in breathing, apnea and subsequently death	Adewusi and Oke (1985)
Amygdalin	MCF-7 (human breast cancer cells)	IC50 = 5,880.00 μg/mL at 24 h	Inhibiting the proliferation	Ramadan et al. (2019)
Amygdalin	Human breast cancer cells MCF-7 and T47D	IC50 = 39 and 45 mM at 72 h, respectively	Inhibiting the proliferation	Abboud et al. (2019)
Amygdalin	Human breast cancer cells MCF-7 and SR-BR-3	IC50 = 14.2 and 13.7 mg/mL at 24 h, respectively	Inhibiting the proliferation	Moradipoodeh et al. (2020)
Amygdalin-Z $_{HER2}$ affibody conjugate	Human breast cancer cells MCF-7 and SR-BR-3	IC50 = 8.27 and 19.8 mg/mL at 24 h, respectively	Inhibiting the proliferation	Moradipoodeh et al. (2020)
Amygdalin-folic acid nanoparticles	MCF-10A (human normal mammary epithelial cells)	IC50 = 180.3 μg/mL at 24 h	Anti-proliferative activity	Askar et al. (2023)
Amygdalin-folic acid nanoparticles	Human breast cancer cells MCF-7 and MDA-MB-231	IC50 = 79.8 and 94.9 μg/mL at 24 h, respectively	Anti-proliferative activity	Askar et al. (2023)
Amygdalin	Huh-7 (human liver cancer cells)	IC50 = 11.587, 1.9, 0.625 mM at 24, 48 and 72 h, respectively	Inhibiting the proliferation in a dose and time-dependent manner	Mamdouh et al. (2021)
Amygdalin	HepG-2 (human liver cancer cells)	IC50 = 41.86, 1.224, 0.089 mM at 24, 48 and 72 h, respectively	Inhibiting the proliferation in a dose and time-dependent manner	Mamdouh et al. (2021)
Amygdalin	HepG-2 (human liver cancer cells)	IC50 = 2,691.54 μg/mL at 24 h	Inhibiting the proliferation	Ramadan et al. (2019)
Amygdalin	HepG-2 (human liver cancer cells)	IC50 = 458.10 mg/mL at 48 h	Inhibiting the proliferation	Zhou et al. (2012)
Amygdalin+ β-D-glucosidase	HepG-2 (human liver cancer cells)	IC50 = 3.2 mg/mL at 24 h	Inhibiting the proliferation	Zhou et al. (2012)
Amygdalin	HCT116 (human colon cancer cells)	IC50 = 6,309.57 μg/mL at 24 h	Inhibiting the proliferation	Ramadan et al. (2019)
CuO-TiO_2-Chitosan-Amygdalin Nanocomposites	MOLT4 (human acute lymphoblastic leukemia cells)	IC50 = 38.41 μg/mL at 24 h	Inhibiting the proliferation	Elderdery et al. (2022)
Amygdalin	PC12 (rat pheochromocytoma cells) and MDCK (Madin Darby canine kidney cells)	IC50 = 38.53 and 63.97 μM at 48 h, respectively	Inhibiting the proliferation	Song et al. (2016)
Amygdalin+ β-D-glucosidase	PC12 (rat pheochromocytoma cells) and MDCK (Madin Darby canine kidney cells)	IC50 = 5.97 and 3.93 μM at 48 h, respectively	Inhibiting the proliferation	Song et al. (2016)

suggesting that amygdalin may have undergone degradation before reaching the intestinal tract. In contrast, prunasin exhibited a higher bioavailability of 64.91% ± 6.30% when administered orally. These findings indicate that amygdalin undergoes deglycosylation metabolism (Zhang et al., 2022b).

Changes in the oral dose of amygdalin lead to variations in its pharmacokinetic parameters. For instance, when rats were orally administered 5 mg/kg of amygdalin, the following parameters were observed: T_{max} was 14 min, C_{max} was 23.08 ng/mL, area under the plasma concentration curve (0-t) (AUC_{0-t}) was 1,391.77 ng min/mL, area under the plasma concentration curve (0-∞) ($AUC_{0-\infty}$) was 1,569.22 ng min/mL, $t_{1/2}$ was 28.76 min, and MRT was 53.33 min (Zhang et al., 2022b). However, when the dosage was increased to 100 mg/kg, the following parameters were observed:

TABLE 8 Pharmacokinetic profiles of ASA.

Animal	Drug administrated	Dose	Compound	Pharmacokinetic parameters	Reference
Male SD rats	ASA aqueous extracts (oral administration)	3 g/kg	Amygdalin	T_{max}(h): 0.50 ± 0.00	Song et al. (2015)
				C_{max}(ng/mL): 223.6 ± 32.1	
				AUC_{0-t}(ng·h/mL): 286.5 ± 66.8 $t_{1/2}$(h): 1.15 ± 0.26	
				MRT_{0-t}(h): 1.33 ± 0.23	
				Vz/F(L/kg): 196.8 ± 47.8	
				CLz/F(L/kg·h): 121.1 ± 31.4	
			D-Amygdalin	T_{max}(h): 0.50 ± 0.00	
				C_{max}(ng/mL): 112.1 ± 14.9	
				AUC_{0-t}(ng·h/mL): 147.8 ± 34.9 $t_{1/2}$(h): 1.37 ± 0.48	
				MRT_{0-t}(h): 1.34 ± 0.22	
				Vz/F(L/kg): 231.6 ± 74.6	
				CLz/F(L/kg·h): 120.4 ± 31.5	
			L-Amygdalin	T_{max}(h): 0.50 ± 0.00	
				C_{max}(ng/mL): 111.5 ± 18.4	
				AUC_{0-t}(ng·h/mL): 138.7 ± 32.4 $t_{1/2}$(h): 1.18 ± 0.28	
				MRT_{0-t}(h): 1.31 ± 0.24	
				Vz/F(L/kg): 200.4 ± 62.6	
				CLz/F(L/kg·h): 119.9 ± 31.9	
			Prunasin	T_{max}(h): 0.58 ± 0.20	
				C_{max}(ng/mL): 5,212.8 ± 777.1	
				AUC_{0-t}(ng·h/mL): 6919.9 ± 1,455.7 $t_{1/2}$(h): 2.21 ± 0.52	
				MRT_{0-t}(h): 1.57 ± 0.22	
				Vz/F(L/kg): 15.9 ± 3.0	
				CLz/F(L/kg·h): 5.1 ± 0.9	
			D-Prunasin	T_{max}(h): 0.58 ± 0.20	
				C_{max}(ng/mL): 1,674.8 ± 227.1	
				AUC_{0-t}(ng·h/mL): 2101.4 ± 453.0 $t_{1/2}$(h): 2.20 ± 0.64	
				MRT_{0-t}(h): 1.51 ± 0.22	
				Vz/F(L/kg): 27.8 ± 9.5	
				CLz/F(L/kg·h): 8.8 ± 1.5	
			L-Prunasin	T_{max}(h): 0.62 ± 0.21	
				C_{max}(ng/mL): 3561.2 ± 619.8	
				AUC_{0-t}(ng·h/mL): 4811.1 ± 1,056.9 $t_{1/2}$(h): 2.22 ± 0.62	
				MRT_{0-t}(h): 1.59 ± 0.23	
				Vz/F(L/kg): 11.1 ± 2.7	
				CLz/F(L/kg·h): 3.5 ± 0.7	

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TABLE 8 (*Continued*) Pharmacokinetic profiles of ASA.

Animal	Drug administrated	Dose	Compound	Pharmacokinetic parameters	Reference
Male and female SD rats	Amygdalin (oral administration)	100 mg/kg	Amygdalin	T_{max}(h): 0.25	Qin et al. (2021)
				C_{max}(ng/mL): 93.871	
				AUC_{0-t}(ng·h/mL): 73.595	
				$AUC_{0-\infty}$(ng·h/mL): 74.133 $t_{1/2}$(h): 1.21	
				MRT(h): 1.91	
Male Wistar rats	Amygdalin (oral administration)	5 mg/kg	Amygdalin	T_{max}(min): 14.00 ± 10.84	Zhang et al. (2022b)
				C_{max}(ng/mL): 23.08 ± 5.08	
				AUC_{0-t}(1,569.22): 1,391.77 ± 560.91	
				$AUC_{0-\infty}$(ng·min/mL): 1,569.22 ± 650.62 k_e(/min): 0.030 ± 0.010	
				$t_{1/2}$(min): 28.76 ± 7.25	
				MRT(min): 53.33 ± 10.05	
				Vd(mL/kg): 140,028.28 ± 27,425.92	
				CL(mL/min/kg): 3636.14 ± 1,375.61	
				F (%): 0.19 ± 0.08	
			Prunasin	T_{max}(min): 22.00 ± 4.47	
				C_{max}(ng/mL): 1835.12 ± 268.09	
				AUC_{0-t}(ng·min/mL): 99,732.22 ± 17,256.2	
				$AUC_{0-\infty}$(ng·min/mL): 103,913.17 ± 14,202.48 k_e(/min): 0.015 ± 0.002	
				$t_{1/2}$(min): 47.79 ± 5.72	
				MRT(min): 51.27 ± 2.62	
				Vd(mL/kg): 3336.43 ± 741.42	
				CL(mL/min/kg): 48.19 ± 7.58	
	Amygdalin (intravenous administration)	5 mg/kg	Amygdalin	T_{max}(min): 2.00 ± 0.00	
				C_{max}(ng/mL): 34,763.84 ± 18,057.68	
				AUC_{0-t}(ng·min/mL): 731,268.98 ± 109,541.87	
				$AUC_{0-\infty}$(ng·min/mL): 731,909.80 ± 109,917.01 k_e(/min): 0.010 ± 0.004	
				$t_{1/2}$(min): 67.93 ± 24.72	
				MRT(min): 39.42 ± 5.95	
				Vd(mL/kg): 680.71 ± 257.40	
				CL(mL/min/kg): 6.97 ± 1.12	
			Prunasin	T_{max}(min): 69.00 ± 29.24	
				C_{max}(ng/mL): 88.64 ± 22.18	
				AUC_{0-t}(ng·min/mL): 6754.24 ± 1,304.65	
				$AUC_{0-\infty}$(ng·min/mL): 81,926.10 ± 8557.72 k_e(/min): 0.004 ± 0.002	
				$t_{1/2}$(min): 214.40 ± 96.83	
				MRT(min): 281.70 ± 103.83	

(Continued on following page)

TABLE 8 (*Continued*) Pharmacokinetic profiles of ASA.

Animal	Drug administrated	Dose	Compound	Pharmacokinetic parameters	Reference
				Vd(mL/kg): 186,646.54 ± 74,474.47	
				CL(mL/min/kg): 638.28 ± 167.77	
	Prunasin (oral administration)	5 mg/kg	Prunasin	T_{max}(min): 16.00 ± 5.48	
				C_{max}(ng/mL): 2912.06 ± 433.45	
				AUC_{0-t}(ng·min/mL): 134,797.34 ± 13,091.48	
				$AUC_{0-\infty}$(ng·min/mL): 135,731.78 ± 12,982.12 k_e(/min): 0.013 ± 0.005	
				$t_{1/2}$(min): 63.48 ± 31.17	
				MRT(min): 46.80 ± 4.65	
				Vd(mL/kg): 3369.66 ± 1,532.62	
				CL(mL/min/kg): 37.02 ± 3.80	
				F (%): 64.91 ± 6.30	
	Prunasin (intravenous administration)	5 mg/kg	Prunasin	T_{max}(min): 2.00 ± 0.00	
				C_{max}(ng/mL): 6926.50 ± 1952.91	
				AUC_{0-t}(ng·min/mL): 207,670.12 ± 22,295.20	
				$AUC_{0-\infty}$(ng·min/mL): 208,663.12 ± 22,869.27 k_e(/min): 0.010 ± 0.004	
				$t_{1/2}$(min): 69.42 ± 22.68	
				MRT(min): 43.94 ± 3.33	
				Vd(mL/kg): 2469.24 ± 979.45	
				CL(mL/min/kg): 25.05 ± 2.29	
3 males and 4 females aged 63.3 ± 9.1 years	Almond skin polyphenols (oral administration)	450 mg	Catechin	T_{max}(h): 1.4 ± 0.2	Chen et al. (2019)
				C_{max}(ng/mL): 44.3 ± 15.6	
			Naringenin	T_{max}(h): 3.3 ± 0.5	
				C_{max}(ng/mL): 19.3 ± 8.2	
			total flavonoids	T_{max}(h): 1.7 ± 0.3	
				C_{max}(ng/mL): 82.3 ± 17.6	

Notes: T_{max}, time to peak concentration; AUC_{0-t}, area under the plasma concentration curve (0-t); $AUC_{0-\infty}$, area under the plasma concentration curve (0-∞); CL, body clearance; CLz/F, clearance/bioavailability; C_{max}, maximum concentration; F (%), bioavailability; ke, elimination rate constant; MRT, mean residence time; $t_{1/2}$, elimination half-time; Vd, volume of distribution; Vz/F, volume of distribution/bioavailability.

T_{max} was 0.25 h, C_{max} was 93.871 ng/mL, AUC_{0-t} was 73.595 ng h/mL (equivalent to 4415.7 ng min/mL), $AUC_{0-\infty}$ was 74.133 ng h/mL (equivalent to 4447.98 ng min/mL), t1/2 was 1.21 h, and MRT was 1.91 h (Yang et al., 2021). Notably, there is little difference in T_{max} between the doses of 5 mg/kg and 100 mg/kg, suggesting that the absorption speed of amygdalin may not be affected by dosage. However, as the dose increases, C_{max}, AUC, $t_{1/2}$, and MRT of amygdalin significantly increase. This indicates that higher doses lead to higher peak concentrations of amygdalin and slower elimination, resulting in a longer presence of amygdalin in the body.

Different drug-delivery routes have a significant impact on the absorption, distribution, and elimination of amygdalin. When amygdalin is injected intravenously at a dose of 5 mg/kg, it reaches its T_{max} within 2 min, while oral administration takes 14 min. The C_{max} after intravenous injection is 34,763.84 ± 18,057.68 ng/mL, compared to only 23.08 ng/mL with oral administration. These indicate that amygdalin is absorbed more rapidly and reaches higher peak plasma concentrations when administered intravenously. Furthermore, the volume of distribution (Vd) for intravenous injection and oral administration is 680.71 ± 257.40 mL/kg and 140,028.28 ± 27,425.92 mL/kg, respectively. This suggests that when amygdalin is administered intravenously, it is primarily distributed in the plasma, whereas after oral administration, it becomes more

concentrated. Additionally, the $t_{1/2}$ of intravenous administration (67.93 ± 24.72 h) is longer than that of oral administration (28.76 ± 7.2 h), and the MRT of intravenous administration (39.42 ± 5.95 min) is shorter than that of oral administration (53.33 ± 10.05 min). These findings indicate that amygdalin remains in the body for a longer duration when administered intravenously (Zhang et al., 2022b).

In addition, the pharmacokinetics of flavonoids in ASA were also investigated. After orally administering 450 mg of ASA skin polyphenols, the plasma was found to contain catechin and naringenin. The T_{max} and C_{max} values for catechin were 1.4 ± 0.2 h and 44.3 ± 15.6 ng/mL, respectively. For naringenin, the T_{max} and C_{max} values were 3.3 ± 0.5 h and 19.3 ± 8.2 ng/mL, respectively. Moreover, the C_{max} of total flavonoids was 82.3 ± 17.6 ng/mL, which exceeded the levels of catechin and naringenin. This suggests the presence of other unidentified flavonoids in ASA.

8 Conclusion and future perspectives

Natural medicinal plants have shown significant benefits in treating a range of diseases, including COVID-19 (Setayesh et al., 2022), respiratory diseases (Hajimonfarednejad et al., 2023), mental health disorders such as anxiety and insomnia (Motti and de Falco, 2021), hyperlipidemia (Hashempur et al., 2018), and common fungal infections (Amini et al., 2023). These plants are characterized by their multi-component and multi-target nature, making them vital in the treatment of various illnesses. ASA, a Chinese herbal medicine with a long history of medicinal use, is rich in phytochemical ingredients, active substances, and nutrients. It serves as both a medicinal drug and nutraceutical, with great potential for broad application.

Here, we comprehensively reviewed the phytochemical composition, pharmacological activities, clinical applications, toxicology, and pharmacokinetics studies of ASA. The present study offers a comprehensive summary of the phytochemical composition of ASA, categorizing it into distinct structural types for the first time. It also provides a systematic overview of the pharmacological activities and mechanism of action of ASA. Moreover, the study includes a novel compilation of various detoxification methods before ASA administration, along with an analysis of the alterations in pharmacokinetic parameters after ASA administration. The current research primarily focuses on assessing the anticancer potential of various extracts of ASA and its main component, amygdalin. To date, researchers have successfully isolated and identified 170 chemical components from different ASA extracts. Extensive *in vivo* and *in vitro* pharmacological studies have revealed that amygdalin and polyphenols in ASA possess a wide range of pharmacological activities. Furthermore, ASA fatty oil and volatile oil also exhibit specific pharmacological activities in the treatment of certain diseases.

However, there are some aspects worth noting and requiring further research. 1) Amygdalin in ASA exhibits excellent anti-cancer activity in various cell lines. However, most studies conducted so far have been *in vitro*, with only a few *in vivo* experiments. Therefore, more preclinical research and translation into clinical studies are needed. 2) It is important to note that ASA is toxic, as amygdalin can be metabolized by β-D-glucosidase, leading to cyanide poisoning. There is limited research on detoxification methods of ASA, and current methods may result in the loss of some active ingredients. Therefore, future research should focus on developing efficient detoxification methods that also preserve the therapeutic properties of ASA. 3) While more than 170 chemical components have been identified in ASA, the pharmacological evaluation has been limited to a few compounds such as amygdalin, its metabolites, total polyphenols, and total volatile oils. Thus, there is an urgent need for in-depth studies on the phytochemistry and pharmacological properties of ASA, particularly the mechanism of action of its bioactive components. 4) ASA and its compounds have shown promising therapeutic effects in the treatment of respiratory diseases in both *in vivo* and *in vitro* studies. Some ASA-containing formula preparations have been included in the 2020 edition of the Chinese Pharmacopoeia. Therefore, further investigation into the pharmacological activities and mechanisms of action of these compounds is warranted. 5) Currently, there is a lack of pharmacokinetic data on different ASA extracts and active compounds. Conducting more pharmacokinetic studies on crude ASA extracts and active compounds is crucial for the rational clinical use and development of new drugs.

ASA, a Chinese herbal medicine, is known for its medicinal and food uses. It is rich in phytochemicals and nutrients, making it clinically valuable and potentially useful for food development. Further research is needed to investigate the pharmacological activities of different components of ASA and understand their underlying mechanisms. This study offers a comprehensive analysis of ASA, providing valuable insights for researchers to improve their understanding of ASA and promote the development of ASA as a clinical drug and healthy food.

Author contributions

ST: Conceptualization, Formal Analysis, Data curation, Investigation, Methodology, Writing–original draft. MW: Investigation, Methodology, Writing–review and editing. YP: Investigation, Methodology, Writing–review and editing. YL: Investigation, Methodology, Writing–review and editing. JL: Investigation, Methodology, Writing–review and editing. QT: Writing–review and editing, Data curation, Formal Analysis. TM: Data curation, Formal Analysis, Writing–review and editing. YS: Writing–review and editing, Investigation, Methodology. CZ: Writing–review and editing, Conceptualization. JG: Conceptualization, Writing–review and editing, Funding acquisition, Project administration. HX: Conceptualization, Funding acquisition, Writing–review and editing, Formal Analysis, Supervision.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was funded by the National Natural Science Foundation of China (No. 81573813), the Science and Technology Department of Sichuan Province of China (No. 2023NSFSC0653), the Sichuan Provincial Administration of Traditional Chinese Medicine of China (Nos. 2021XYCZ007), the Health Commission of Sichuan Province of China (No. 21PJ107), and the

Excellent Talent Program of Chengdu University of Traditional Chinese Medicine of China (No. GJJJ2021003).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 25 January 2024
DOI 10.3389/fphar.2024.1356324

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OPEN ACCESS

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RECEIVED 15 December 2023
ACCEPTED 15 January 2024
PUBLISHED 25 January 2024

CITATION
Yu Y, Liu Y and Meng Z (2024), Role of traditional Chinese medicine in age-related macular degeneration: exploring the gut microbiota's influence.
Front. Pharmacol. 15:1356324.
doi: 10.3389/fphar.2024.1356324

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Role of traditional Chinese medicine in age-related macular degeneration: exploring the gut microbiota's influence

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The pathogenesis of age-related macular degeneration (AMD), a degenerative retinopathy, remains unclear. Administration of anti-vascular endothelial growth factor agents, antioxidants, fundus lasers, photodynamic therapy, and transpupillary warming has proven effective in alleviating symptoms; however, these interventions cannot prevent or reverse AMD. Increasing evidence suggests that AMD risk is linked to changes in the composition, abundance, and diversity of the gut microbiota (GM). Activation of multiple signaling pathways by GM metabolites, including lipopolysaccharides, oxysterols, short-chain fatty acids (SCFAs), and bile acids (BAs), influences retinal physiology. Traditional Chinese medicine (TCM), known for its multi-component and multi-target advantages, can help treat AMD by altering GM composition and regulating the levels of certain substances, such as lipopolysaccharides, reducing oxysterols, and increasing SCFA and BA contents. This review explores the correlation between GM and AMD and interventions for the two to provide new perspectives on treating AMD with TCM.

KEYWORDS

age-related macular degeneration, gut microbiota composition, gut microbiota metabolites, gut-retinal axis, traditional Chinese medicine

1 Introduction

Globally, age-related macular degeneration (AMD) is the third major factor contributing to substantial and irreversible vision impairment following cataracts and glaucoma. Advanced retinal pigment epithelial (RPE) atrophy or choroidal neovascularization can contribute to moderate to severe vision loss in patients with AMD (Ferris et al., 2013). The prevalence of AMD continues to rise worldwide and remarkably increases with age despite significant geographic and lifestyle differences. Currently, the global estimate of AMD prevalence is 196 million, projected to rise to 288 million by 2040 (Xiao et al., 2023), and is anticipated to exceed 498 million by 2050 (Lima-Fontes et al., 2022). The progression of AMD is significantly influenced by factors such as aging, photodamage, obesity (Tian et al., 2023), dyslipidemia, chronic inflammation (Ghosh et al., 2022; de Almeida Torres et al., 2022), hypertension (Zhang et al., 2022), smoking, and internal eye surgery (Ng Yin Ling et al., 2021; Abusharkh et al., 2023; Muraleva and Kolosova, 2023) (Figure 1). In Western medicine, treatments such as anti-vascular endothelial growth factor (anti-VEGF) (Al-Zamil and Yassin, 2017; Williamson et al., 2023), corticosteroids (Kaya et al.,

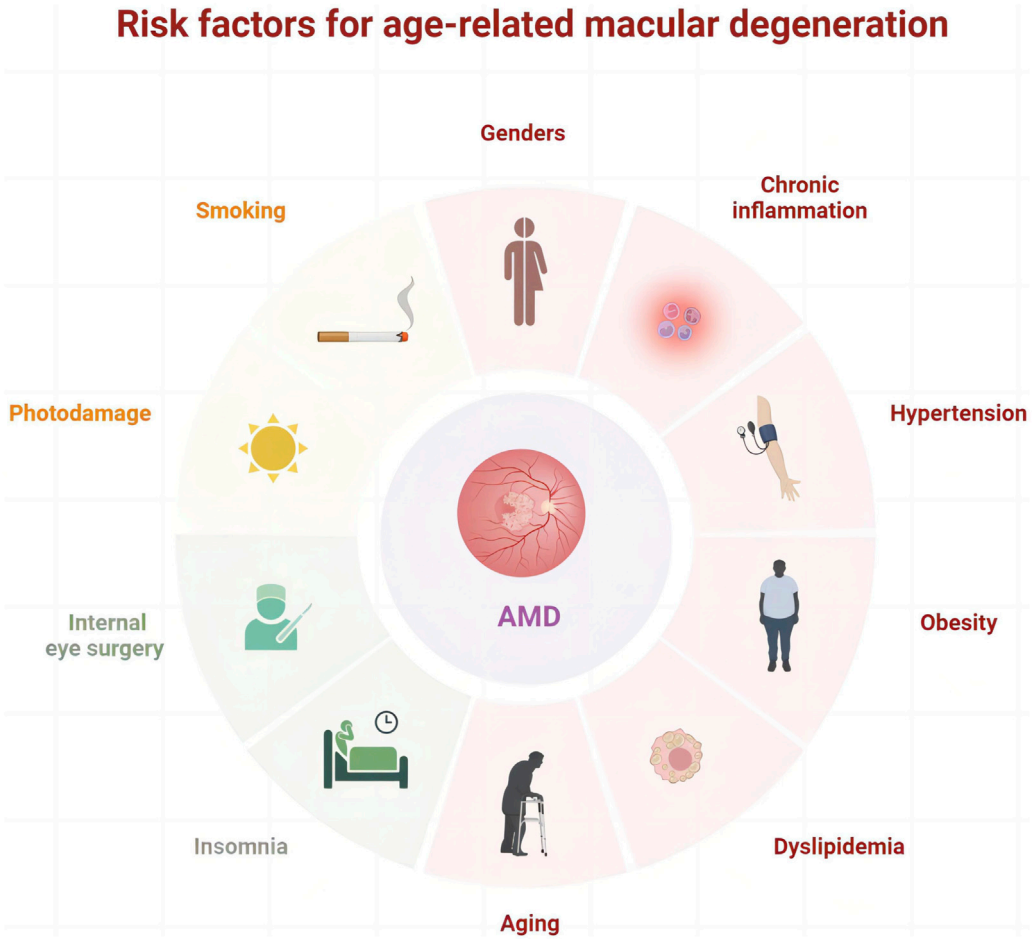

FIGURE 1
Risk factors associated with age-related macular degeneration.

2019), antioxidants (Evans and Lawrenson, 2017; Kushwah et al., 2023), laser therapy (Cohn et al., 2021), and photodynamic therapy (Ando et al., 2023) are utilized. Although these approaches can relieve symptoms, they cannot inhibit the development of AMD, and the treatment options for non-neovascular AMD are particularly limited.

Gut microbiota (GM) coexists with the human body, playing an important role in maintaining the homeostasis of the internal environment and influencing various physiological functions. These functions include metabolism, synthesis of vitamins and other nutrients, antitoxicity, intestinal defense, regulation of immune organ development and maturation, and hematopoiesis (Afzaal et al., 2022; Ahlawat et al., 2021; Saxami et al., 2023). GM colonizes the gastrointestinal tract, influences the intestinal environment, and engages in bi-directional interactions with other organs (Ahlawat et al., 2021). This interaction has given rise to the concept of the "gut-organ axis," which includes gut-brain, gut-liver, gut-lung, gut-cardiac, and gut-ocular axes (Guo et al., 2023a) (Figure 2). GM can convert environmental signals and dietary molecules into metabolite signals, facilitating communication with various organs and tissues of the host via various signaling pathways.

Recently, considerable evidence has accumulated suggesting a correlation between GM and AMD and its associated risks (Liu et al., 2023; Luo and Skondra, 2023; Mao et al., 2023). Regulating the GM is a potential therapeutic strategy for preventing AMD. Moreover, given its multi-component, multi-pathway, multi-target, multi-efficacy nature and low side effects, traditional Chinese medicine (TCM) has been used to manage similar fundus lesions since at least 620 CE (Cao et al., 2022). Recent research (Li et al., 2022a) has revealed that TCM is efficacious in treating AMD by modulating the GM, offering a novel approach to preventing and managing AMD. This review seeks to present the existing association between GM and AMD, evaluate the influence of TCM on GM, and explore the relationship of TCM and GM with AMD, aiming to provide new insights into treating AMD with TCM.

2 GM composition and metabolites influence AMD development

The GM is an intricate environment consisting of numerous microorganisms, such as viruses, bacteria, archaea, fungi, and protozoans, including up to 1,000 bacterial species. Healthy adults have up to 1×10^{14} bacteria in their intestines (Wang and Sun, 2022). Approximately 3.3 million genes exist in the human GM, corresponding to approximately 150 times the number of genes in

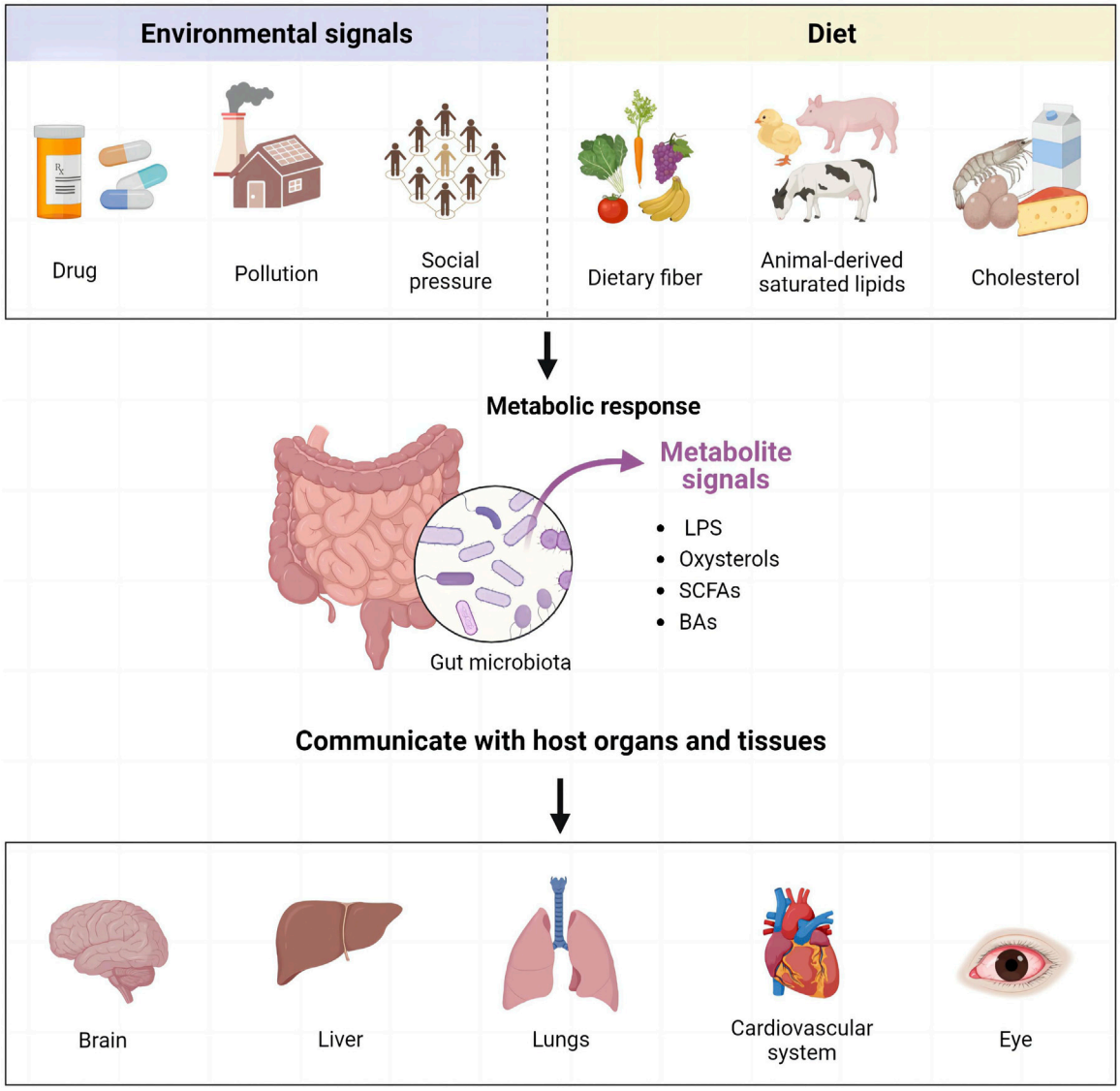

FIGURE 2
Representation of the "gut-organ axis."

the human genome (Rinninella et al., 2018). The GM includes the phyla *Firmicutes*, *Fusobacteria*, *Actinobacteria*, *Bacteroidetes*, *Verrucomicrobia*, and *Proteobacteria*. Among these, *Firmicutes* and *Bacteroidetes* are the most prevalent GM phyla, and the *Firmicutes/Bacteroidetes (F/B)* ratio is an important measure of microbial equilibrium in the whole gut.

The GM components are classified as probiotic, neutrophilic, or pathogenic based on their pathogenicity. The appropriate balance between probiotic and pathogenic bacteria can safeguard the intestinal mucosal barrier, promote digestion and absorption of nutrients, enhance immunity, and impede the invasion of pathogenic microorganisms into the body.

GM can further impact human health via the metabolites they release, such as lipopolysaccharides (LPS) (He et al., 2023a), oxysterols (Lefort and Cani, 2021), short-chain fatty acids (SCFAs), and bile acids (BAs) (Enriquez et al., 2023; Xiang et al., 2023). These metabolites function as signaling molecules that govern metabolic, immune, and inflammatory reactions in individuals with AMD.

2.1 GM composition influences AMD development

The occurrence and progression of AMD are linked to abnormal alterations in the GM composition. Although the relationship is unclear, GM dysregulation may play a role in AMD progression by enhancing the signaling pathway to activate the complement system abnormally (Xue et al., 2023). When the complement system is over-activated or dysregulated, the out-of-control complement system becomes a key link in triggering infection and inflammation. Enteric pathogens can break through the intestinal mucosal barrier, enter the eye via the somatic circulation, and trigger a localized inflammatory reaction, subsequently leading to a rise in the

generation of interleukin (IL)-6, tumor necrosis factor-alpha (TNF-α), and vascular endothelial growth factor (VEGF-A). These cytokines were linked to the development of neovascular AMD (Zinkernagel et al., 2017). Investigation of the intestinal flora of patients with AMD using metagenomic sequencing revealed that the AMD group exhibited a notably diminished presence of *Firmicutes* and an elevated prevalence of *Proteobacteria* and *Bacteroidetes* compared with the control group. Moreover, the AMD group displayed a significant increase in *Escherichia-Shigella* at the genus level compared with that seen in the control group, whereas the proportions of *Blautia* and *Anaerostipes* were lower (Zhang et al., 2023).

Additionally, changes in the GM composition have been linked to oxidative stress, activation of the complement system, inflammation, and altered choroidal hemodynamics. Zinkernagel et al. (2017) conducted sequencing of intestinal macro-genomes in patients with AMD and healthy controls. They observed a higher abundance of *Anaerotruncus*, *Oscillibacter*, *Ruminococcus torques*, and *Eubacterium ventriosum* among individuals with AMD. In mice, these organisms are linked to the activation of proinflammatory chemokines, whereas in humans, they are associated with increased IL-6 and IL-8 levels. In a clinical case-control study (involving 85 patients with advanced AMD and 49 healthy individuals), Lin et al. discovered dysregulation of intestinal ecology that resulted in an increased abundance of *Holdemanella*, *Prevotella*, *Desulfovibrio*, and other bacteria. However, compared to that seen in the control group, *Oscillospira*, *Dorea*, and *Blautia* were reduced in abundance, indicating a correlation between certain bacteria and oxidative stress, inflammation, and heightened intestinal permeability in patients with AMD (Lin et al., 2021). Zysset-Burri et al. (2020) identified *Negativicutes* as a potential biomarker of neovascular AMD and found single nucleotide polymorphisms in the recombinant human complement factor H gene linked to AMD. *Negativicutes* correlated positively with the AMD-associated complement factor H risk allele.

GM-based studies conducted in mouse models have demonstrated a correlation between *Clostridials* and *Firmicutes* with retinal damage, whereas *Bacteroidales* have been associated with AMD repair. A positive correlation was observed between the relative abundance of *Clostridials* and *Firmicutes* unknown species-level genome bins and lesion size, central pit thickness, hemorrhage size, and angiographic leakage. In contrast, a negative correlation was observed between lesion and hemorrhage size and the relative abundance of *Bacteroidales* (Xue et al., 2023).

In addition, alterations in the GM may impact the metabolic risk factors for AMD. *Firmicutes* may promote obesity and chronic inflammation. The elevated abundance of *Firmicute*s following the intake of high-fat foods allows greater energy acquisition, leading to weight gain and obesity (Ley et al., 2006). Feeding mice a high-fat diet (HFD) can contribute to obesity, chronic low-grade inflammation, increased intestinal permeability, and ultimately worsen choroidal neovascularization by increasing the abundance of *Firmicutes* (Xiao et al., 2022). Moreover, *Lactobacillus acidophilus* is negatively associated with obesity. By reducing the *F/B* ratio and controlling the expression of genes associated with lipolysis, lipid synthesis, and energy metabolism, this bacteria effectively counteracts HFD-induced dysbiosis of intestinal ecology and upholds the integrity of the intestinal barrier (Kang et al., 2022).

Additionally, a decrease in the *F/B* ratio signals senescence. Compared to healthy young adults, who have a high proportion of *Firmicutes*, older adults demonstrate a decrease in *Bifidobacteria* and an increase in *Bacteroidetes*, as well as a decline in gut microbial diversity, with a reduction in the relative abundance of core species and an augment in the colonization of opportunistic species (*Betaproteobacteria*) (Biagi et al., 2017). *Bacteroidetes* and *Firmicutes* are the most common bacteria found in the GM of older individuals (Wu et al., 2021). Older individuals have a high percentage of *Bacteroidetes* and an increased relative abundance of *Clostridium* in their GM compared to younger individuals, and there is also a reduced abundance of *Ruminococcus torque* and *Prevotella* in this age group. *Bacteroidetes* constitute the majority (53%) of the primary microbiota in older individuals, in contrast to the reduced level (8%–27%) present in healthy young individuals.

Bifidobacteria are believed to reduce inflammatory responses in older individuals. A randomized controlled double-blind crossover trial (Macfarlane et al., 2013) showed that combined administration of *Bifidobacteria* and the prebiotic inulin led to an increase in *Bifidobacteria* and a reduction in *Proteobacteria* in the host. This regimen also increased butyrate production and significantly decreased TNF-α, a proinflammatory cytokine, in the peripheral blood.

GM dysbiosis is an imbalance between probiotics and pathogenic bacteria. The composition and function of intestinal microorganisms change with host genes, diet, internal environment, and other factors. When the equilibrium of the intestinal microecology is disturbed, the number of foreign microorganisms and pathogenic bacteria in the body increases, inducing intestinal metabolic disorders and immune system dysfunction, leading to morbidity. The body can mobilize more probiotics to restore a balanced and healthy microecological environment to combat these pathogens.

2.2 GM metabolites influence AMD development

2.2.1 Lipopolysaccharides influence AMD development

The outer membrane of Gram-negative bacteria contains LPS, which consist of a hydrophobic domain-endotoxin-lipid A, a hydrophilic O-antigen, and a central polysaccharide (Brodzikowska et al., 2022). Two molecules of 3-hydroxy fatty acids are esterified to the glucosamine backbone of lipid A, and another two are connected via an amide linkage. Lipid A serves as the pathogen-associated molecular pattern of LPS. Increased LPS concentrations act as potent immune activators and toll-like receptor (TLR) 4 (TLR4) ligands, triggering inflammation (Chassaing and Gewirtz, 2014; Scott et al., 2017). Within the eye, various cells, such as perivascular macrophages, microglia, photoreceptors, dendritic cells, and RPE cells, exhibit proinflammatory signals via LPS (Tsioti et al., 2022). LPS-induced retinal explants exhibited a notable neuroinflammatory reaction, marked by deterioration of neurons and increased levels of various cytokines (Ghosh et al., 2018).

Furthermore, emerging evidence strongly suggests that low-grade inflammation caused by LPS plays a role in the progression of AMD. Larsen et al. (2023) observed increased levels of esterified 3-hydroxy fatty acids (indicative of LPS burden) in blood samples, indicating a potential role of LPS exposure in the early stages of AMD pathophysiology. The above suggests that LPS leads to a range of acute and chronic inflammatory responses, generating edema,

exudation, and neovascularization, further affecting the development and progression of AMD (Ibbett et al., 2014).

Researchers have explained the risk of LPS-induced AMD via various mechanisms, such as immune inflammation, peroxidative damage, and RPE senescence. Liu et al. (2019) proposed that LPS promotes the production of inflammatory cytokines and apoptosis of RPE cells by inducing miR-21-3p expression, which can lead to AMD. LPS-induced miR-21-3p overexpression increased the protein and mRNA levels of the inflammatory cytokines monocyte chemoattractant protein-1 (MCP-1) and IL-6 in RPE cells while elevating apoptosis, caspase-3 activity, and levels of cleaved caspase-3 and poly-(ADP-ribose) polymerase proteins, which exacerbate inflammatory responses and apoptosis. LPS not only stimulates the phosphorylation of extracellular signal-regulated kinase (ERK1/2) and nuclear factor kappa-B (NF-κB), but also strongly increases the expression levels of IL-1β, IL-6, IL-12, VEGF, TNF, and TNF-related apoptosis-inducing ligand. Furthermore, exposure to LPS increases the expression of glutathione peroxidase and mitochondrial manganese superoxide dismutase, leading to oxidative stress in the cells. However, Ozal et al. (2018) found that esculetin downregulates the secretion of proinflammatory cytokines and decreases NF-κB activation, ERK1/2 phosphorylation, and VEGF levels, consequently suppressing oxidative stress and inflammation, protecting against LPS-mediated RPE cell death, and preventing AMD development.

LPS production by Gram-negative bacteria in the intestinal flora is involved in HFD-induced obesity. Gram-negative bacteria secrete LPS, which binds to the complex receptor CD4/TLR4 on the surface of immune cells, releasing proinflammatory cytokines and developing inflammatory responses and metabolic disorders, ultimately leading to diseases such as obesity (Davis, 2016). Therefore, therapeutic options that balance LPS levels are of interest in AMD.

2.2.2 Oxysterols influence AMD development

Oxysterols are cholesterol derivatives produced via enzymatic or free radical oxidation associated with oxidative stress, inflammation, and apoptosis (Testa et al., 2018; Zarrouk et al., 2020; de Medina et al., 2022). Several studies have suggested a correlation between retinal degeneration and cholesterol metabolism, specifically the transformation of cholesterol into oxysterols and the degeneration of the retina (Pfeffer et al., 2021; Zhang et al., 2021). Dasari et al. (2011) observed that providing rabbits with a diet high in cholesterol for 12 weeks resulted in heightened β-amyloid (Aβ) levels in the retina, oxidative harm, apoptosis, and elevated accumulation of cholesterol and oxysterols (24-hydroxycholesterol (24-OH), 27-hydroxycholesterol (27-OH), 4β-hydroxycholesterol, 7α-hydroxycholesterol, 25-hydroxycholesterol (25-OH), 7-ketocholesterol (7KC), and 7β-hydroxycholesterol (7β-OH)). A prior study (Dasari et al., 2010) revealed that 27-OH induced toxicity in RPE cells via the stimulation of Aβ1–42 peptide production, elevation of stress markers specific to the endoplasmic reticulum (cysteinyl asparaginase-12 and C/EBP homologous protein), reduction in dysregulation of Ca^{2+} homeostasis, mitochondrial membrane potential, oxidative stress (as indicated by glutathione depletion and reactive oxygen species (ROS) production), and apoptosis. Studies have demonstrated that cholesterol metabolism and its oxidation byproducts, including 7KC, can adversely affect RPE cells, as 7KC triggers oxidative stress and cell death. Various alterations have been noted in RPE cells in the presence of 7KC, such as damage to mtDNA, mitochondrial dysfunction, increased production of ROS/reactive nitrogen species (Gramajo et al., 2010), and activation of caspase-8, -12, and -3 (Neekhra et al., 2007). 7β-OH induces a caspase-3-independent mode of cell death related to lysosomal destabilization, which plays a significant role in the signaling pathways resulting in cell death (Malvitte et al., 2008). These findings collectively demonstrate the association between oxysterols and AMD.

Furthermore, oxysterols cause a rise in inflammatory cytokines in retinal cells. The presence of 25-OH (20–30 μg/mL) in human retinal pigment epithelium (ARPE-19) cells triggered the release of IL-8 (via the MEK/ERK1/2 pathway), VEGF, and MCP-1. The administration of 25-OH upregulated IL-8 transcription and secretion, facilitated by ERK1/2 and phosphoinositol-3 kinase activities and the involvement of transcription factor activator protein 1 and NF-κB. The presence of 7KC and 7β-OH elevated the secretion of IL-1β and IL-6 in ARPE-19 cells (Dugas et al., 2010). Huang et al. (2012) also demonstrated that 7KC (8 μM) enhanced levels of TNF-α, IL-1β, IL-6, IL-8, transforming growth factor beta 1 (TGF-β1), and VEGF by a mechanism that may involve endoplasmic reticulum stress. Larrayoz et al. (2010) found that 7KC induced cytokine production via the kinase signaling pathways ERK, p38MAPK, and AKT-PKCζ-NF-κB via interactions in the plasma membrane. The activation of NF-κB was linked to the MAPK/ERK pathway. These findings illustrate that oxysterols, particularly 25-OH, 7βOH, and 7KC, can trigger oxidative stress, apoptosis, and inflammation in RPE cells, thereby inducing retinal degeneration. Therefore, lowering oxysterol levels may be beneficial in preventing and treating AMD.

2.2.3 Other GM metabolites influence AMD development

Organic fatty acids with a carbon chain length of less than six, known as short-chain fatty acids (SCFAs), are generated by bacteria in the gut, including 41 families, such as Lactobacillaceae, Clostridiaceae, Christensenellaceae Bifidobacteriaceae, Lachnospiraceae, and *Akkermansia muciniphila*. The main components of SCFAs are acetate, propionate, and butyrate. Acetate, constituting 50%–60% of SCFAs, is produced by *Bifidobacteria* and *Lactobacilli*, along with bacteria such as *Clostridium* spp., *Anaerotruncus*, *Lachnospira*, *Akkermansia Muciniphila*, and *Streptococcus* spp. The *Bacteroidetes* and Negativicutes class of *Firmicutes* synthesize propionate via a succinate route, utilizing vitamin B12 to transform succinate into propionate. Other Negativicutes bacteria form propionate from lactate via the succinate, such as *Veillonella* spp. or acrylate pathways, such as *Coprococcus catus*, Lachnospiraceae, and *Megasphaera elsdenii*. *Eubacterium rectale* and *Roseburia* spp., members of the *Clostridium coccoides* group (clostridial cluster XIVa), along with *Faecalibacterium prausnitzii*, a member of the *Clostridium leptum* group (clostridial cluster IV), are capable of producing butyrate. Besides the two prevalent human clusters, clostridial clusters I, III, XV, and XVI can also produce butyrate. SCFAs enter the bloodstream via the intestine, directly influencing the functioning of peripheral tissues and metabolic processes. SCFAs are crucial in regulating gene expression, controlling host metabolic processes (cell proliferation, diversification, and apoptosis),

inflammatory reactions, and energy supply (Abdalkareem Jasim et al., 2022; Fillier et al., 2022; Rekha et al., 2022; Anachad et al., 2023; Ney et al., 2023). The metabolic responses mediated by SCFA receptors also influence obesity, contributing to AMD (Tian et al., 2023). Propionic acid increases peptide YY and glucagon-like peptide 1 (GLP-1) production and secretion, which helps to control obesity. Propionate and butyrate have the potential to combat obesity by stimulating intestinal gluconeogenesis, thereby enhancing metabolic wellbeing. Butyric acid upregulates the lipocalin-mediated AMP-activated protein kinase (AMPK) pathway, promoting mitochondrial biosynthesis and fatty acid oxidation by inhibiting histone deacetylases and augmenting peroxisome proliferator-activated receptor alpha (PPAR-α). SCFAs further ameliorate obesity by decreasing PPAR-α expression, increasing adipose tissue metabolism, and reducing body fat accumulation (Anachad et al., 2023).

Additionally, SCFAs may exert anti-inflammatory effects. SCFAs can traverse the blood–ocular barrier via the circulatory system. Chen et al. (2021) demonstrated the ability of high-dose, intraperitoneally administered SCFAs to reach the eye and impede LPS-induced endophthalmitis. SCFAs hinder TNF-α, IL-6, and the chemokines C-X-C motif chemokine ligand 1 and C-X-C motif chemokine ligand 12 when exposed to inflammatory stimuli *in vitro*, including ligands for TLR and IL-17. SCFAs reduce inflammatory mediators produced by LPS-stimulated retinal astrocytes and enhance the ability of retinal astrocytes to activate T cells, thereby treating AMD. Growing evidence indicates that SCFAs help to improve AMD and its associated risk factors. However, further clinical trials and animal experiments are required to validate these findings.

Bile acids (BAs) are synthesized from cholesterol within hepatocytes. The gallbladder stores BAs, which are then released into the intestine, aiding in the absorption of dietary fats and vitamins. Research indicates that GM significantly influences BA metabolism (Wahlström et al., 2016). Primary BAs—cholic acid and chenodeoxycholic acid—are produced from liver cholesterol, whereas GM generates secondary BAs via hydroxylation, deconjugation, epimerization, or oxidation processes. Both primary and secondary BAs in the liver are typically linked to glycine or taurine. Research has demonstrated that BAs positively impact experimental models studying retinal disorders. Warden et al. (2020) found that tauroursodeoxycholic acid promotes the phagocytosis of outer segments of photoreceptor cells by RPE cells, inhibits human retinal endothelial cell proliferation *in vitro*, and inhibits choroidal neovascularization. The BA taurocholic acid shields HRPEpiC primary retinal epithelial cells from oxidative stress-induced damage to their structure and function while also inhibiting VEGF-induced angiogenesis in choroidal endothelial cells. Moreover, glycine-bound BAs protect RPE cells from oxidative harm and impede VEGF-triggered angiogenesis in choroidal endothelial cells. Glycine-bound BAs offer protection against both atrophic and neovascular AMD.

In summary, LPS and oxysterols can cause oxidative stress, cellular senescence, and various acute and chronic inflammatory responses that further affect the occurrence and development of AMD (Figure 3). SCFAs improve AMD symptoms by regulating cell proliferation, differentiation, and apoptosis, inhibiting inflammatory responses, and providing energy. BAs improve lipid metabolism, protect oxidative stress-induced RPE cells, and inhibit choroidal neoangiogenesis. Consequently, the progression of AMD can be significantly slowed and postponed via the regulation of intestinal flora metabolites, including LPS balance, oxysterol reduction, and an increase in SCFAs and BAs (Guo et al., 2023b).

3 Mechanisms by which the GM plays a role in TCM on AMD

The spleen and stomach are important organs in the digestive system of the human body, with the functions of digesting and absorbing food and maintaining normal metabolism and energy supply. TCM theory suggests that the spleen and stomach have physiological functions analogous to those of the GM. After preliminary digestion by the stomach, filtering, and absorption by the small intestine, food is transformed into subtle substances, that is, qi and blood, by the spleen. These substances are subsequently circulated throughout the body to sustain the regular physiological functions of other organs and tissues. Proper spleen and stomach functioning facilitates sustained energy metabolism, and improves immunity against pathogen invasion (Wu et al., 2020; Ye et al., 2022). Studies have shown that when the "transformation" and "defense" functions of the spleen and stomach are normal, they are similar to the role of the intestinal flora on the body's metabolism and immune function. When the intestinal flora maintains homeostasis, the body's nutrient metabolism functions normally, and the spleen and stomach transform food into nutrients. In contrast, when the intestinal microecology is disturbed, the host is susceptible to pathogens, and the spleen and stomach malfunction, leading to qi and blood deficiencies, as well as poisonous metabolites.

The defining feature of AMD is the degeneration of the macular region. According to the principles of TCM, the spleen and stomach are intricately linked to this disease, and AMD is characterized by weakness in the spleen and stomach. Food preferences, improper rest, stress, anxiety, and old age can lead to malfunction of the spleen and stomach, making it difficult to generate qi and blood. Weak qi and blood impede nutrient supply to the eye tissues, fostering disease. The poisonous metabolites produced accumulate in tissue cells over time and the functional structure of the retina is impaired (Yang and Jia, 2013; Li, 2022).

The metabolism of the intestinal flora is intricately linked to the functions of the spleen and stomach. LPS and oxysterols are biomarkers of poisonous metabolites in TCM. The GM converts LPS and oxysterols from food and is associated with oxidative stress, various acute and chronic inflammatory responses, and apoptosis. Dysbiosis of the microbiota and an increase in pathogenic bacteria can be regarded as "external toxins." In addition, the dysfunction of microbiota-derived metabolites can be seen as "internal toxins," leading to inflammation, oxidative stress, dyslipidemia, obesity, aging, and VEGF production. This is also the pathological basis of AMD, which explains the poisonous metabolites of TCM from a modern microbiological perspective. Furthermore, GM metabolites, such as SCFAs and BAs, can protect the retinal pigment epithelium from oxidative damage, inflammatory stimuli, and apoptosis, which can have a protective effect against retinal damage, such as light-induced retinopathy and AMD. This suggests that TCM can be used

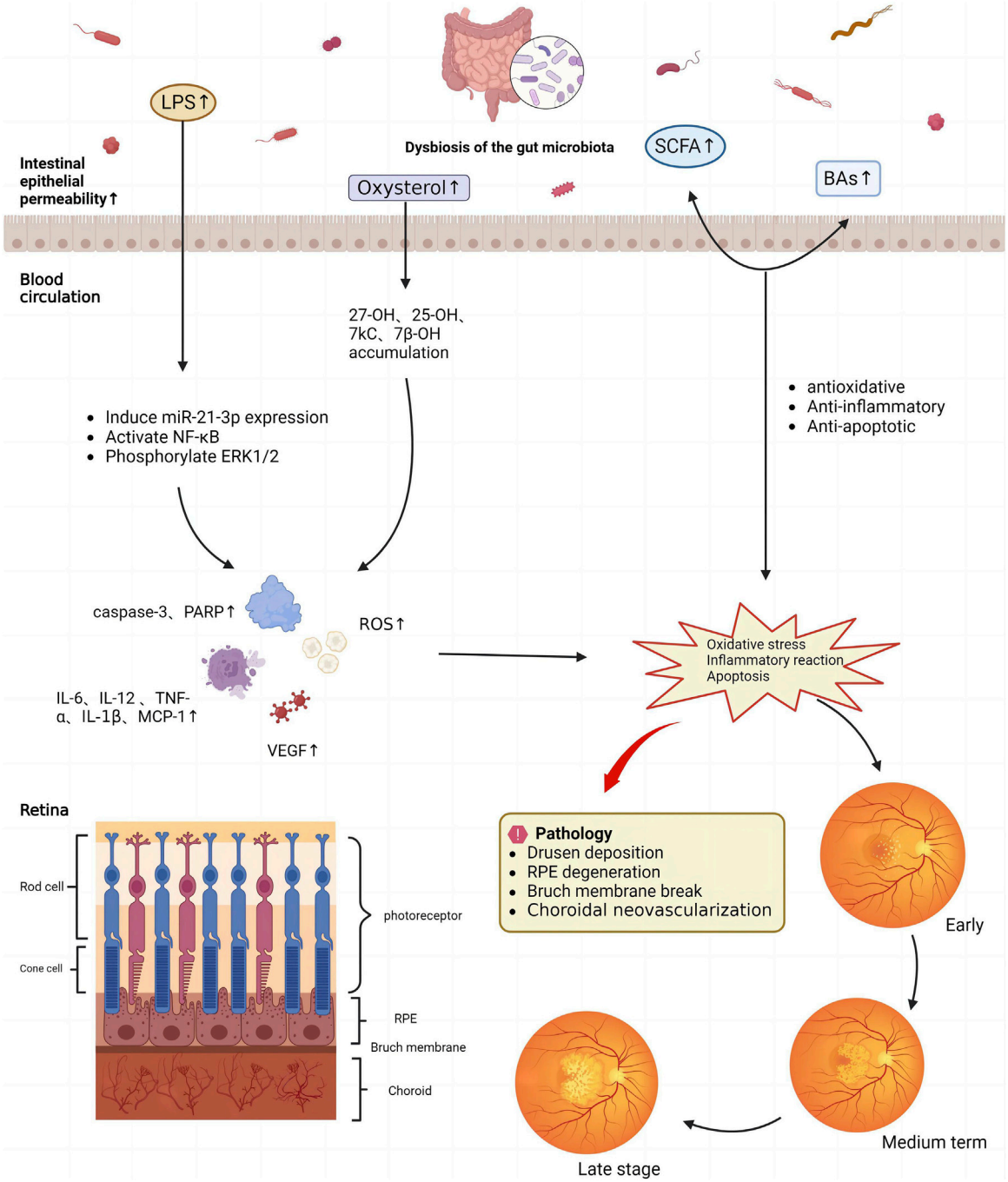

FIGURE 3
Mechanism underlying the effects of gut microbiota metabolites on age-related macular degeneration.

to intervene in AMD by adjusting the intestinal flora, increasing SCFAs and BAs, and ameliorating oxidative damage, apoptosis, inflammation, and altered choroidal hemodynamics.

4 Therapeutic intervention for AMD with TCM

Most botanical drugs are orally ingested and absorbed through the digestive system. The components of these botanical drugs are metabolized or transformed by intestinal microorganisms, producing new biologically active molecules that facilitate drug absorption into the bloodstream. The composition and structure of the GM are affected by TCM metabolites, which in turn affect the function of diseased organs and tissues via the action of the intestinal flora. Research has indicated that a single botanical drug's components and metabolites may improve AMD symptoms and risk factors by modulating intestinal microorganisms. Below, we review therapeutic interventions using botanical drugs based on categorizing natural chemical components, single botanical drugs, and botanical drug decoctions.

4.1 Natural chemical components

4.1.1 Flavonoids

Flavonoids are compounds that are abundantly found in plants (Table 1). They have different phenolic structures consisting of two benzene rings with phenolic hydroxyl groups, primarily in glycosidic and free forms. These compounds exhibit antioxidative, anti-inflammatory (Guo et al., 2023c), antimicrobial, anti-apoptotic, neovascularization inhibiting, and central nervous and cardiovascular system protective effects (Lan et al., 2023a). Typically, flavonoid glycosides contain glucose bonds. Glucosides with water-soluble sugar components have low pharmacological activity and are difficult to absorb in the intestine, leading to a lack of bioavailability. Nevertheless, enzymatic degradation, hydrolysis, reduction, dehydroxylation, and other reactions involving intestinal microbiota can convert most flavonoids into simple phenolic acids, leading to their absorption and subsequent bioavailability enhancement.

Flavonoids can be divided into two primary groups depending on how they control the GM. Some flavonoids can be metabolized by the GM and act as substrates for various catalytic reactions involving various enzyme systems produced by the GM. Consequently, the bacteria responsible for these reactions tend to propagate (Feng et al., 2018). In contrast, some flavonoids influence the cell membranes of specific bacteria (such as *Staphylococcus aureus* and *Escherichia coli*), either by directly disrupting the lipid bilayer of the cell membrane or by modifying the permeability of the cell membrane, ultimately hindering the proliferation of these bacteria (Xie et al., 2015). Flavonoids modulate the relative abundance of probiotic and harmful bacteria. Pan et al. (2023) found that certain flavonoids (particularly hesperidin-7-O-glucoside, prunin, and isoquercitrin) possess bactericidal, antiviral, and anti-inflammatory properties.

Moreover, flavonoids also modulate the abundance and number of intestinal microbiota, leading to a considerable reduction in total SCFA production. By using *in vitro* simulated fermentation technology to study the fecal microbial composition of healthy individuals, it has been demonstrated that hesperidin-7-O-glucoside, prunin, and isoquercetin increase *Bifidobacterium* and result in a decrease in the relative abundance of *Bilophila, Lachnoclostridium*, promoting intestinal mucosal absorption and digestion, biobarrier function (Odamaki et al., 2016), and immunomodulation (Milani et al., 2017). Flavonoids reduce the effects of harmful bacteria. Hesperidin-7-O-glucoside, naringenin, and lisin all significantly reduce the levels of *Haemophilus* and the relative abundance of harmful bacteria, such as *Clostridium pullulans* and *Bacteroides*, suggesting that flavonoids can regulate signaling pathways, such as TLR4/NF-κB, NOD-like receptor family pyrin domain containing 3, and MAPK, along with the expression of the genes encoding matrix metalloproteinase-9, IL-1β, IL-6, IL-8, soluble intercellular adhesion molecule-1, MCP-1, and other

TABLE 1 Natural chemical components and gut microbiota.

Natural chemical components	Physiological function related to gut microbiota	Gut microbiota	References
Flavonoids	(1) Regulate the number and abundance of gut microbiota	Increased: Bifidobacterium Decreased: Bilophila, Lachnoclostridium, and Haemophilus, Bacteroides and Clostridium pullulans	Pan et al. (2023), Odamaki et al. (2016), Sun et al. (2023), Carrera-Quintanar et al. (2018)
	(2) Regulate inflammatory factor gene expression and inhibit inflammatory responses		
	(3) Reduce ROS production, alter AKT/GSK-3β and NrF2/ARE signaling pathways, and regulate antioxidant gene expression		
	(4) Inhibit adipogenesis to regulate obesity		
Berberine	(1) Inhibits the LPS/NF-κB signaling pathway to improve the metabolic and inflammatory state of organisms	Increased: Ruminococcus, Desulfovibrio vulnificus, Lactobacillus, and Akkermansia muciniphila Decreased: *Aspergillus*, *Trematode spirochetes*, and the ratio of *Firmicutes* to *Bacteroidetes*	Zhang et al. (2012), Li et al. (2018), Liao et al. (2020), Shen et al. (2021), Yang et al. (2022)
	(2) Activates AMPK pathway to combat oxidative stress		
	(3) Promotes the production of SCFAs		
	(4) Corrects the imbalance of the GM ratio		
Resveratrol	(1) Promotes the metabolism of SCFAs and increases the level of butyric acid	Increased: *Bacillus anthropophilus, Lactobacillus, Bifidobacterium*, and the ratio of *Firmicutes* to *Bacteroidetes* Decreased: *Firmicutes* and *Enterococcus faecalis*	Qiao et al. (2014), Zhang et al. (2015), Chen et al. (2016), Maugeri et al. (2018), Alrafas et al. (2019), Dull et al. (2019), Li et al. (2022b)
	(2) Anti-inflammatory and anti-metabolic disorder effects through various pathways, such as NF-κB, arachidonic acid, AP-1, or AHR		
	(3) Reduces the extent of oxidative stress damage in RPE cells		
	(4) Activates SIRT1 and downregulates HIF-1α expression and VEGF secretion in RPE cells		
	(5) Improves hyperlipidemia		

inflammatory factors, suppressing the inflammatory response (Sun et al., 2023). The presence of flavonols, a type of flavonoid, facilitates the proliferation of beneficial bacteria, such as *Lactobacillus* and *Bifidobacterium*, while diminishing the presence of *Clostridium* spp. Taken together, hesperidin-7-O-glucoside, lisinin, and isoquercitrin, which are flavonoid monoglycosides containing only one glucose group in their structures, can lead to an increase in beneficial intestinal bacteria and a decline in harmful intestinal bacteria. This action helps ameliorate intestinal dysbiosis and diminish the impact of other factors on the intestinal flora.

Natural flavonoid products also have antioxidant properties that reduce oxidative stress and inhibit the activation of various signaling pathways to ameliorate inflammatory diseases. By improving the dysregulation of intestinal dynamic homeostasis *in vivo*, ROS production is reduced, the AKT/glycogen synthase kinase-3-beta (GSK-3β) and Nuclear factor erythroid-derived-2-like 2 (Nrf2)/antioxidant response element (ARE) signaling pathways are directly altered, and the expression of antioxidant genes, such as *NAD(P)H quinone oxidoreductase 1, heme oxygenase-1, glutamate-cysteine ligase modifier, glutathione*, and *superoxide dismutase* (*SOD*), are regulated (Sun et al., 2023).

Increasingly, flavonoid metabolites, which are biologically active compounds, play a role in influencing the gut microbiome and reducing signs of obesity by hindering adipogenesis. Flavonoids, with their anti-inflammatory and antioxidant properties, impede the production of ROS and the cyclooxygenase (COX)-2 and NF-κB signaling pathways, thereby influencing obesity development and inflammatory responses (Carrera-Quintanar et al., 2018). Detaram et al. (2021) suggested that intake of higher levels of certain flavonoids may improve vision outcomes in patients with AMD. These findings were consistent with those of the Blue Mountains Eye Study, which revealed a connection between the overall consumption of flavonoids and reduced probability of AMD.

These findings suggest that flavonoids help control the development of AMD by modulating the gut microbial composition to slow inflammation, oxidative stress, dyslipidemia, and obesity.

4.1.2 Berberine

Berberine (BBR) or BBR hydrochloride is an isoquinoline alkaloid derived from *Coptis chinensis* Franch. [Ranunculaceae; coptidis rhizoma]. It exhibits pharmacological properties, including anti-inflammatory, cardioprotective, and hypoglycemic effects (Miao and Cui, 2022; Saha et al., 2023). Intestinal flora enhances the bioavailability of orally administered BBR. The intestinal microbiota transforms BBR into absorbable dihydroberberine, which is five times more absorbable than BBR. Dihydroberberine is not stable in solution and can be reoxidized to BBR in intestinal tissues (Cheng et al., 2021).

BBR may inhibit inflammatory responses, oxidative stress, and apoptosis by altering the growth of various intestinal bacteria. It can reduce pathogenic bacteria and improve the metabolic and inflammatory state of organisms by inhibiting the LPS/NF-κB signaling pathway to promote the production of SCFAs, improving the relative abundance of GM, augmenting the relative abundance of SCFA-producing flora, and diminishing endotoxins (Shen et al., 2021). Yang et al. (2022) found that BBR modulates the GM and inhibits the initiation of the TLR4 signaling pathway, as well as the release of the nucleotide-binding oligomerization domain-like receptor protein 3 inflammasome and its cytokines. Li et al. (2018) also found that BBR inhibits oxidative damage caused by hydrogen peroxide (H_2O_2) in the human D407 RPE cell line. Pretreatment of D407 cells with BBR effectively inhibited apoptosis caused by H_2O_2 by rectifying irregular alterations in the nuclear structure, impeding the reduction in mitochondrial membrane potential, decreasing lactate dehydrogenase release, and impeding the activity of caspase 3/7 induced by H_2O_2. Western blot analysis revealed that BBR induced the phosphorylation and activation of AMPK in D407 cells in a manner that is dependent on the amount of time and dosage administered. In contrast, the action of BBR was inhibited by treating cells with compound C or reducing AMPK levels with specific siRNAs. Primary cultured human RPE cells yielded comparable outcomes. The collective findings indicate that BBR can protect RPE cells against oxidative stress via AMPK pathway activation, thereby addressing and slowing the progression of AMD.

Moreover, BBR can correct dyslipidemia by regulating the GM to improve AMD. BBR can boost the presence of *Ruminococcus, Desulfovibrio vulnificus, Lactobacillus*, and *A. muciniphila*, decline pathogenic bacteria, such as *Aspergillus* and *Trematode spirochetes*, promote the production of intestinal microbial metabolites such as SCFAs, and restore the breakdown and assimilation of glycolysis, amino acid metabolism, and carbohydrates (Liao et al., 2020), rectifying the imbalance in the rat intestinal flora ratio caused by a HFD, reducing the proportion of *Firmicutes* to *Bacteroidetes*, safeguarding the intestinal mucosal barrier, improving intestinal permeability, and preventing hyperlipidemia (Zhang et al., 2012).

4.1.3 Resveratrol

Resveratrol is a polyphenolic compound with a low molecular weight naturally found in grapes, berries, and mulberries. It is recognized as a natural anti-inflammatory agent with a broad spectrum of medicinal effects, including anti-inflammatory, antimicrobial, antioxidant, immune-modulatory, anti-cardiovascular, and hepatocyte-protective activities (Boldyreva et al., 2023; Ghavidel et al., 2023; Santos et al., 2023). However, its notably low bioavailability poses a challenge in explaining the material basis of its *in vivo* pharmacodynamic effects. Several recent studies have shown that the exertion of its pharmacodynamic effects is associated with intestinal flora. Regarding intestinal flora regulation, resveratrol reduces the number of *Bacteroidetes*, promotes the metabolism of SCFAs, increases the level of butyric acid, and restores the intestinal flora to a homeostatic level (Alrafas et al., 2019).

Resveratrol has the potential to regulate the abundance of GM, thereby exerting antioxidant, anti-inflammatory, and anti-VEGF properties. Dull et al. (2019) showed that resveratrol can regulate the composition of gut bacteria, such as raising the amount of *Bifidobacterium*, *Bacillus anthropophilus*, and *Lactobacillus* and decreasing the amount of *Enterococcus faecalis* and *Firmicutes*. It may also exert anti-metabolic and anti-inflammatory effects via various pathways, such as NF-κB, arachidonic acid, activator protein-1, or aryl hydrocarbon receptor (AHR), diminishing proinflammatory elements and moderate granulocyte infiltration, functioning as an anti-proliferative and anti-inflammatory agent (Li et al., 2022b). Simultaneously, resveratrol can diminish the amount

of oxidative stress damage in RPE cells by scavenging excess ROS, enhancing antioxidant enzyme activity, improving mitochondrial function, agonizing PPAR-α and peroxisome proliferator-activated receptor-δ, and up-regulating the mRNA expression of antioxidant genes *BCL2* and *HO1*, thereby inhibiting the development of AMD. Maugeri et al. (2018) found that resveratrol restored the methylation levels of long-interspersed nuclear element-1 and reduced the oxidative stress and inflammatory response in ARPE-19 cells by regulating the functions of Sirtuin 1 and DNA methyltransferase. Resveratrol can also inhibit the phosphorylation and activation of vascular endothelial growth factor receptor 2 in endothelial cells and exert anti-AMD effects by activating SIRT1, as well as down-regulating the expression level of hypoxia-inducible factor-1α (HIF-1α) and VEGF secretion in RPE cells (Zhang et al., 2015).

Resveratrol can ameliorate intestinal flora dysbiosis caused by a HFD in mice, increase the amount of *Bacteroidetes* and *Firmicutes*, stimulate the propagation of *Lactobacillus* and *Bifidobacterium,* restore the imbalance in lipid metabolism, and significantly boost the expression of *FIAF* to reduce the number of blood lipids (Qiao et al., 2014). Chen et al. (2016) observed the impact of resveratrol on the GM and trimethylamine-N-oxide (TMAO) levels in apolipoprotein E knockout and choline-fed C57BL/6J mice. They found that plasma trimethylamine and TMAO levels decreased in the choline-fed resveratrol-treated group. Additionally, resveratrol intervention caused a decrease in *Firmicutes* and an increase in *Bacteroidetes* in the intestinal tracts of these mice, which indicated that resveratrol could reduce the abundance of detrimental bacteria, as well as effectively increase the relative abundance of advantageous microorganisms and the amount of intestinal pathogenic metabolites. These actions significantly enhance the hyperlipidemic state, restore the normal level of blood lipids, and could aid in preventing and controlling AMD by regulating its risk factors.

4.2 Single botanical drugs

4.2.1 *Poria cocos* (Schw.) Wolf [*Polyporaceae*; Poria]

Poria is a common botanical drug (Table 2). Its main chemical components are polysaccharides, triterpenoids, sterols, and trace elements, such as calcium, iron, zinc, selenium, potassium, sodium, and phosphorus. *Poria* polysaccharides and triterpenoids have major pharmacological activities. Contemporary pharmacological research has demonstrated that *Poria* coccinea has antioxidant, immunomodulatory, anti-inflammatory, and intestinal microbiota-regulatory effects (Xu et al., 2023). *Poria* increases the beneficial bacteria *Lactobacillus* and *Bifidobacterium* and decreases *Vibrio desulfuricans*, inflammation-associated bacteria *Mucor* spp., and *Staphylococcus* to attenuate oxidative stress, inflammatory responses, and apoptosis (Ye et al., 2023). Huai et al. (2023) evaluated the unique ability of *Poria* to promote water metabolism in the body for the treatment of diabetic macular edema. They ranked it high among the therapeutic drugs for proliferative diabetic retinopathy.

Further, a computerized search of clinical cases related to the treatment of macular edema in the Chinese full-text journal database and Wanfang database ranked *Poria* in first place, with a recurrence rate of 60 times. Kim et al. (2018) reported improved visual acuity and disappearance of retinal and optic disk hemorrhages in patients with non-proliferative glycoconjugate network after administration of *Poria*-containing Modified-Goshajinkigan (Niucheshenqiwan in Chinese). Lan et al. (2023b) found that *Poria* selectively modulated the abundance of *Odoribacter*, *Muribaculum*, *Oscillibacter*, *E. coli*, and *Turicibacter*, leading to a reduction of the inflammation levels caused by dextran sodium sulfate (DSS) in mice. *Poria* aqueous extract improves metabolic homeostasis and inflammation by restoring intestinal homeostasis, controlling the relationship between GM and host metabolites, modulating hypothalamic neurotransmitters, decreasing proinflammatory cytokines, and inhibiting the expression of TNF-α/NF-κB signaling pathway proteins. Additionally, *Poria* can improve AMD risk factors, such as hyperlipidemia and obesity, by regulating intestinal bacterial communities.

Moreover, water-insoluble *Poria* polysaccharides could improve intestinal mucosal integrity by regulating the intestinal bacterial community and increasing the butyrate-producing biomass of *Clostridium perfringens* (Sun et al., 2020a), thereby promoting glucose-stimulated lipid metabolism, alleviating hyperlipidemia and reducing inflammation and steatosis. Zhu et al. (2022) investigated the effects of *Poria* oligosaccharides on glucose and lipid metabolism disorders in HFD-induced obese mice. Compared to controls, *Poria* oligosaccharides improved insulin resistance and glucose intolerance and reduced insulin and blood glucose levels in HFD-fed mice. Additionally, *Poria* oligosaccharides treatment inhibited the mRNA expression of fatty acid synthesis regulators in epididymal fat and the expression of proinflammatory factors, such as TNF-α, IL-1β, IL-6, and MCP-1. Moreover, *Poria* oligosaccharides partially rectified the imbalance of GM in HFD-fed mice, accompanied by decreased various gut metabolites of significant importance in impairing the intestinal barrier, such as BAs, SCFAs, and tryptophan.

4.2.2 *Panax ginseng* C.A.Mey. [*Araliaceae*; ginseng radix]

Ginseng, derived from the desiccated root and rhizome of *Panax ginseng* C. A. Meyer, a member of the Wujiaceae family, possesses several health benefits. It comprises a diverse range of active components, including ginsenosides, polysaccharides, and volatile oils (Su et al., 2023; Zhou et al., 2023), significantly affecting immunity, oxidative stress, inflammation, apoptosis, and coagulation (Zhou et al., 2023). Lee et al. (2015) utilized the ginsenoside targeted transport pathway to improve nutrient exchange in Bruch's membrane of human donors, delaying its aging and preventing the onset and progression of AMD. Betts et al. (2012) reported that ginsenoside Rb1 from *ginseng* root extract increases the number of cultured adult ARPE-19 cells while decreasing the release of the angiogenic factor VEGF produced by ARPE-19 cells, suggesting that Rb1 plays a role in the prevention of angiogenic ophthalmopathies such as AMD. Cho et al. (2001) demonstrated that ginsenosides inhibited TNF-α production in mouse or human macrophages stimulated by LPS, suggesting that *ginseng* has a preventive effect on AMD.

Additionally, *ginseng* ameliorates AMD risk factors by modulating gut microbes. The ginsenoside Rg5 significantly lowered the *F/B* ratio and markedly attenuated inflammatory responses caused by metabolic

TABLE 2 Single botanical drugs and gut microbiota.

Single botanical drugs	Active ingredients	Physiological function related to gut microbiota	Gut microbiota	References
Poria	Poria polysaccharides	Regulates intestinal bacterial communities	Increases the butyrate-producing biomass of *Clostridium perfringens*	Sun et al. (2020a)
		Promotes lipid metabolism		
		Reduces inflammatory response		
	Poria oligosaccharides	Restores intestinal homeostasis, modulates intestinal flora–host metabolite interactions to improve body metabolic homeostasis and inflammation	Modulates the abundance of Odoribacter, Muribaculum, Oscillibacter, *Escherichia coli*, and Turicibacter	Lan et al. (2023b)
		Restores imbalances in the gut microbiota	Increased: Lactobacillus and Clostridium difficile Decreased: Helicobacter, Lachnospiraceae family, Alistipes, Ruminococcus, Faecalibacterium, Desulfovibrio, and Helicobacter mucosus	Zhu et al. (2022)
		Promotes the production of BAs, SCFAs, and tryptophan metabolites		
		Improves disorders of glucose–lipid metabolism to combat obesity		
Ginseng	Ginsenoside	Increases SCFA levels	Increased: Proteobacteria and Bacteroidetes Decreased: *Verrucomicrobia* and the ratio of *F*/B	Zhuang et al. (2021)
		Intervenes in obesity by regulating the composition of the intestinal flora, improving glycolipid metabolism and inflammatory responses		
	Ginseng pectin	Regulates intestinal flora	Increased: Akkermansia, Bifidobacterium, Bacteroides, and Prevotella	Ren et al. (2023)
		Increases levels of acetic, propionic, and butyric acids and of valine		
		Activation of the AMPK pathway, improving dyslipidemia and obesity		
	White ginseng (WEWG)	Improves gut dysbiosis to fight obesity	Increased: Lactobacillus and Parabacteroides Decreased: *F*/B ratio, *Ruminiclostridium*	Zhou et al. (2020)
	Red ginseng (WERG)	Improves intestinal flora dysbiosis to fight obesity	Increased: Bifidobacterium, Lactobacillus, Akkermansia, and Gastrococcus Decreased: Desulfovibrio and *Escherichia coli*	Guo et al. (2015), Zhou et al. (2020), Peng et al. (2021), Lee et al. (2022), Eun et al. (2023)
		Increases probiotic content for anti-aging effects		
		Improves the structure and composition of the intestinal microbiota to reduce the inflammatory response		
Astragalus	Astragalus polysaccharide (APS)	Improves immunity by improving gut microbiota and increasing body weight and immune organ indices	Increased: Parasutterella, Parabacteroides, Clostridium perfringens XIVb, Butyricicoccus, Dorea, Lactobacillus, Bifidobacterium, Rousselaeria, and Desulfovibrio Decreased: Pseudoflavonifractor, Parapovella, Tyzzerella, and Lachnoclostridium	Li et al. (2023), Wei et al. (2023), Zhao et al. (2023)
		Improvement of gut microbiota, lowering of IL-1β, IL-6, and endotoxin levels, and inhibition of the TLR4/NF-κB pathway to reduce inflammatory responses		
		Modulates gut microbial abundance, increases SCFA production, and promotes anti-inflammatory bacteria		
	Fermented Astragalus	Intervenes in the inflammatory state by regulating the balance of Th1/Th2/Th17/Treg-related cytokines	Alters the structure of the intestinal microbiota and enriches *Akkermansia* and *Aristichthys* species	Li et al. (2022c)
Atractylodes macrocephala	Atractylodes macrocephala volatile oil	Reduces inflammatory stimuli	Increased: Enterorhabdus, Parvibacter, and Akkermansia Decreased: Turicibacter, Parasutterella, and Erysipelatoclostridium	Cheng et al. (2023)
	Atractylodes polysaccharide	Increases the abundance and diversity of gut microbiota	Increased: Relative abundance of potentially beneficial bacteria, such as *Bifidobacterium bifidum*	Kai et al. (2022)

(Continued on following page)

TABLE 2 (*Continued*) Single botanical drugs and gut microbiota.

Single botanical drugs	Active ingredients	Physiological function related to gut microbiota	Gut microbiota	References
		Reduces proinflammatory cytokine overexpression to achieve anti-inflammatory effects	Decreased: Proportion of harmful bacteria, such as *Clostridium strictum* 1 and *Shigella coli*	
		Decreases systemic LPS	Increased: Lactobacillus and Rhodococcus Decreased: *Firmicutes*, *Clostridium difficile*, and *Escherichia coli*	He et al. (2023b)
		Increases levels of tryptophan metabolites		
		Regulates the composition of intestinal flora		
		Regulates disorders of glucose and lipid metabolism		

endotoxemia. Moreover, Rg5 markedly decreased the abundance of *Firmicutes* and *Verrucomicrobia*. It augmented the abundance of *Proteobacteria* and *Bacteroidetes* in a mouse model of diabetes at the phylum level, ameliorating diabetes-associated dysbiosis and metabolic disorders of the intestinal microbiota. *Ginseng* saponins inhibit obesity and its complications by improving the metabolism of endogenous substances in the gut, reducing inflammation, and altering the composition of the GM. *Ginseng* pectin, a mixed pectin containing the structural domains of rhamnogalacturonan-I and homogalacturonan, improves the intestinal flora by increasing *Bifidobacterium, Akkermansia, Prevotella*, and *Bacteroides,* which increases the levels of propionic, acetic, and butyric acids and valine. These, in turn, participate in cinnamonoside-, 10-hydroxy-8-fen-2-fenuglone glucoside-, leucovorin-, 24-propylcholesterol-3-ol-, and other lipid regulation-related pathways in serum metabolite alterations, and activation of the AMP-activated protein kinase pathway, to ameliorate lipid disorders in obese rats (Zhuang et al., 2021; Ren et al., 2023).

Processed *ginseng* is categorized into white (dried *ginseng*) (WEWG) and red (steamed *ginseng*) (WERG). In HFD-induced obese mice, aqueous extracts of WEWG and WERG demonstrated the ability to alleviate intestinal dysbiosis and exhibited anti-obesity effects, particularly the aqueous extract of WEWG. WEWG markedly decreased the *F/B* ratio and the amount of *Ruminiclostridium* while augmenting the amount of *Parabacteroides* and *Lactobacillus*. WERG decreased the amount of *Desulfovibrio* and augmented the amounts of S24-7 and *Gastrococcus* (Zhou et al., 2020). A separate *in vitro* experiment demonstrated that WERG stimulated the growth of the probiotics *Lactobacillus* and *Bifidobacterium bifidum* and curbed the overgrowth of *E. coli*, thereby enhancing the microbiological structure of the intestinal tract and reducing *in vivo* inflammation (Guo et al., 2015). Peng et al. (2021) revealed that WERG treatment caused a more pronounced enhancement of probiotic levels, such as those of *B. bifidum* and *Akkermansia*, than WEWG, indicating the increased anti-aging efficacy of WERG.

Additionally, research on elderly Korean women showed that the consumption of fermented red *ginseng* (RG) modified 20 distinct bacterial species, thereby enhancing overall wellbeing via its impact on defecation, biochemical parameters, and metabolism (Lee et al., 2022). Eun et al. (2023) found that the addition of WERG and its subsequent microbial conversion via the fermentation of RG resulted in alterations in the composition of the GM, exhibiting characteristics of both RG and fermented RG, which were linked to an improved obesity phenotype and glucose balance. These modifications were also linked to the enhanced integrity of the gut barrier, thus safeguarding against inflammation caused by heart failure at both local and systemic levels.

4.2.3 *Astragalus mongholicus* Bunge [*Fabaceae*; astragali radix praeparata cummelle]

Astragalus is the dried root of *Astragalus membranaceus*. Its main components are polysaccharides, saponins, flavonoids, amino acids, and other compounds. Additionally, it contains alkaloids, glucuronic acid, iodine, silicon, zinc, and other trace elements. *Astragalus* polysaccharide (APS) (Zheng et al., 2020) and astragaloside (Yang et al., 2022) are the main active components of *Astragalus*. It has antioxidant, anti-inflammatory, anti-aging, anti-apoptosis, immunomodulatory, intestinal mucosa protective, and intestinal flora regulatory effects and modulates signaling pathways (Zheng et al., 2020; Liang et al., 2023). Li et al. (2002) studied a mouse model of retinal photoreceptor degeneration induced by exposure to bright light and the DNA alkylating agent methyl methanesulfonate. They found that astragaloside A reduced the expression of genes involved in necroptosis and inflammatory responses, inhibited microglia activation, and attenuated retinal oxidative stress and inflammation, thereby protecting photoreceptor cells. Similarly, Sun et al. (2020b) discovered that ultrasmall astragaloside-loaded lipid nanocapsule eye drops improved retinal morphology and function in sodium iodate (NaIO3)- induced dry AMD mice and protected retinal function from oxidative stress and apoptosis.

Astragalus can exert anti-inflammatory, antioxidant, and immunomodulatory effects by regulating GM abundance. APS altered its composition, enhancing the variety of GM, leading to the reduction in the relative prevalence of *Pseudoflavonifractor* and *Parapovella* and the increase of *Parabacteroides, Parasutterella, Butyricicoccus, Clostridium perfringens* XIVb, and *Dorea*. APS also increased the immune organ indexes and body weights, reduced IL-6, IL-1β, and endotoxin levels, inhibited the TLR4/NF-κB pathway, and improved immune disorders in rats via modulating their GM, particularly certain bacteria implicated in immune and inflammatory responses and production of SCFAs (Zhao et al., 2023). Wei et al. (2023) found that APS regulated GM abundance, enhanced the abundance of anti-inflammatory bacteria, generation of SCFAs, reversed the aberrant expression of NF-κB,

NrF2, and their downstream factors in the brain-gut axis, and exerted anti-inflammatory and antioxidant effects on neuronal cells. Li et al. (2023) found that APS significantly activated intestinal TLR4 and MAPK pathways, reversing intestinal flora disorders in immunocompromised mice. APS resulted in an increase in bacterial populations at the genus level, including *Lactobacillus, Bifidobacterium, Rousselaeria*, and *Desulfovibrio*, and a decrease in the abundances of *Tyzzerella* and *Lachnoclostridium*. However, APS did not enhance the immune system of immunocompromised mice with GM depletion. Li et al. (2022c) discovered that supplementing mice with fermented *Astragalus* (FA) controlled abnormal activation of the intestinal immune barrier, resulting in decreased levels of MPO and immunoglobulin E and increased levels of immunoglobulin A. The levels of TNF-α, IL-1β, IL-6, and IL-17 were downregulated in the intestines, and those of TGF-β and IL-10, which are anti-inflammatory, were increased, indicating that FA may affect the inflammatory process by controlling Th1/Th2/Th17/Treg-related cytokines.

Furthermore, fatty acid supplementation modified the composition of the GM and enhanced the presence of *Akkermansia* and *Aristochthys* spp., both of which were associated with the generation of SCFAs. Fermented *Astragalus*-induced microorganisms and their metabolites play a role in preserving the intestinal mucosal barrier's integrity by affecting intestinal mucosal immunity. Mice supplemented with FA exhibited greater expression of intestinal tight junction protein and mucous-secreting protein ZO-1 and occludin, as well as MUC2 genes, than those supplemented with unfermented *Astragalus*. Repair of the intestinal mucosal barrier by FA has been validated by regulating apoptosis in intestinal epithelial cells Li et al. (2022c).

4.2.4 *Atractylodes macrocephala* Koidz. [*Asteraceae*; atractylodis macrocephalae rhizoma]

Atractylodes macrocephala, commonly referred to as "Yuzhu," "Xizhu," or "Wuzhu," functions as the desiccated rhizome of *A. macrocephala* within the Asteraceae family. *Atractylodes macrocephala* contains sesquiterpenes, lactones, polysaccharides, flavonoids, and other chemical components that have antioxidant, anti-apoptosis, and anti-inflammatory effects, improve gastrointestinal function and immunity, and lowers blood lipids (Xie et al., 2023). In a study involving a macular edema formula containing *A. macrocephala*, the total volume of the macular center area in diameter of the patients was significantly reduced, and the visual acuity and visual field were significantly improved (Zhu et al., 2016). In addition, *Atractylodes* may improve AMD risk factors by modulating gut microbes to act as an anti-inflammatory and lipid-lowering agents. *Atractylodes macrocephala* volatile oil reduces harmful bacteria, such as *Parasutterella, Turicibacter*, and *Erysipelatoclostridium*. It increases the number of beneficial bacteria, such as *Parvibacter, Enterorhabdus,* and *Akkermansia*, attenuating the inflammatory response in DSS-induced mouse models (Cheng et al., 2023). Kai et al. (2022) found that *Atractylodes* polysaccharide modulates the intestinal flora by increasing the abundance and diversity of intestinal flora, decreasing the proportion of harmful bacteria, such as *Clostridium strictum* 1 and *Shigella coli*, and increasing the relative abundance of potentially beneficial bacteria, such as *B. bifidum*, thereby alleviating DSS-induced weight loss. It also prevented the overexpression of proinflammatory cytokines TNF-α, IL-1β, and IL-6, reduced neutrophil infiltration, and upregulated the expression of MUC2 and the tight junction protein claudin-1, thus attenuating DSS-induced intestinal mucosal barrier damage in mice. In a rat model administered *A. macrocephala,* He et al. (2023b) observed an abundance of *Lactobacillus* and *Rhodococcus* species in the intestine, as well as an increase in tryptophan metabolites, such as indole, indole-3-propionic acid, and tryptophan. This, in turn, increased the expression of the intestinal AHR. The activation of AHR caused the upregulation of GLP-1 and IL-22 at proteins and mRNA levels, decreased systemic LPS, improved gut barrier function, activated the hepatic STAT3/IL-22R/peroxisomal acyl-coenzyme A oxidase 1 (ACOX1) and pancreatic GLP-1R/p-CREB signaling pathways, and improved lipid metabolism and insulin resistance.

4.3 Botanical drug decoction

4.3.1 Shenling Baizhu powder

Shenling Baizhu powder comprises *ginseng*, *Poria*, *A. macrocephala*, *Glycyrrhiza glabra* L. [Fabaceae; radix et rhizoma glycyrrhizae], *Dioscorea polystachya* Turcz [Dioscoreaceae; dioscoreae rhizoma], *Coix lacryma-jobi* var. ma-yuen (Rom.Caill.) Stapf [Poaceae; coicis semen], *Nelumbo nucifera* Gaertn. [Nelumbonaceae; lotus seed], *Lablab purpureus subsp.* Purpureus [Fabaceae; semen lablab album], *Wurfbainia villosa* (Lour.) Skornick. & A.D.Poulsen [Zingiberaceae; amomi fructus], and *Platycodon grandiflorus* (Jacq.) A.DC. [Campanulaceae; platycodonis radix]. Recent research has shown that this powder possesses anti-inflammatory, antiviral, and antioxidative properties, improves immune function, regulates blood lipids, balances intestinal flora, and safeguards the intestinal mucosal barrier. *Ginseng* and Atractylodes Maculatus San protect the blood-retinal barrier, inhibit the expression of inflammatory factors, balance the ecological stability of the intestinal tract by regulating the intestinal bacterial flora, and improve the risk factors for macular edema (Bai et al., 2023). Polysaccharides of Shenling Baizhu powder alleviate inflammation by regulating tryptophan metabolism in *Bacteroides, B. bifidum*, and *Ruminococcus*. The tryptophan metabolite kynurenine activates the expression of polypeptide 1 (CYP1A1) and AHR and promotes the expression of anti-inflammatory IL-10 (Lv et al., 2022). Botanical drugs that strengthen the spleen and benefit qi have demonstrated a significant impact in regulating intestinal flora and enhancing immunity in animal models of spleen deficiency and clinical studies (Lima-Fontes et al., 2022). Shenling Baizhu powder has the potential to enhance the prevalence of the SCFA-producing bacteria *Puccinia* and *Treponema* and decrease the prevalence of the opportunistic pathogens *Vibrio desulfuricans* and *Cholera* spp. It significantly reduces the level of myeloperoxidase, increases the levels of catalase and SOD in the serum of rats, and exerts antimicrobial effects by enhancing the antioxidant capacity and modulating the intestinal microbiota (Gu et al., 2021).

5 Conclusion

The human gut is home to hundreds of millions of bacteria and is assumed to be the "second human genome." Presently, an

unhealthy lifestyle and the improper use of antibiotics can result in intestinal flora dysbiosis. The disruption of the GM and alterations in metabolites, including LPS, oxysterols, SCFAs, and BAs, have been linked to various factors impacting AMD, including oxidative stress, apoptosis, aging, inflammation, dyslipidemia, obesity, and modified choroidal hemodynamics.

Exploring the relationship between TCM and GM could be highly beneficial. This investigation could provide insight into how TCM works to prevent and manage AMD, thus broadening the scope of TCM theory. In recent years, there have been fewer reports of TCM treating AMD by modulating GM. This review focused on the correlation between GM and its metabolites and AMD-related risk factors, drawing upon previous research. Furthermore, we demonstrated the possible ways TCM modulates the GM to improve AMD based on classification. While organizing the data, we found that the diversity of intestinal flora may be associated with host species, dietary preferences, and lifestyle habits. Consequently, we hypothesized that different parts of the intestinal tract harbor different types and amounts of dominant GM within the same individual. However, the relationship between various gut bacteria and the precise mechanisms by which the GM influences AMD remain unclear. Further studies are required to identify the precise pharmacodynamics, target pathway, and mechanism of action of TCM. Consequently, future research should concentrate on the biotransformation of TCM active components via the GM and whether these biotransformed metabolites positively or negatively influence TCM's efficacy in treating AMD. Further animal trials and clinical randomized, controlled, multicenter, large-sample studies should be conducted to confirm the effectiveness and safety of TCM in treating AMD via GM regulation and to identify novel therapeutic objectives for AMD.

Scope statement

This review focuses on the mechanism of action of Chinese medicine in treating AMD, as well as previous related research. Literature suggests that gut flora-related mechanisms may be a plausible explanation. All these are related to the fields of ethnopharmacology, intestinal flora, molecular mechanisms, and neurodegenerative diseases, which are the focus of your journal, and we hope to communicate with and learn from the general readers of your journal through your journal.

Author contributions

YY: Conceptualization, Writing–original draft, Writing–review and editing. YL: Supervision, Writing–review and editing. ZM: Writing–review and editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

Figures in this review were created using the BioRender platform. We extend our gratitude to Editage (www.editage.cn) for their expertise in English language editing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 26 January 2024
DOI 10.3389/fphar.2023.1317620

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OPEN ACCESS

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RECEIVED 10 October 2023
ACCEPTED 27 December 2023
PUBLISHED 26 January 2024

CITATION
Chen J, Yu D, Li X, Deng Q, Yang H, Chen L and Bai L (2024), A review of *Brucea javanica*: metabolites, pharmacology and clinical application.
Front. Pharmacol. 14:1317620.
doi: 10.3389/fphar.2023.1317620

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A review of *Brucea javanica*: metabolites, pharmacology and clinical application

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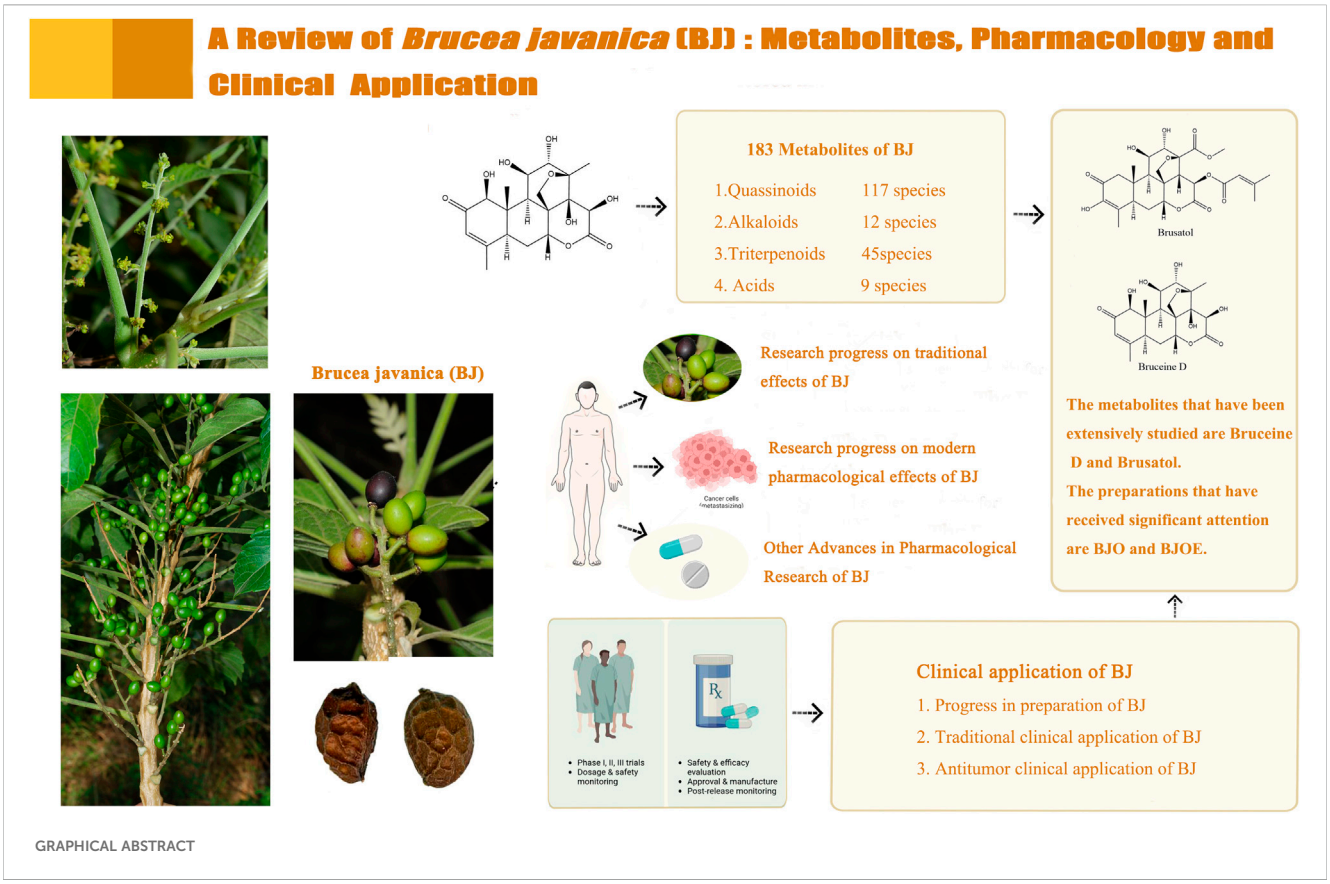
This review examines advances in the metabolites, pharmacological research, and therapeutic applications of the medicinal fruit of *Brucea javanica* (L.) Merr. *Brucea javanica* (BJ) is derived from the fruit of the *Brucea javanica* (L.) Merr. There are nearly 200 metabolites present in BJ, and due to the diversity of its metabolites, BJ has a wide range of pharmacological effects. The traditional pharmacological effects of BJ include anti-dysentery, anti-malaria, etc. The research investigating the contemporary pharmacological impacts of BJ mainly focuses on its anti-tumor properties. In the article, the strong monomeric metabolites among these pharmacological effects were preliminarily screened. Regarding the pharmacological mechanism of action, current research has initially explored BJ's pharmacological agent and molecular signaling pathways. However, a comprehensive system has yet to be established. BJ preparations have been utilized in clinical settings and have demonstrated effectiveness. Nevertheless, clinical research is primarily limited to observational studies, and there is a need for higher-quality research evidence to support its clinical application. There are still many difficulties and obstacles in studying BJ. However, it is indisputable that BJ is a botanical drugs with significant potential for application, and it is expected to have broader global usage.

KEYWORDS

***Brucea javanica*, metabolites, traditional pharmacological effects, modern pharmacological effects, clinical application**

1 Introduction

Traditional Chinese Medicine (TCM) has been essential to Chinese healthcare. Chinese botanical drugs (CBD) that incorporate natural compounds are also employed in contemporary medicine to treat several ailments. CBD mainly comprises plant, animal, and mineral medicines, of which plant medicine accounts for the majority. CBD has been widely used in China, some Asian countries, and Europe (Lee et al., 2018; Li et al., 2019a). *Brucea javanica* (L.) Merr. is a well-known CBD belonging to the Simaroubaceae family, an evergreen shrub widely distributed in southeast Asia and northern Australia (Liu et al., 2012a; Zhang et al., 2022), validated by the Plant List database (https://mpns.science.kew.org/mpns-portal/). *Brucea javanica* (BJ) is the fruit of the *Brucea javanica* (L.) Merr., also



known as *Yadanzi*, uses ripe dried fruit as medicine. The ripe fruit is black or brown, has a cold nature, and tastes bitter (Chen et al., 2013b; Zhang et al., 2021).

The conventional utilization of BJ in Southeast Asian countries, such as Indonesia and Cambodia, includes its use as an anti-malarial, anti-trypanosomal, and hypoglycemic drug (Murnigsih et al., 2005; NoorShahida et al., 2009; Bawm et al., 2010). In Australia, it is used as an analgesic (Yeshi et al., 2022), while in China, it is used to treat dysentery, malaria, corn, and skin warts (Kitagawa et al., 1994; Yin and Du, 2000; Huang et al., 2017a). The traditional use of BJ has been confirmed through observational studies in numerous research studies (Qiang, 1999; Ge, 2001; Caiyong et al., 2019). There are few studies on the mechanism of action of BJ in traditional applications, and there is a lack of comprehensive literature reviews. With the development of analytical technology, more than 100 metabolites have been isolated from BJ, including quassinoids, triterpenoids, alkaloids, etc. (Kim et al., 2004a; Chen et al., 2011; Chen et al., 2013a; He et al., 2021). In recent decades, extensive studies have demonstrated that the metabolites of BJ possess a diverse range of pharmacological properties, such as anti-cancer, anti-inflammatory, and anti-viral activities (Yang et al., 2013; Ryu et al., 2017; Xie et al., 2021). Most of the research on the metabolites of BJ focuses on quassinoids, which have demonstrated anti-tumor activity in modern research (Cheng et al., 2017; Lee et al., 2020). It is worth noting that alkaloids and acidic metabolites exhibited cytotoxic effects in the study (Toyota et al., 1990; Samat et al., 2017), whereas terpenoids demonstrated anti-inflammatory activity (Hu et al., 2022). This suggests that future research on the metabolites of BJ should not be biased only towards quasinoids. Modern research on the pharmacological mechanisms of action primarily focuses on the anti-tumor effects. Brusatol and Bruceine D are metabolites that have been extensively researched and exert anti-tumor effects through multiple signaling pathways (Xiao et al., 2014; Ye et al., 2018). Notably, multiple metabolites in BJ demonstrate anti-tumor effects through various signaling pathways (Mata-Greenwood et al., 2002; Lu et al., 2021). This indicates that we should not only focus on the mechanisms of well-known metabolites such as Brusatol and Bruceine D. The clinical research on the traditional pharmacological effects of BJ mainly focuses on treating dysentery, skin warts, and corns. The contemporary scientific investigation and practical implementation of BJ primarily focus on its potential as an anti-neoplastic agent. Multiple clinical studies have demonstrated that the co-administration of BJ with other pharmacological agents in individuals with cancer can enhance therapeutic efficacy, improve patients' quality of life, and mitigate adverse reactions (Shan et al., 2011; Liu et al., 2013; Lu et al., 2013). However, there is a lack of comprehensive literature summarizing clinical research in both traditional and modern applications of BJ.

This review will elaborate on the metabolites of BJ, research on its pharmacological effects, and progress in clinical application research. This paper summarizes the status and challenges of BJ research and suggests future research directions. Fifteen metabolites with research potential were initially identified through current research data.

2 Materials and methods

2.1 Document retrieval

Search the SciFinder, PubMed, and CNKI databases using the following search methods: SciFinder, PubMed: "All Fields: *Brucea javanica* OR *Yadanzi*," "All Fields: *Brucea javanica* (L.) Merr. AND *Yadanzi*," CNKI: "Keywords: Metabolites of *Brucea javanica* (L.) Merr. OR Mechanism of action of *Brucea javanica* (L.) Merr. OR Clinical application of *Brucea javanica* (L.) Merr.," "Keywords: Metabolites of *Brucea javanica* AND Mechanism of action of *Brucea javanica* AND Clinical application of *Brucea javanica*."

2.2 Inclusion and exclusion criteria

Literature inclusion criteria: research papers and reviews related to BJ. Exclusion criteria: repeatedly published documents, documents with missing critical information such as authors, popular science articles, conference abstracts, news reports, etc. The retrieved documents were imported into the literature manager EndNote software. After deduplication, two researchers screened, read, and analyzed the records according to the inclusion and exclusion criteria and eliminated documents that did not meet the requirements.

2.3 Search results

A total of 291 journal articles were obtained from the SciFinder and PubMed databases, while an additional 600 journal articles were collected from CNKI. After removing duplicate articles, excluding popular scientific publications, and articles with missing information, a total of 198 articles were included.

3 Metabolites of BJ

This chapter analyses BJ's metabolites and structure, which contains 183 metabolites. The metabolites include quassinoids, alkaloids, triterpenes, and acids. We will elaborate on the classification of metabolites of BJ.

3.1 Quassinoids of BJ

Currently, quassinoids are considered highly significant active metabolites in BJ. Notably, the most notable quassinoids are categorized as C20-quassinoids (Duan et al., 2021). The elemental composition of the core structure includes three hexagonal rings and a lactone ring. Quassinoids exhibit a wide range of pharmacological effects, including traditional applications such as anti-malarial, anti-dysentery, and anti-insect properties (Guo et al., 2005; Bawm et al., 2008; Zhou et al., 2018). In modern research, they also have anti-tumor and anti-muscular atrophy effects (Mata-Greenwood et al., 2002; Baek et al., 2019). In this section, the molecular formulas of quassinoids are presented in tables. There are many quassinoids in BJ. This section classifies and lists the quassinoids based on their naming characteristics. Figure 1 shows the structural diagram of the mother nucleus of quassinoids. Figure 2 displays the structural diagram of quassinoids named with the prefix "Bru". Figure 3 illustrates the structural diagram of quassinoids named with the prefix "Java". Figure 4 depicts the structural diagram of quassinoids named with the prefix "Yadanzi". Lastly, Figure 5 presents the structural diagram of other quassinoids in BJ. Table 1 displays the molecular formula of quassinoids named with the prefix "Bru". Table 2 illustrates the molecular formula of quassinoids named with the prefix "Java". Table 3 depicts the molecular formula of quassinoids named with the prefix "Yadanzi". Lastly, Table 4 presents the molecular formula of other quassinoids in BJ.

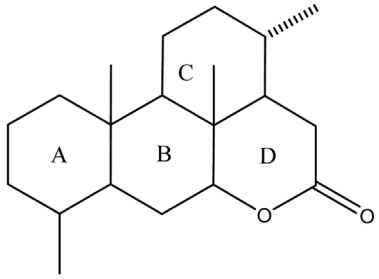


FIGURE 1
Nuclear parent of quassinoids from BJ.

3.2 Alkaloids of BJ

Currently, 12 alkaloids have been discovered in BJ. Most alkaloids have no documented pharmacological activity. Nevertheless, several alkaloids, such as Canthin-6-one and Bruceolline J, have been discovered to exhibit cytotoxic properties (Samat et al., 2017). Table 5 shows the molecular formula of the alkaloids in BJ. Figure 6 displays the corresponding molecular structure.

3.3 Triterpenoids of BJ

Another vital metabolite found in BJ is terpenoids. This paper presents a comprehensive list of 45 types of terpenoids documented in the literature. Most of these terpenoids are categorized as pentacyclic triterpenoids, with a lesser fraction falling under tetracyclic triterpenoids. The terpenoids known as brujavanoids A-U have been found to possess anti-inflammatory properties (Hu et al., 2022). Table 6 displays the chemical formulae of terpenoids found in BJ. The molecular structures are seen in Figure 7.

3.4 Acids from BJ

Additionally, this part also lists nine acids found in BJ. Among these acids, Bruceanic acid D (Toyota et al., 1990) exhibited significant cytotoxicity in leukemia. Table 7 provides the molecular structures of the types of acids in BJ. Figure 8 displays the molecular structure.

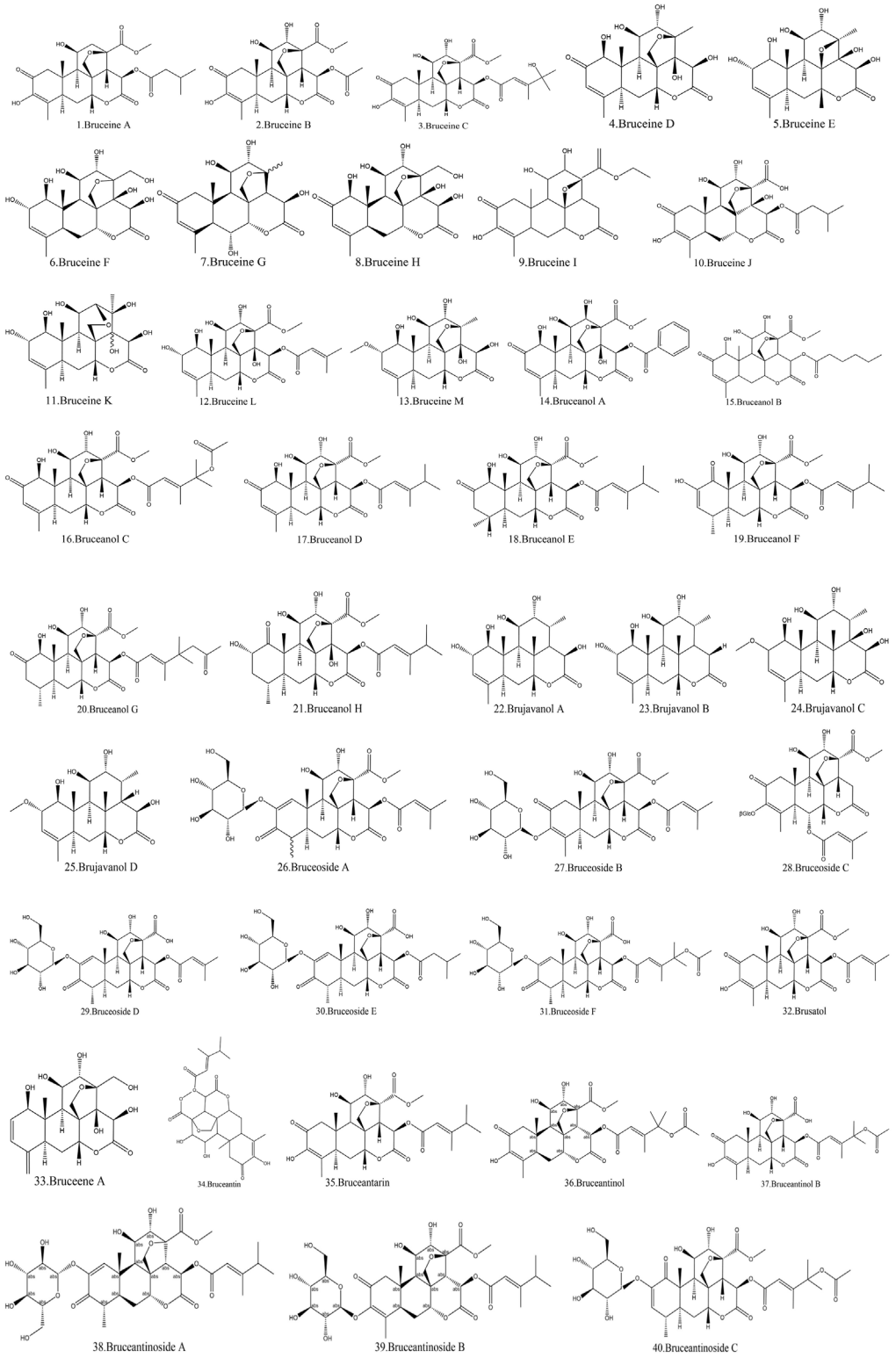


FIGURE 2
Quassinoids structure diagram from BJ, named with the prefix "Bru."

4 Advances in BJ's pharmacology

The metabolites of BJ are diverse and exhibit a wide range of pharmacological effects. This review summarizes the research progress on the pharmacological mechanism of BJ in traditional usage and its molecular mechanism of action in modern applications. We initially addressed the research advancements regarding the traditional pharmacological properties of BJ,

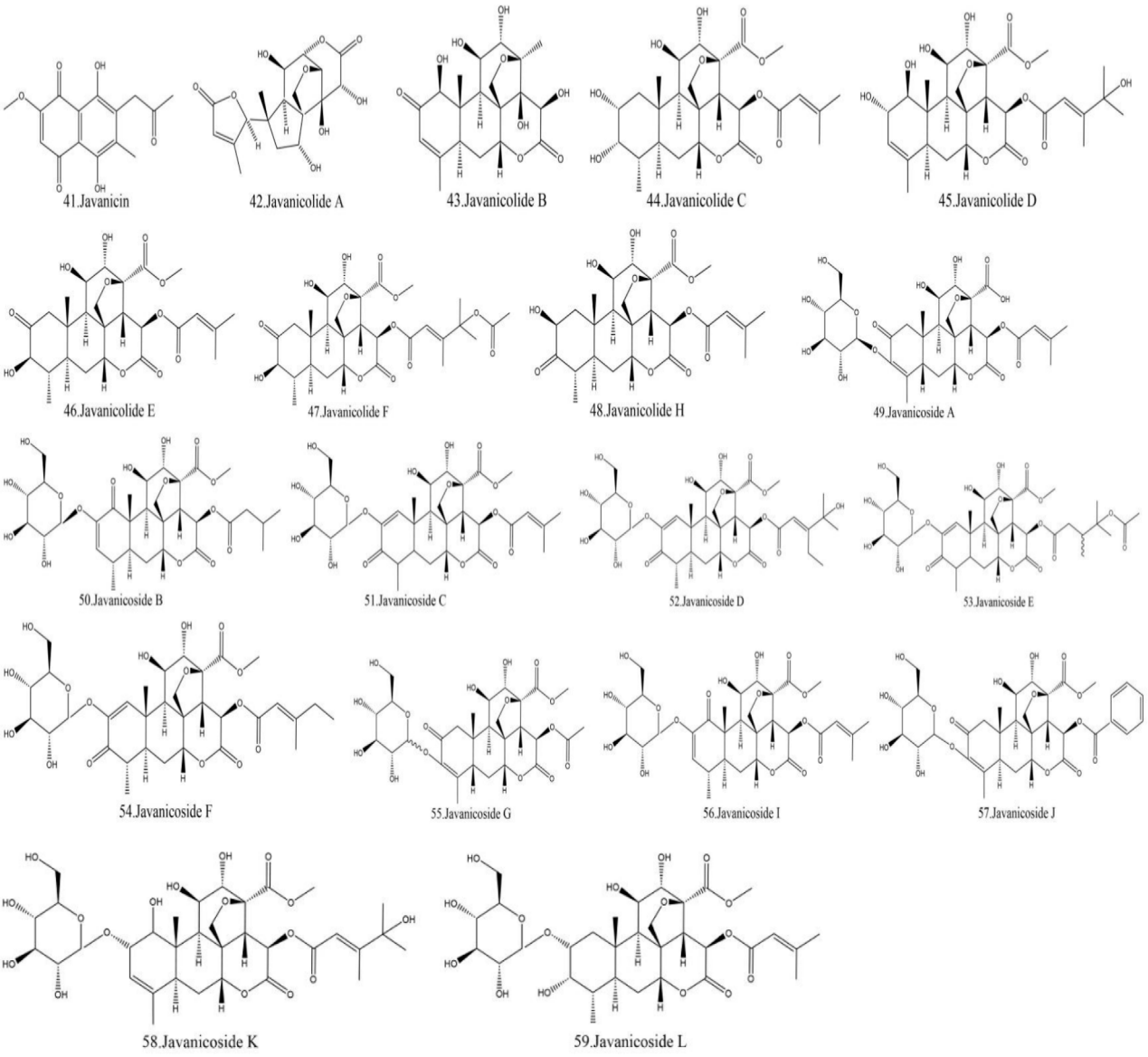


FIGURE 3
Quassinoids structure diagram from BJ, named with the prefix "Java."

including its anti-dysentery, anti-malaria, insecticidal, skin wart, and corn-fighting actions. Then, we discussed the latest advancements in research on the contemporary pharmacological effects of BJ, specifically focusing on its anti-tumor properties. The objective is to provide a research foundation for its future clinical utilization.

4.1 Research progress on traditional effects of BJ

In this section, we will describe the research progress on the traditional pharmacological effects of BJ. These effects include its anti-dysentery, anti-malarial, and insecticidal, as well as its use in the treatment of skin warts and corns. We have initially screened out compounds with pharmacologically active effects in traditional applications and their corresponding pharmacological action mechanisms, hoping to provide valuable information for future research. Research progress on the pharmacological mechanism of BJ's traditional application is depicted in Figure 9. It should be noted that the traditional pharmacological mechanism of BJ, whether it is used for anti-inflammatory diseases, reducing blood sugar levels, or addressing skin conditions such as warts and corns, still requires further understanding. Current research is primarily limited to *in vitro* and animal studies, and there is a lack of high-quality clinical research evidence in this area. Therefore, it is necessary to further study the mechanisms behind the traditional pharmacological effects of BJ and conduct scientific and rigorous clinical research. This is the cornerstone for further promoting BJ on a global scale.

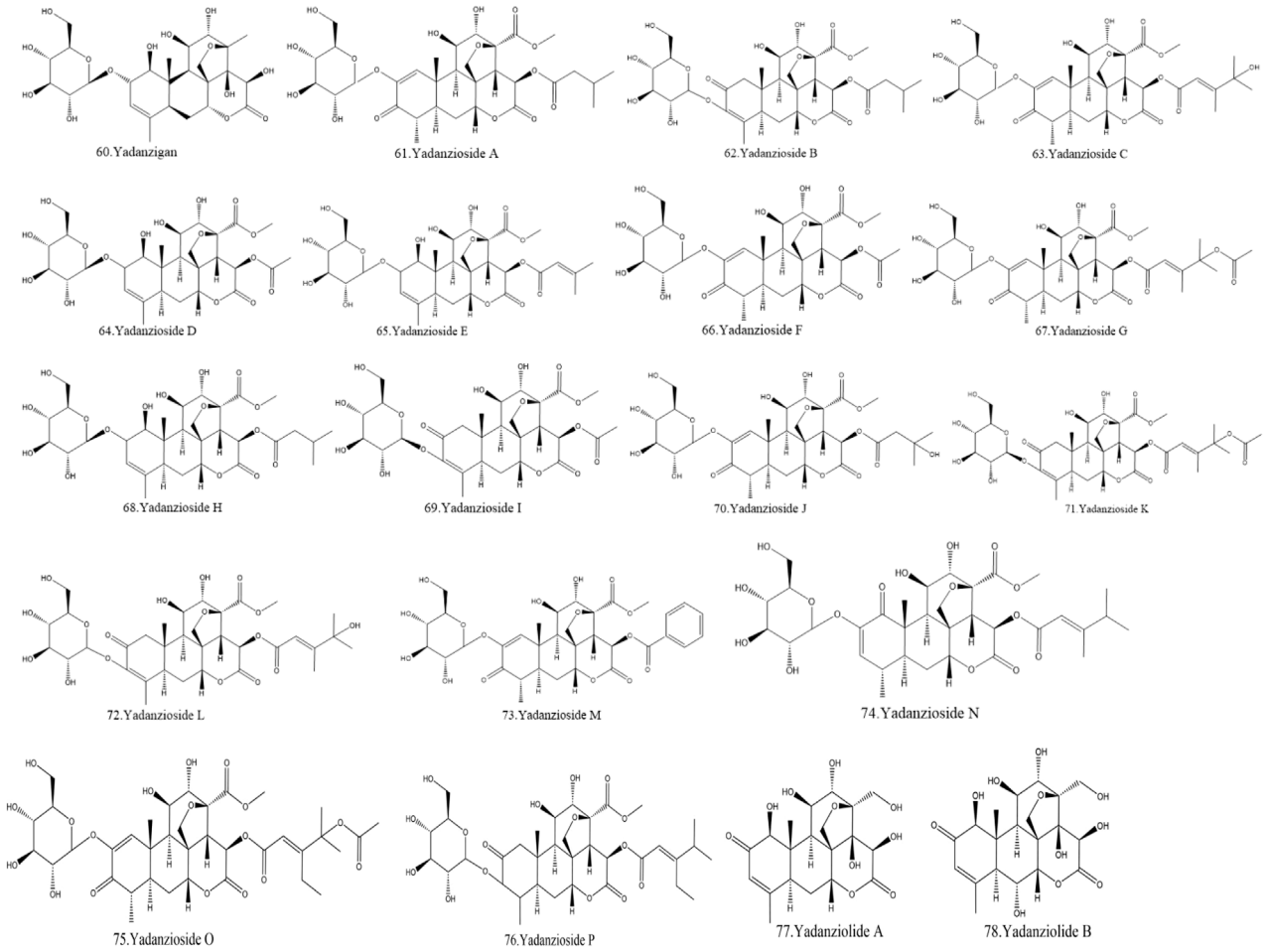


FIGURE 4
Quassinoids structure diagram from BJ, named with the prefix "Yadanzi."

4.1.1 Anti-dysentery effect of BJ

BJ, as a remedy for diarrhea, is used in traditional Southeast Asian medicine. In modern medicine, "dysentery" encompasses ulcerative colitis and bacillary dysentery (Yu et al., 2023). The primary causes of dysentery are bacterial and amoebic infections (Siciliano et al., 2021), which primarily result in gastroenteritis and ulcerative gastroenteritis. The potential efficacy of BJ in treating dysentery may stem from its antibacterial and anti-inflammatory characteristics. In this section, we will present the metabolites of BJ used for treating dysentery and provide an overview of the advancements in pharmacological research in this field.

Numerous metabolites present in BJ have exhibited significant anti-inflammatory properties in various research studies. Studies have been undertaken to investigate the underlying mechanism responsible for the anti-inflammatory activities exhibited by these metabolites. Brusatol (Zhou et al., 2018) has been shown to reduce inflammation in RAW264.7 cells stimulated by lipopolysaccharide and in rats with colitis induced by 2,4,6-trinitrobenzenesulfonic acid. This effect may be ascribed to the decrease in levels of inflammatory cytokines, which is associated with the suppression of the NF-κBNF-KB signaling pathway and the modulation of Nrf2-mediated oxidative stress. Brusatol-enriched in Brucea javanica oil (BJO) (Zheng et al., 2021) improved dextran sodium sulfate-induced enteritis, which may be related to the inhibition of NF-KB and ras homolog gene families, member A/rho-associated kinase (RhoA/ROCK) signaling pathways. The anti-inflammatory benefits of Brucea javanica oil emulsion (BJOE) on ulcerative colitis in mice are significant. This may be attributed to its ability to block the NF-KB signal transduction pathway and reduce the expression of inflammatory mediators (Huang et al., 2017a; Li et al., 2018). Huang et al. (2019) further revealed that BJOE was superior to sulfasalazine and azathioprine in the treatment of colitis induced by 2,4,6-trinitrobenzene sulfonic acid in rats. The observed phenomenon could be linked to the suppression of the toll-like receptor 4 (TLR4)-associated NF-κB signaling pathway, which results in a subsequent decrease in inflammatory mediators.

Furthermore, BJ exhibits pharmacological effects against amoeba. The amoeba infiltrates the human intestinal tract, resulting in the onset of a condition known as amoebic dysentery. A study conducted in Indonesia revealed that bruceine A and bruceantinol have demonstrated significant anti-amoeba activity *in vitro*, as evidenced by their IC50 values of 0.01 and 0.02 μmol/L, respectively (Subeki et al., 2007).

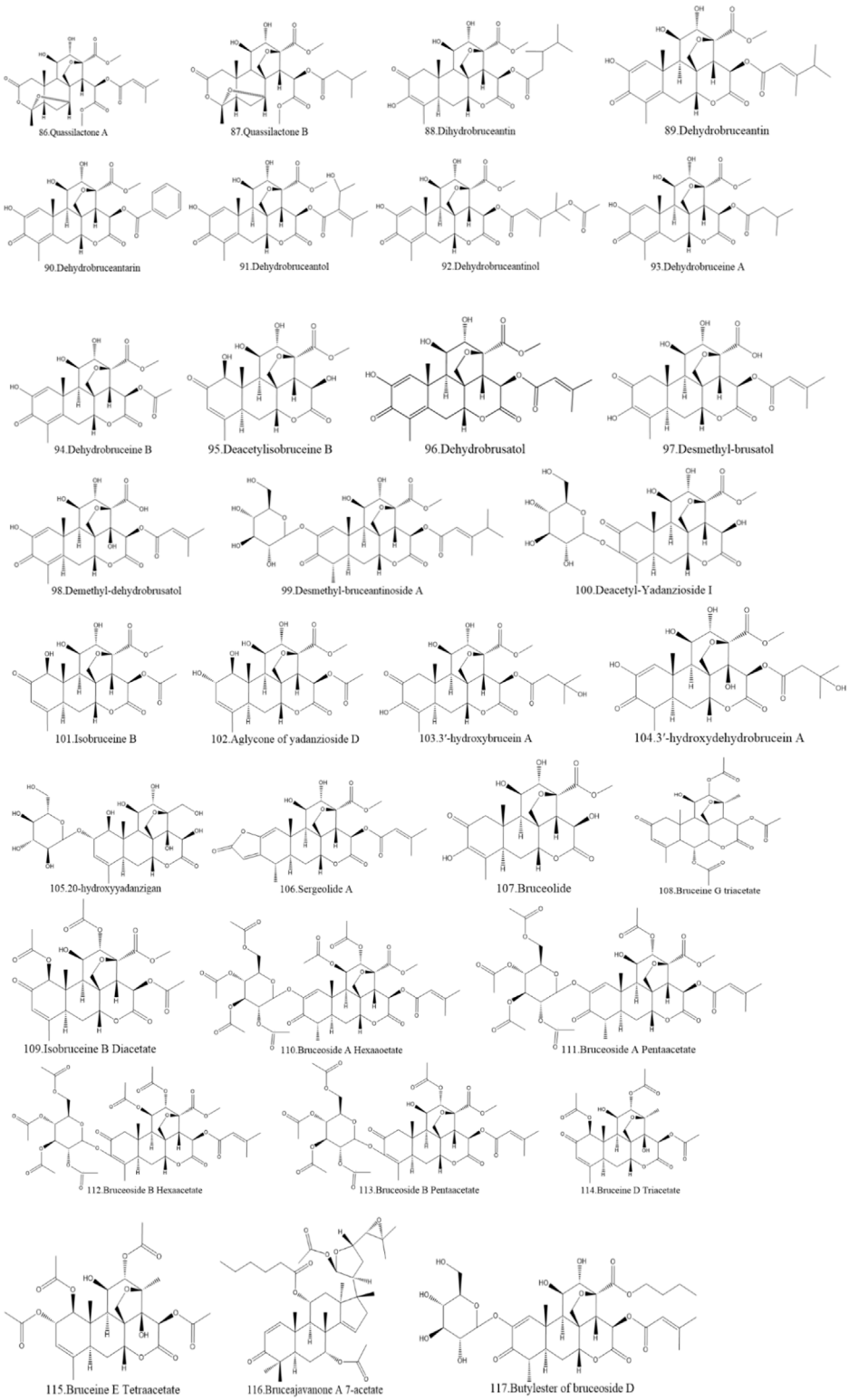


FIGURE 5
Other quassinoids structure diagram from BJ.

In summary, the monomeric metabolites of BJ, including Brusatol, Bruceine A, and Bruceantinol (Subeki et al., 2007; Zhou et al., 2018), have shown promising properties as anti-dysenteric agents. Among them, Brusatol has initially been explored for its mechanism of action in research on inflammatory diseases. Qian et al. (2011) demonstrated that the aqueous extract of BJ (AE-BJ) exhibits a more potent anti-inflammatory effect compared to the alcohol-extracted metabolite and shows a good dose-effect

TABLE 1 Quassinoids from BJ named with the prefix "Bru."

Name	Formula	Ref.	Name	Formula	Ref.
Bruceine A	$C_{26}H_{34}O_{11}$	Polonsky et al. (1967)	Bruceine B	$C_{23}H_{28}O_{11}$	Polonsky et al. (1967)
Bruceine C	$C_{28}H_{36}O_{12}$	Polonsky et al. (1967)	Bruceine D	$C_{20}H_{26}O_{9}$	Lee et al. (1979)
Bruceine E	$C_{20}H_{28}O_{9}$	Lee et al. (1979)	Bruceine F	$C_{20}H_{28}O_{10}$	Yu and Li (1990)
Bruceine G	$C_{20}H_{26}O_{8}$	Duncan and Henderson (1968)	Bruceine H	$C_{20}H_{26}O_{10}$	Zhao et al. (2011a)
Bruceine I	$C_{22}H_{28}O_{9}$	Yu and Li (1990)	Bruceine J	$C_{25}H_{32}O_{11}$	Subeki et al. (2007)
Bruceine K	$C_{20}H_{28}O_{9}$	Zhao et al. (2011b)	Bruceine L	$C_{26}H_{34}O_{11}$	Zhao et al. (2011b)
Bruceine M	$C_{21}H_{30}O_{9}$	Su et al. (2013a)	Bruceanol A	$C_{28}H_{30}O_{11}$	Okano et al. (1985)
Bruceanol B	$C_{27}H_{36}O_{11}$	Okano et al. (1985)	Bruceanol C	$C_{30}H_{38}O_{13}$	Fukamiya et al. (1988)
Bruceanol D	$C_{28}H_{36}O_{11}$	Imamura et al. (1993)	Bruceanol E	$C_{28}H_{38}O_{11}$	Imamura et al. (1993)
Bruceanol F	$C_{28}H_{36}O_{11}$	Imamura et al. (1993)	Bruceanol G	$C_{30}H_{40}O_{13}$	Imamura et al. (1995)
Bruceanol H	$C_{28}H_{38}O_{11}$	Imamura et al. (1995)	Brujavanol A	$C_{20}H_{30}O_{7}$	Chumkaew and Srisawat (2016)
Brujavanol B	$C_{20}H_{30}O_{6}$	Chumkaew and Srisawat (2016)	Brujavanol C	$C_{21}H_{33}O_{8}$	Chumkaew et al. (2017)
Brujavanol D	$C_{21}H_{32}O_{7}$	Chumkaew et al. (2017)	Bruceoside A	$C_{32}H_{42}O_{16}$	Lee et al. (1977)
Bruceoside B	$C_{32}H_{42}O_{16}$	Lee et al. (1979)	Bruceoside C	$C_{32}H_{42}O_{16}$	Fukamiya et al. (1992)
Bruceoside D	$C_{31}H_{40}O_{16}$	Ohnishi et al. (1995)	Bruceoside E	$C_{31}H_{42}O_{16}$	Ohnishi et al. (1995)
Bruceoside F	$C_{35}H_{46}O_{18}$	Ohnishi et al. (1995)	Brusatol	$C_{26}H_{32}O_{11}$	Sim et al. (1968)
Bruceene A	$C_{20}H_{26}O_{9}$	Ye et al. (2015)	Bruceantin	$C_{28}H_{36}O_{11}$	Kupchan et al. (1973)
Bruceantarin	$C_{28}H_{30}O_{11}$	Kupchan et al. (1973)	Bruceantinol	$C_{30}H_{36}O_{12}$	Kupchan et al. (1975)
Bruceantinol B	$C_{29}H_{35}O_{13}$	Subeki et al. (2007)	Bruceantinoside A	$C_{34}H_{46}O_{16}$	Okano et al. (1981)
Bruceantinoside B	$C_{34}H_{46}O_{16}$	Okano et al. (1981)	Bruceantinoside C	$C_{36}H_{48}O_{18}$	Fukamiya et al. (1987)

TABLE 2 Quassinoids from BJ named with the prefix "Java."

Name	Formula	Ref.	Name	Formula	Ref.
Javanicin	$C_{24}H_{28}O_{12}$	Lin et al. (1990)	Javanicolide A	$C_{19}H_{24}O_{9}$	Kim et al. (2003)
Javanicolide B	$C_{20}H_{26}O_{10}$	Kim et al. (2003)	Javanicolide C	$C_{26}H_{36}O_{11}$	Kim et al. (2004b)
Javanicolide D	$C_{28}H_{38}O_{12}$	Kim et al. (2004b)	Javanicolide E	$C_{26}H_{34}O_{11}$	Yan et al. (2010)
Javanicolide F	$C_{30}H_{40}O_{13}$	Yan et al. (2010)	Javanicolide H	$C_{26}H_{34}O_{11}$	Liu et al. (2012a)
Javanicoside A	$C_{32}H_{42}O_{16}$	Kim et al. (2003)	Javanicoside B	$C_{32}H_{44}O_{16}$	Kim et al. (2004b)
Javanicoside C	$C_{32}H_{40}O_{16}$	Kim et al. (2004b)	Javanicoside D	$C_{35}H_{48}O_{17}$	Kim et al. (2004b)
Javanicoside E	$C_{36}H_{50}O_{18}$	Kim et al. (2004b)	Javanicoside F	$C_{33}H_{44}O_{16}$	Kim et al. (2004b)
Javanicoside G	$C_{29}H_{38}O_{16}$	He et al. (2021)	Javanicoside I	$C_{32}H_{42}O_{16}$	Kim et al. (2004a)
Javanicoside J	$C_{34}H_{40}O_{16}$	Kim et al. (2004a)	Javanicoside K	$C_{34}H_{48}O_{17}$	Kim et al. (2004a)
Javanicoside L	$C_{32}H_{46}O_{16}$	Kim et al. (2004a)			

relationship. Based on the studies, Brusatol and the aqueous extract of BJ may serve as indicators of BJ's anti-inflammatory effect. Nonetheless, existing research mainly consists of *in vitro* and animal studies, and research on the molecular mechanism of action is still in the early stages of exploration. It is still necessary to further study the mechanism of BJ's anti-dysentery effect and elucidate its potential mechanisms to lay the foundation for subsequent research in this field.

4.1.2 Antimalarial and insecticidal effects of BJ

BJ has anti-plasmodium activity and is often used in traditional applications for the treatment of malaria. The anti-insect effects of

TABLE 3 Quassinoids from BJ named with the prefix "Yadanzi."

Name	Formula	Ref.	Name	Formula	Ref.
Yadanzigan	$C_{26}H_{38}O_4$	Zhang et al. (1983)	Yadanzioside B	$C_{32}H_{44}O_{16}$	Sakaki et al. (1985)
Yadanzioside A	$C_{32}H_{44}O_{16}$	Sakaki et al. (1985)	Yadanzioside D	$C_{23}H_{30}O_{11}$	Sakaki et al. (1985)
Yadanzioside C	$C_{34}H_{46}O_{17}$	Sakaki et al. (1985)	Yadanzioside F	$C_{29}H_{38}O_{16}$	Yoshimura et al. (1985)
Yadanzioside E	$C_{32}H_{44}O_{16}$	Sakaki et al. (1985)	Yadanzioside H	$C_{32}H_{46}O_{16}$	Sakaki et al. (1985)
Yadanzioside G	$C_{36}H_{48}O_{18}$	Sakaki et al. (1985)	Yadanzioside J	$C_{32}H_{44}O_{17}$	Yoshimura et al. (1985)
Yadanzioside I	$C_{29}H_{38}O_{16}$	Yoshimura et al. (1985)	Yadanzioside L	$C_{34}H_{46}O_{17}$	Yoshimura et al. (1985)
Yadanzioside K	$C_{36}H_{48}O_{18}$	Sakaki et al. (1986b)	Yadanzioside N	$C_{34}H_{46}O_{16}$	Sakaki et al. (1986b)
Yadanzioside M	$C_{34}H_{40}O_{16}$	Sakaki et al. (1986b)	Yadanzioside P	$C_{34}H_{46}O_{16}$	Sakaki et al. (1986a)
yadanzioside O	$C_{31}H_{41}O_{12}$	Sakaki et al. (1986c)	Yadanziolide B	$C_{20}H_{26}O_{11}$	Yoshimura et al. (1985)
Yadanziolide A	$C_{20}H_{26}O_{10}$	Yoshimura et al. (1985)	Yadanziolide D	$C_{19}H_{24}O_9$	Yoshimura et al. (1988)
Yadanziolide C	$C_{20}H_{26}O_9$	Yoshimura et al. (1985)	Yadanziolide S	$C_{20}H_{28}O_9$	Su et al. (2002)
Yadanziolide E	$C_{20}H_{26}O_9$	He et al. (2021)	Yadanziolide U	$C_{26}H_{38}O_{13}$	Chen et al. (2011)
Yadanziolide T	$C_{20}H_{28}O_8$	Chen et al. (2011)	Yadanziolide V	$C_{19}H_{26}O_8$	Chen et al. (2011)

TABLE 4 Other quassinoids in BJ.

Name	Formula	Ref.	Name	Formula	Ref.
Quassilactone A	$C_{26}H_{34}O_{12}$	Su et al. (2020)	Quassilactone B	$C_{26}H_{36}O_{12}$	Su et al. (2020)
Dihydrobruceantin	$C_{28}H_{38}O_{11}$	Kupchan et al. (1973)	Dehydrobruceantarin	$C_{34}H_{34}O_{14}$	Kupchan et al. (1975)
Dehydrobruceantin	$C_{28}H_{40}O_{14}$	Kupchan et al. (1975)	Dehydrobruceantinol	$C_{30}H_{36}O_{13}$	Sakaki et al. (1985)
Dehydrobruceantol	$C_{28}H_{34}O_{12}$	Kupchan et al. (1975)	Dehydrobruceine B	$C_{26}H_{26}O_{11}$	Kupchan et al. (1975)
Dehydrobruceine A	$C_{29}H_{32}O_{11}$	Phillipson and Darwish (1981)	Dehydrobrusatol	$C_{26}H_{30}O_{11}$	Sakaki et al. (1985)
Deacetylisobruceine B	$C_{21}H_{26}O_{10}$	Okano et al. (1985)	Demethyl-dehydrobrusatol	$C_{25}H_{28}O_{11}$	He et al. (2021)
Desmethyl-brusatol	$C_{25}H_{30}O_{11}$	Rahman et al. (1999)	Deacetyl-Yadanzioside I	$C_{27}H_{36}O_{15}$	He et al. (2021)
Desmethyl-bruceantinoside A	$C_{33}H_{44}O_{16}$	Rahman et al. (1999)	Aglycone of yadanzioside D	$C_{23}H_{30}O_{11}$	Kim et al. (2004b)
Isobruceine B	$C_{21}H_{24}O_9$	Okano et al. (1985)	3′-hydroxydehydrobrucein A	$C_{26}H_{35}O_{13}$	Lahrita et al. (2019)
3′-hydroxybrucein A	$C_{26}H_{35}O_{12}$	Lahrita et al. (2019)	Sergeolide A	$C_{28}H_{32}O_{11}$	Yang et al. (2022)
20-hydroxyyadanzigan	$C_{26}H_{38}O_{15}$	Zhan et al. (2020)	Bruceine G triacetate	$C_{22}H_{28}O_9$	Duncan and Henderson (1968)
Bruceolide	$C_{21}H_{26}O_{10}$	Polonsky et al. (1967)	Bruceoside A Hexaaoetate	$C_{44}H_{54}O_{22}$	Lee et al. (1979)
Isobruceine B Diacetate	$C_{27}H_{32}O_{13}$	Kupchan et al. (1975)	Bruceoside B Hexaacetate	$C_{44}H_{54}O_{22}$	Lee et al. (1979)
Bruceoside A Pentaacetate	$C_{42}H_{52}O_{21}$	Lee et al. (1979)	Bruceine D Triacetate	$C_{26}H_{32}O_{12}$	Lee et al. (1979)
Ruceoside B Pentaacetate	$C_{42}H_{52}O_{21}$	Lee et al. (1979)	Bruceajavanone A 7-acetate	$C_{40}H_{58}O_9$	Pan et al. (2009)
Bruceine E Tetraacetate	$C_{28}H_{36}O_{13}$	Lee et al. (1979)	Butylester of bruceoside D	$C_{35}H_{48}O_{16}$	Rahman et al. (1999)

BJ mainly include anti-Plasmodium, anti-Babesia, anti-Trypanosoma, and the ability to kill Culex fumigatus. Among the metabolites of BJ, Brusatol exhibits various functions, including anti-malarial, anti-trypanosome, and mosquito-killing properties against Culex mosquitoes. Among the metabolites investigated, Bruceantin exhibits the most potent antimalarial activity (Guo et al., 2005), consistent with previous findings from the University of London School of Pharmacy (Oneill et al., 1988). Based on current research, it is speculated that Bruceantin may serve as a potential antimalarial metabolite for BJ. Table 8 lists metabolites of BJ with insecticidal properties.

4.1.3 Skin warts and corn treatment of BJ

Warts are benign skin growths caused by a deoxyribonucleic acid (DNA) virus known as the human papillomavirus (HPV), which infects the outer layer of the skin and enters the epithelial

TABLE 5 Alkaloids of BJ.

Name	Chemical formula	Ref.
Bruceolline C	$C_{16}H_{12}N_2O_4$	Ouyang et al. (1995)
Bruceolline G	$C_{20}H_{18}N_2O_7$	Ouyang et al. (1995)
Bruceolline H	$C_{13}H_{11}NO_3$	Chen et al. (2011)
Bruceolline I	$C_{13}H_{13}NO_3$	Chen et al. (2011)
Bruceolline J	$C_{13}H_{13}NO_2$	Chen et al. (2011)
Bruceolline K	$C_{19}H_{23}NO_7$	Chen et al. (2011)
Bruceolline L	$C_{13}H_{15}NO_2$	Chen et al. (2011)
Bruceolline M	$C_{19}H_{25}NO_7$	Chen et al. (2011)
Bruceolline N	$C_{19}H_{27}NO_9$	Chen et al. (2011)
Bruceolline E	$C_{13}H_{11}NO_2$	Jordon et al. (2012)
Canthin-6-one	$C_{14}H_8N_2O$	Samat et al. (2017)
Bruceacanthinoside	$C_{26}H_{28}O_{12}$	Kitagawa et al. (1994)

cells. BJ monomer metabolites and extracts have shown potential effects in treating genital warts in research studies. The mechanisms of action of BD and brusatol for treating genital warts have been demonstrated in in vitro studies. BD and brusatol may be effective metabolites for the future treatment of genital warts. Corns are keratoproliferative lesions that appear as localized cone-shaped growths on the skin of the feet (Yuchen et al., 2017). BJ has been found to possess inhibitory effects on HPV and a corrosive impact on the skin, making it a potential treatment option for skin warts and corns.

Lianna et al. (2014) demonstrated the potential of BJOE for treating condyloma acuminatum. They observed that the injection of BJOE increased the levels of serum interleukin-2, interleukin-10, interleukin-18, and interferon-r. Bruceine D (BD) has shown inhibitory effects on HPV-16 infection in multiple studies (Liuliu et al., 2015; Jingli et al., 2017). BD may hinder the continued growth of virus-infected cells by reducing the expression of E6 and E7 mRNA in cells infected with high-risk HPV16. In addition, Brusatol can regulate the expression of Toll-like receptor 4 (TLR4) and inhibit the proliferation of genital warts keratinocytes, thereby regulating its progression (Weiqi et al., 2023). BJ is also utilized for the treatment of corn. It is crushed and applied topically to the affected area (Xiongzhang and Guang, 2000). However, the specific mechanism of action has not been reported.

4.1.4 Diabetes and diabetic nephropathy application of BJ

In the context of traditional medicinal practices in Malaysia, it has been observed that BJ is used as a therapeutic intervention for managing diabetes. In research investigations, various active compounds found in BJ have been shown to possess

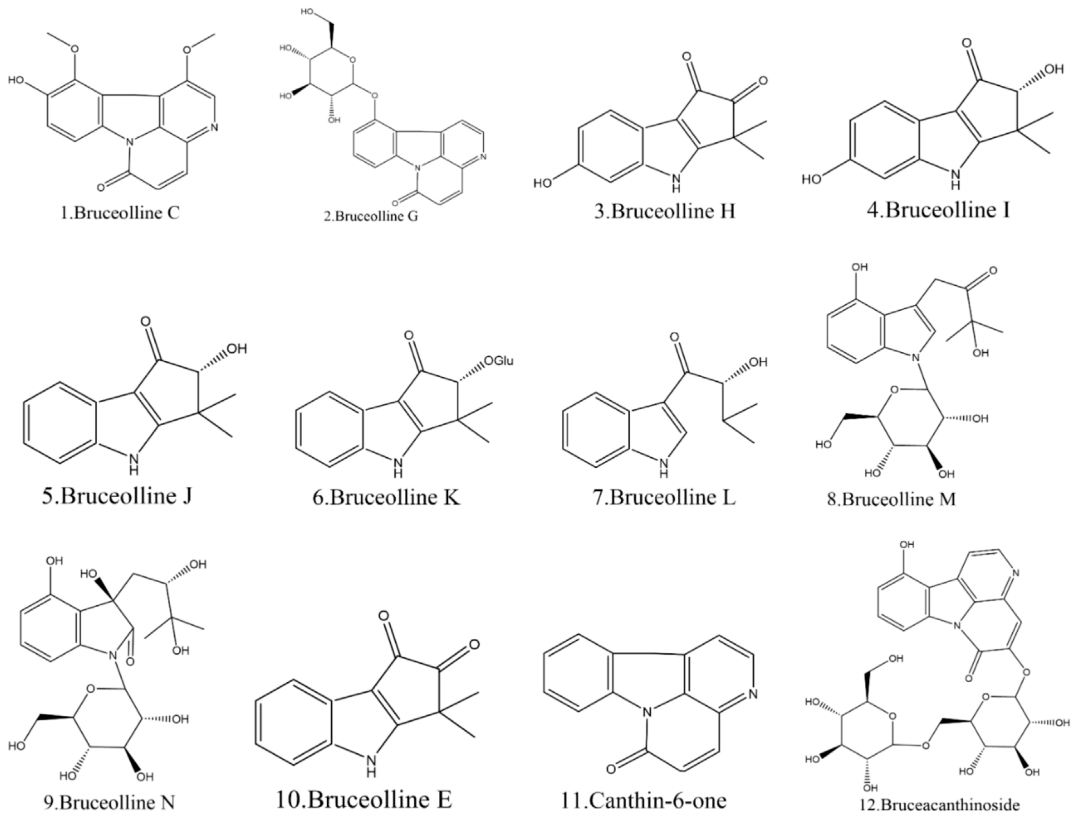


FIGURE 6
Structural diagram of the alkaloids in BJ.

TABLE 6 Triterpenoids of BJ.

Name	Formula	Ref.	Name	Formula	Ref.
Bruceajavanin A	$C_{34}H_{48}O_7$	Kitagawa et al. (1994)	Bruceajavanin B	$C_{33}H_{48}O_6$	Kitagawa et al. (1994)
Dihydrobruceajavanin A	$C_{34}H_{50}O_7$	Kitagawa et al. (1994)	Bruceajavanone A	$C_{36}H_{56}O_8$	Pan et al. (2009)
Bruceajavanone B	$C_{39}H_{54}O_9$	Pan et al. (2009)	Bruceajavanone C	$C_{38}H_{56}O_9$	Pan et al. (2009)
Bruceajavaninone A	$C_{37}H_{54}O_8$	Pan et al. (2009)	Brumollisols A	$C_{30}H_{48}O_5$	Chen et al. (2013a)
Brumollisols B	$C_{30}H_{50}O_5$	Chen et al. (2013a)	Brumollisols C	$C_{32}H_{52}O_6$	Chen et al. (2013a)
Brujavanone A	$C_{34}H_{48}O_8$	Dong et al. (2013)	Brujavanone B	$C_{33}H_{48}O_7$	Dong et al. (2013)
Brujavanone C	$C_{32}H_{46}O_6$	Dong et al. (2013)	Brujavanone D	$C_{33}H_{50}O_8$	Dong et al. (2013)
Brujavanone E	$C_{32}H_{48}O_8$	Dong et al. (2013)	Brujavanone F	$C_{34}H_{52}O_8$	Dong et al. (2013)
Brujavanone G	$C_{33}H_{48}O_7$	Dong et al. (2013)	Brujavanone H	$C_{33}H_{50}O_7$	Dong et al. (2013)
Brujavanone I	$C_{33}H_{52}O_7$	Dong et al. (2013)	Brujavanone J	$C_{33}H_{52}O_7$	Dong et al. (2013)
Brujavanone K	$C_{32}H_{50}O_7$	Dong et al. (2013)	Brujavanone L	$C_{32}H_{48}O_6$	Dong et al. (2013)
Brujavanone M	$C_{39}H_{62}O_{10}$	Dong et al. (2013)	Brujavanone N	$C_{34}H_{54}O_8$	Dong et al. (2013)
Brujavanoid A	$C_{31}H_{50}O_6$	Hu et al. (2022)	Brujavanoid B	$C_{34}H_{54}O_9$	Hu et al. (2022)
Brujavanoid C	$C_{33}H_{52}O_9$	Hu et al. (2022)	Brujavanoid D	$C_{33}H_{48}O_8$	Hu et al. (2022)
Brujavanoid E	$C_{34}H_{50}O_8$	Hu et al. (2022)	Brujavanoid F	$C_{35}H_{56}O_8$	Hu et al. (2022)
Brujavanoid G	$C_{35}H_{56}O_8$	Hu et al. (2022)	Brujavanoid H	$C_{34}H_{54}O_8$	Hu et al. (2022)
Brujavanoid I	$C_{34}H_{54}O_8$	Hu et al. (2022)	Brujavanoid J	$C_{34}H_{54}O_8$	Hu et al. (2022)
Brujavanoid K	$C_{34}H_{54}O_8$	Hu et al. (2022)	Brujavanoid L	$C_{34}H_{54}O_8$	Hu et al. (2022)
Brujavanoid M	$C_{34}H_{52}O_7$	Hu et al. (2022)	Brujavanoid N	$C_{34}H_{52}O_8$	Hu et al. (2022)
Brujavanoid O	$C_{33}H_{50}O_8$	Hu et al. (2022)	Brujavanoid P	$C_{34}H_{52}O_8$	Hu et al. (2022)
Brujavanoid Q	$C_{33}H_{50}O_8$	Hu et al. (2022)	Brujavanoid R	$C_{34}H_{52}O_8$	Hu et al. (2022)
Brujavanoid S	$C_{33}H_{50}O_8$	Hu et al. (2022)	Brujavanoid T	$C_{34}H_{54}O_7$	Hu et al. (2022)
Brujavanoid U	$C_{34}H_{54}O_7$	Hu et al. (2022)			

pharmacological actions that lower blood glucose levels. BD and Bruceine E (NoorShahida et al., 2009) were found to significantly reduce glucose levels in both streptozotocin-induced diabetic rats and normoglycemic mice. The reduction observed in diabetic rats was approximately 40.07% ± 11.45%, while in normoglycemic mice, it was approximately 48.82% ± 13.34%. Additionally, the blood glucose decreases curves observed for BD and Bruceine E were similar to those of the positive control, glibenclamide. The blood glucose level of rats was shown to decrease upon administration of the ethyl acetate fraction (EAF) of BJ at a dosage of 125 mg/kg (Ablat et al., 2014). The blood glucose level decreased by 39.91% after 30 min, followed by a further decline of 28.89% and 20.29% at 60 min and 90 min, respectively. When exposed to high glucose stress, Bruceine A has been found to inhibit galectin-1 in rat mesangial HBZY-1 cells. This finding implies that Bruceine A has the potential to function as a renoprotective agent in a murine model of diabetic nephropathy (Li et al., 2022).

The monomer metabolites of BJ, BD, Bruceine E, Bruceine A, and the EAF of BJ all exhibit hypoglycemic effects. Among them, the hypoglycemic effects of BD and Bruceine E are stronger than the EAF of BJ. It is speculated that BD and Bruceine E may be potential compounds for future hypoglycemic effects.

4.2 Research progress on modern pharmacological effects of BJ

Modern pharmacological research on BJ has found various pharmacological effects, including anti-tumor, anti-tuberculosis, anti-muscle atrophy, and lipolysis. Among them, the anti-tumor properties are of the most significant interest. In this section, we screen out compounds in BJ that have pharmacological activities, such as anti-tumor and anti-muscle atrophy, and at the same time, clarify the research progress of the corresponding pharmacological mechanism of action, laying the foundation for future research.

4.2.1 Lymphocytic leukemia

Individual metabolites of BJ, including Bruceanol D, Brusatol, Bruceantin, and Yadanzioside O, have shown potential for further exploration in lymphocytic leukemia. The primary focus of research

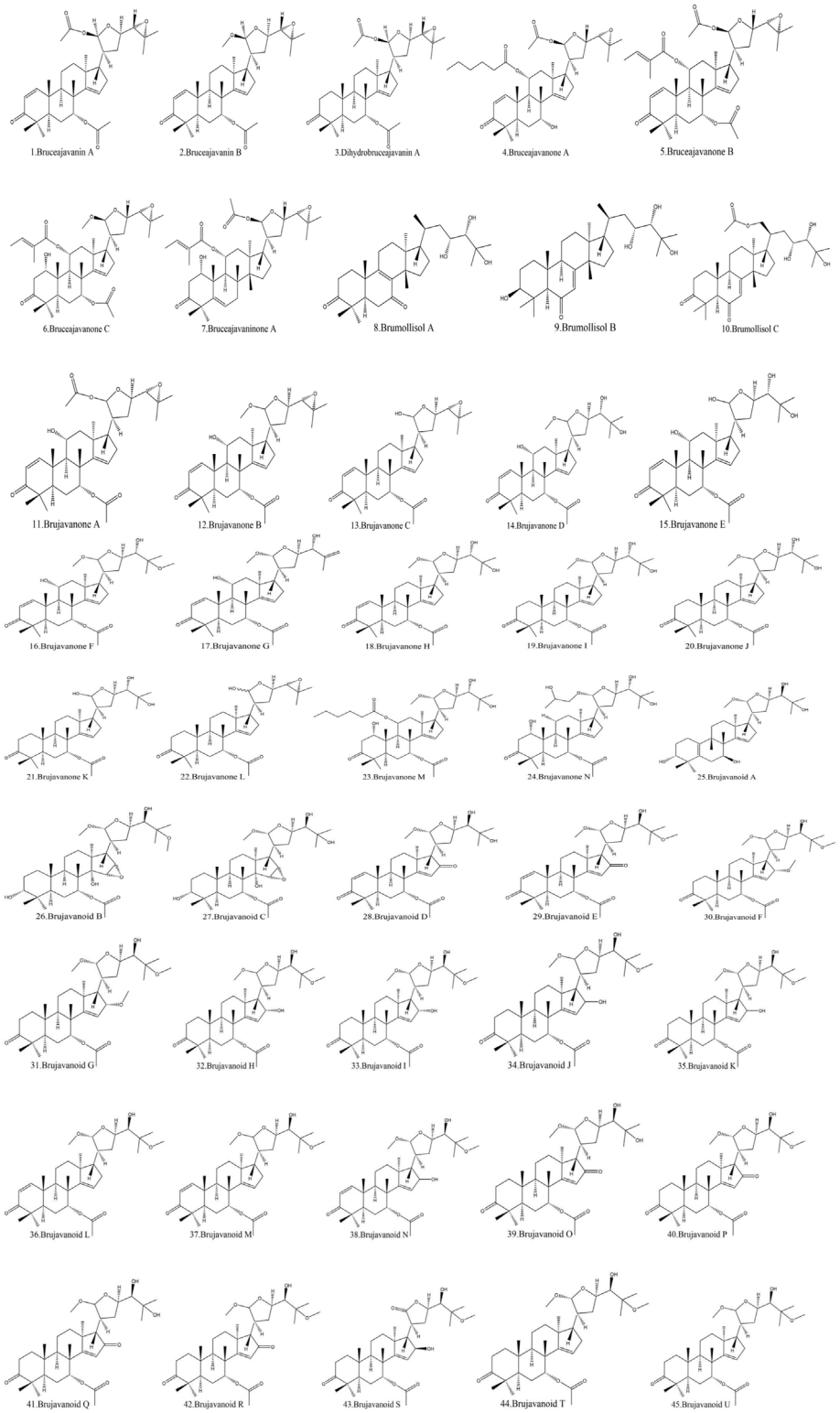


FIGURE 7
Structural diagram of triterpenoids in BJ.

on compound preparations predominantly revolves around BJO. *In vitro* P-388 lymphocytic leukemia cells, Bruceoside C (Fukamiya et al., 1992), Yadanzioside G (Fukamiya et al., 1987), Bruceantinoside C (Fukamiya et al., 1987), and Yadanzioside N (Fukamiya et al., 1987) demonstrated potent cytotoxicity with ED50 values of 3.15–7.49 μmol/L. Bruceanic acid D (Toyota et al., 1990), Bruceanol C (Fukamiya et al., 1988), Bruceanol E, Bruceanol F, and Bruceanol D (Imamura et al., 1993) demonstrated

TABLE 7 Acids of BJ.

Name	Chemical formula	Ref.
Bruceaketolic acid	$C_{22}H_{26}O_{11}$	Lin et al. (1990)
Bruceanic acid A	$C_{27}H_{36}O_{12}$	Toyota et al. (1990)
Bruceanic acid B	$C_{27}H_{30}O_{12}$	Toyota et al. (1990)
Bruceanic acid C	$C_{29}H_{38}O_{14}$	Toyota et al. (1990)
Bruceanic acid D	$C_{27}H_{36}O_{13}$	Toyota et al. (1990)
Bruceanic acid E	$C_{25}H_{32}O_{12}$	Liu et al. (2012a)
Bruceanic acid F	$C_{24}H_{30}O_{12}$	Liu et al. (2012a)
Javanic acid A	$C_{26}H_{34}O_{13}$	Liu et al. (2012a)
Javanic acid B	$C_{27}H_{36}O_{13}$	Liu et al. (2012a)

significant cytotoxicity with ED50 values of 0.16–1.36 μmol/L. From the above metabolites, Bruceanol D has a higher safety rating. Javanicoside I, J, K, and L (Kim et al., 2004a) and Javanicoside B (Kim et al., 2004b) showed moderate cytotoxic activity against P-388 murine leukemia cells, with IC50 values ranging from 0.68 to 0.77 μmol/L. Through *in vitro* tests on the HL-60 cell line, Liu et al. demonstrated that Brusatol and Bruceine B (Liu et al., 2011) exhibited significant inhibitory activity against HL-60 cells, with IC50 values of 0.06 and 0.27 μmol/L, respectively. Brusatol and Bruceantin (Mata-Greenwood et al., 2002) exerted significant cytotoxic effects on several leukemic cell lines, showing IC50 values in the range of 0.01–0.19, 0.003–0.096 μmol/L, respectively. The results showed that Brusatol and Bruceantin (Mata-Greenwood et al., 2002) had the strongest cytotoxicity against the Daudi cell line, with IC50 values of 0.01 ± 0.001 ng/mL and 0.003 ± 0.0002 μmol/L. In the compound extract study, BJO exhibited cytotoxicity towards Hl-60 and U937 cells, with IC50 values of 312.7 and 265.4 μg/mL, respectively (Zhang et al., 2011).

In vivo studies of mouse P388 lymphocytic leukemia, Yadanzioside A, B, C, D, E, and G showed antileukemic activity at a dose level of 10 mg/kg, with increased lifespan (ILS) values in experimental mice of 2.0%–9.2% (Sakaki et al., 1985). Yadanzioside P (Sakaki et al., 1986a) exhibited antileukemic activity at doses of 5 and 10 mg/kg/day, increasing ILS values of 15.5% and 28.9% in mice, respectively. Yadanzioside O exhibited antileukemic activity at 2 mg/kg/day doses and 4 mg/kg/day, resulting in a 37.1% and 47.2% increase in mouse ILS values, respectively (Sakaki et al., 1986b). Yadanzioside O, the monomeric metabolite of BJ, showed the best survival advantage in animal studies.

In the investigation of mechanisms of action, Brusatol and Bruceantin (Mata-Greenwood et al., 2002) inhibited cell proliferation by primarily blocking G0/G1 phase cells. They may trigger cell cycle arrest by downregulating the expression of the cellular myeloma viral oncogene (c-MYC), thereby exerting anti-leukemia effects. Additionally, Brusatol can downregulate the expression of c-MYC in normal lymphocytes. The mechanism of action of BJO and BJOE in leukemia has been partially confirmed in research. This includes inhibiting the activation of the phosphoinositide 3-kinase/protein kinase B (PI3K/Akt) signaling pathway and

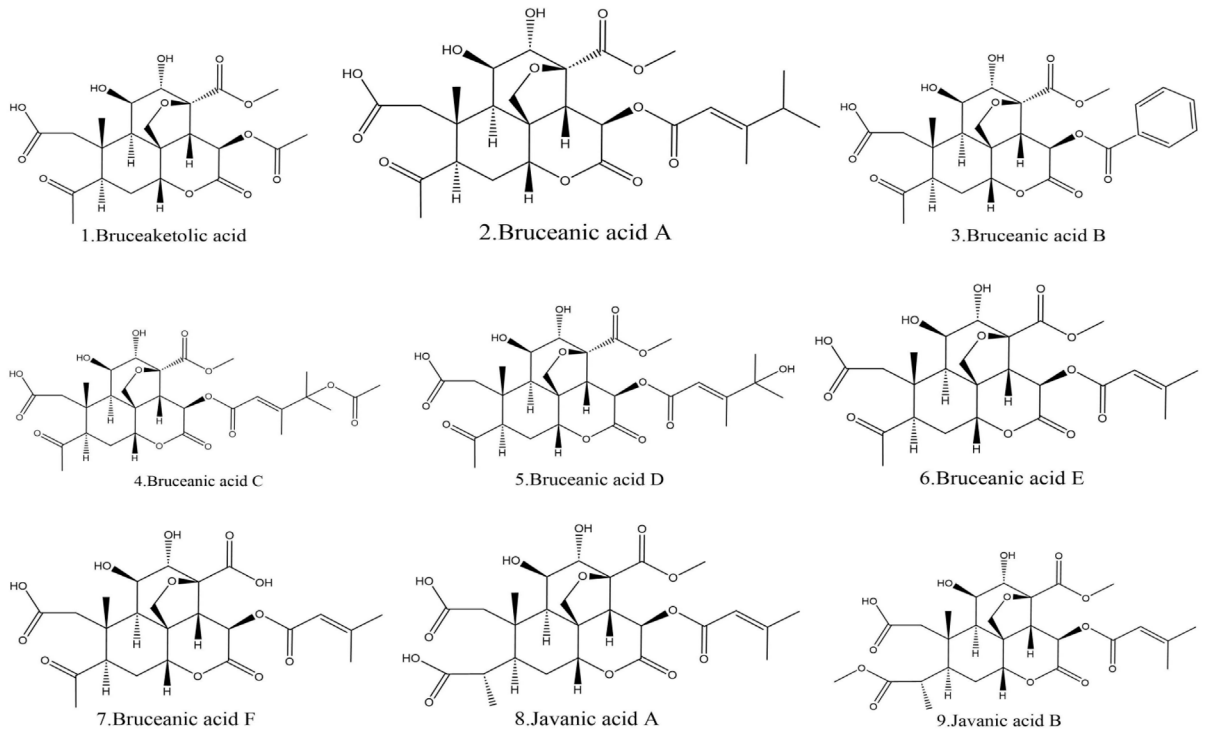


FIGURE 8
Structural diagram of acids in BJ.

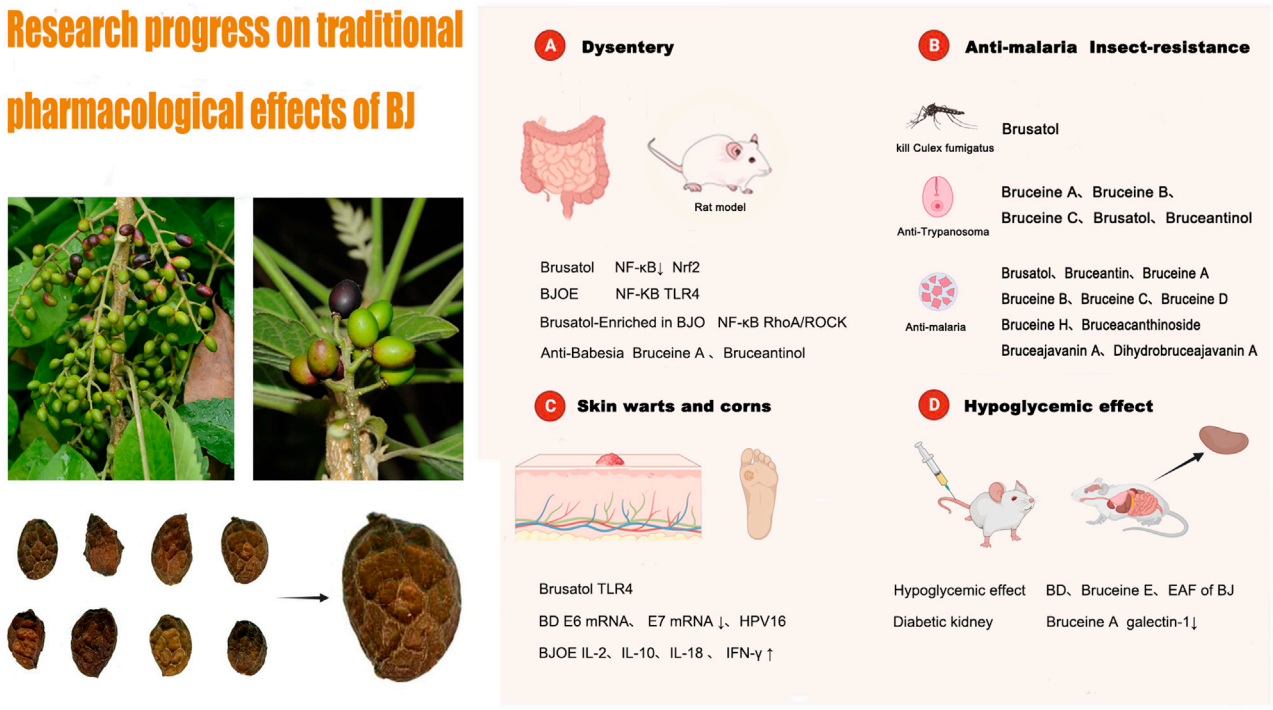


FIGURE 9
The pharmacological mechanism of BJ's traditional application.

TABLE 8 Metabolites in BJ with anti-insect and anti-malarial effects.

Pharmacological effect	Metabolites	Type of study	Quantitative indicators	Ref.
Anti-malaria	Bruceine D	Plasmodium K1	1.41 μmol/L/IC50	Chumkaew and Srisawat (2016)
	Bruceine H	Plasmodium K1	1.06 μmol/L/IC50	Chumkaew et al. (2017)
	Bruceajavanin A	Plasmodium K1	1.10 μmol/L/IC50	Kitagawa et al. (1994)
	Dihydrobruceajavanin A	Plasmodium K1	2.50 μmol/L/IC50	Kitagawa et al. (1994)
	Bruceacanthinoside	Plasmodium K1	25.00 μmol/L/IC50	Kitagawa et al. (1994)
	Bruceine A	P. falciparum	0.02 μmol/L/IC50	Guo et al. (2005)
	Bruceine B	P. falciparum	0.02 μmol/L/IC50	Guo et al. (2005)
	Bruceine C	P. falciparum	0.01 μmol/L/IC50	Guo et al. (2005)
	Brusatol	P. falciparum	0.01 μmol/L/IC50	Guo et al. (2005)
	Bruceantin	P. falciparum	0.002 μmol/L/IC50	Guo et al. (2005)
Anti-Trypanosoma	Bruceine A	Vitro	2.90 μmol/L/IC50	Bawm et al. (2008)
	Bruceine B	Vitro	17.80 μmol/L/IC50	Bawm et al. (2008)
	Bruceine C	Vitro	4.80 μmol/L/IC50	Bawm et al. (2008)
	Brusatol	Vitro	13.60 μmol/L/IC50	Bawm et al. (2008)
	Bruceantinol	Vitro	6.50 nmol/L/IC50	Bawm et al. (2008)
Kill Culex fumigatus	Brusatol	Culex fumigatus	0.654 ± 0.081/ppm/LC90	Dwi Sutiningsih et al. (2019)

upregulating its downstream targets, such as p53 and forkhead box genes, to initiate apoptosis., In addition, apoptosis was induced through the mitochondrial and death receptor apoptosis pathways by downregulating the expression of c-FLIP(L/S), myeloid cell leukemia-1, Bcl-2 (B-cell lymphoma-2), and XIAP (Zhang et al., 2011; Zhang et al., 2021).

4.2.2 Lung cancer

Several metabolites found in BJ exhibit notable cytotoxic properties against lung cancer. The substances Brusatol, Bruceloside C, and BD exhibit promise for further investigation in the context of lung cancer. Additionally, the composite extract AE-BJ demonstrates potential for further exploration in the field of lung cancer research.

Bruceanol E, D, F (Imamura et al., 1993), Bruceanol C (Fukamiya et al., 1988), and Bruceoside C (Fukamiya et al., 1992) exhibited cytotoxicity against A549 lung carcinoma, with ED50 values of 0.65–6.82 μmol/L. Bruceloside C has a minimum ED50 of 0.65 μmol/L, suggesting that it is a safer option compared to other monomers. Bruceosides D, E, and F (Ohnishi et al., 1995) showed selective cytotoxicity in non-small cell lung cancer cell lines, with log GI50 values ranging from −4.14 to −5.66. Quassilactones A and B (Su et al., 2020) exhibited cytotoxic activity against A549 cells with IC50 values of 66.43 ± 0.15 and 75.67 ± 0.10 μmol/L, respectively. BD (Tan et al., 2019) inhibited the viability of H460 and A549 cells with IC50 values of 0.5 and 0.6 μmol/L, respectively. Brusatol (Liu et al., 2011), Bruceine B (Liu et al., 2011), 24-epipiscidinol A (Chen et al., 2013a), and Yadanziolide B (Chen et al., 2011) demonstrated cytotoxicity against human A549 cells. Brusatol exhibited the highest level of cytotoxicity among these metabolites, with IC50 values of less than 0.0064 μmol/L. Studies on the preparation of BJ, Ethanol Extract of BJ, Petroleum Ether Extract of BJ and Ethyl acetate Extract of BJ (BJE), and n-butyl alcohol Extract of BJ (Su et al., 2013b) showed significant cytotoxicity against A549 cell lines, with an IC50 value ranging from 0.02 to 17.47 μg/mL. The warmed water extract of BJ (Lau et al., 2005) induced cell death in non-small-cell lung carcinoma (NSCLC) A549 with an IC50 of 50 ug/mL. The crude extract and its metabolites (Bruceacanthinones A, B et al.) (Makong et al., 2019) exhibited cytotoxicity, with IC50 values ranging from 50.0 ± 5.2 to 80.5 ± 1.8 μg/mL, with a single metabolite showing less cytotoxicity than the crude extract. Aqueous BJ extract (Kim et al., 2016) inhibited the growth of H1975 cells with an IC50 of 2,000 μg/mL while being the specific in NSCLC cells bearing L858R/T790M epidermal growth factor receptor (EGFR).

The investigation into the metabolites of BJ in lung cancer demonstrated that brusatol and dehydrobruceine B (DHB) effectively increased the concentration of reactive oxygen species (ROS) by activating the NRF2 pathway (Zhao et al., 2016; Xie et al., 2021). Furthermore, it activates the pro-apoptotic signal through the mitochondrial intrinsic pathway, resulting in the initiation apoptosis. BD is another monomer metabolite that can induce lung cancer cells through the same mechanism (Xie et al., 2019). BD also promotes apoptosis in lung cancer cells via the JNK pathway (Yang et al., 2013). In addition, it was observed that the AE-BJ [101] exhibited inhibitory effects on the proliferation of H1975 spheroid cells. AE-BJ was also found to reduce the proliferation of stem cells and enhance their apoptotic characteristics (Kim et al., 2021). It has been demonstrated that the metabolite exhibited inhibitory effects on the growth of implanted xenograft H1975 tumors, while simultaneously preserving the overall wellbeing of the animals. The observed inhibition may have something to do with apoptosis and stopping the growth of tumours, specifically in (NSCLC) with somatic epidermal EGFR mutations (Kim et al., 2016). Another preparation of BJ, BJO, induced apoptosis in A549 and H446 lung cancer cells, possibly molecularly mediated by the mitochondrial/caspase pathway by increasing ROS production (Wang et al., 2016a).

4.2.3 Cancer of the digestive system

4.2.3.1 Hepatocellular carcinoma (HCC)

Studies on liver cancer have shown that Brusatol, BD, and Bruceine B exhibit potent cytotoxic effects. Among them, the mechanisms of action of Brusatol and BD have also been preliminarily studied and are valuable for further research. The preparations BJO and AE-BJ also have the potential for further research.

The metabolites of BJ in liver cancer cells have primarily been investigated in Bel-7404, HEPG2, and SMMC 7721 cell lines. Among the metabolites tested, Brusatol exhibited the most potent cytotoxic effect in the SMMC 7721 cell line. Yadanziolide T and Yadanziolide B (Chen et al., 2011) exhibited cytotoxicity against Bel-7402 cells, with IC50 values of 3.5–4.5 μmol/L. Bruceantinol (Su et al., 2013a) had significant growth-inhibiting activity on Bel-7404 with an IC50 < 10 μmol/L. Javanicolide H, Bruceine B, Bruceine E, Bruceine H, Dehydrobrusatol, and Javanicolide E (Liu et al., 2012a) exhibited cytotoxicity against HEPG2 with IC50 values between 0.81 and 3.3 μmol/L. BD demonstrated notable cytotoxicity in HEPG2 cells, with an IC50 value of 1.2 μmol/L (Liu et al., 2012a). Bruceine B (Liu et al., 2011) had significant inhibitory activity against SMMC 7721 hepatocellular carcinoma cells with an IC50 of 0.15 μmol/L. Brusatol demonstrated inhibitory effects on cell viability in SMMC-7721, Huh7, and Hep3B liver cancer cells, with IC50 values of less than 0.064, 0.34, and 0.69 μmol/L, respectively (Liu et al., 2011; Ye et al., 2018). Additionally, it exhibited inhibitory effects on tumor invasion and migration. The IC50 value of BJ's aqueous extract (Chen et al., 2016) on Hep3B cells was found to be 4 mg/mL. It is worth noting that this extract not only promoted apoptosis in hepatoma cells but also inhibited the growth of derived stem cell-like cells. BJ water extract (Lau et al., 2005) was found to induce cell death in Hep3b cells with an IC50 of approximately 50 ug/mL.

Studies have found that BD and Brusatol exert anti-liver cancer effects through multiple signaling pathways. BD significantly inhibited tumor growth in mice with Hep3B, possibly by regulating the expression of miR-95 (Xiao et al., 2014). Another study found that BD affects the breakdown of proteins and the removal of β-catenin. It may also work as a Wnt/Notch crosstalk inhibitor, which means it works better with sorafenib to fight tumors (Cheng et al., 2017). Brusatol has exhibited notable effects in inhibiting proliferation, invasion, and metastasis in a murine model of liver transplantation tumor in humans. These effects are thought to happen through the PI3K/Akt/mTOR pathway and by messing with STAT3 (Ye et al., 2018; Lee et al., 2020).

In the preparation research of BJ, AE-BJ induced death of hepatocarcinoma cells may be related to the mitochondria-dependent pathway associated with caspase 3 activation (Lau et al., 2005). BJO (Shi et al., 2015) inhibited tumor growth in H22 mice, which could be linked to the degradation of energy metabolism, tumor proliferation, and immunomodulatory activity mediated by Akt. Another BJO preparation, Brusatol-BJO (Wang

et al., 2021) significantly increased the survival time of H22 ascites tumor-bearing mice, which could be linked to miRNA-29b and the mitochondria-associated pathway.

4.2.3.2 Other cancers of the digestive system

In gastrointestinal cancer research, several metabolites have demonstrated noteworthy cytotoxic effects and show promise for further investigation. Specifically, Bruceanol G has shown potential in oral cancer, while Brusatol has demonstrated promise in laryngeal cancer. Additionally, BD and Javanicolide H have displayed significant cytotoxic effects in gastric cancer. In pancreatic cancer, both Bruceine A and Brusatol have demonstrated potential therapeutic effects. BD and Brusatol have been investigated for their mechanisms of action in colorectal cancer, while the primary focus of research on preparations revolves around BJOE.

BJ phytochemicals have shown cytotoxic effects on oral cancer, laryngeal cancer, gastric cancer, and intestinal cancer cell lines *in vitro* studies, which we elaborated on in different tumors. Brujavanol A and B (Chumkaew and Srisawat, 2016) showed significant cytotoxicity to human oral cancer cells, with IC50 values of 3.40 and 6.45 μmol/L, respectively. Brujavanol E (Chumkaew et al., 2019) and Bruceanol G (Imamura et al., 1995) exhibited cytotoxic activity against human oral squamous cell carcinoma with IC50 values of 5.54 and 0.55 μmol/L, respectively. BJ extract (Majid et al., 2014) had anti-proliferative activity on KB and ORL-48 cells with IC50 of 24.37 ± 1.75 and 6.67 ± 1.15 ug/mL, respectively. Bruceanol G demonstrated excellent cytotoxicity in oral cancer, while BJ extract demonstrated superior anti-proliferative activity.

In vitro studies of throat cancer, Brusatol (Zhou et al., 2021) demonstrated substantial cytotoxicity to the human laryngeal cancer cell line Hep-2 in the investigation of throat cancer, with an IC50 of 0.53 μmol/L. The IC50 of AE-BJ (Lau et al., 2005) on esophageal squamous cell carcinoma SLMT-1 was around 50 ug/mL.

In gastric cancer cells, Yadanziolides T, BD, yadanziolide B (Chen et al., 2011), Javanicolide H, Bruceine B, D, E, H, Dehydrobrusatol, Javanicolide E (Liu et al., 2012a), and 24-epipiscidinol A (Chen et al., 2013a) exhibited cytotoxicity against BGC-823 cells with IC50 values of 0.52–4.85 μmol/L. Among the monomers, Javanicolide H showed the strongest effect on the BGC-823 gastric cancer cell line, with an IC50 of 0.52 μmol/L.

In vitro studies of pancreatic cancer cells, Bruceine A (Lu et al., 2021) exhibited cytotoxicity to MIA PaCa-2 cells with IC50 values of 0.029 μmol/L. Brusatol exhibited cytotoxicity to PANC-1 and SW1990 cell lines with IC50 values of 0.36 and 0.10 μmol/L, respectively (Zhao et al., 2011a). BD exerted cytotoxic effects on Hs68 cells with IC50 values > 30 μmol/L (Lau et al., 2009) and inhibited proliferation of Capan-2 cells with IC50 values of 1.1 μmol/L (Liu et al., 2012b). Among the above metabolites, Bruceine A showed the strongest cytotoxic effect in the *in vitro* study of pancreatic cancer.

In intestinal cancer cells, Bruceanol D, E, F (Imamura et al., 1993) exhibited cytotoxicity against HCT-8 ileocecal adenocarcinoma with ED50 values of 0.16–0.67 μmol/L. Brusatol, Bruceine B, BD, and Yadanziolide A (Liu et al., 2011) were evaluated for cytotoxicity against SW480 cell lines with IC50 values 0.1–28.5 μmol/L. Yadanziolides T, Yadanziolide B, Bruceine B, D, E, H (Chen et al., 2011; Liu et al., 2012a) exhibited cytotoxicity against HCT-8 with IC50 values of 1.3–6.7 μmol/L. Among the monomers, Bruceine H had the lowest IC50, indicating that its cytotoxicity was the most significant. In the preparation of BJ, Brucea javanica ethanolic extract (BJEE) exhibited cytotoxicity against HCT-116 and HT29 cells with IC50 values of 8.9 ± 1.32 ug/mL (Bagheri et al., 2018a), 48 ± 2.5 ug/mL, respectively (Bagheri et al., 2018b).

In the study of the mechanism of action, Brusatol exerted anti-tumor effects through multiple mechanisms of action in gastrointestinal tumors. Brusatol (Zhou et al., 2021) reversed the Hep-2 cell epithelial-mesenchymal transition (EMT) process, abolishing the Janus kinase 2 (JAK2)/STAT3 signaling pathway in laryngeal cancer cell line. Brusatol (Chen et al., 2021) induced SGC-7901 cell apoptosis in gastric cancer through the PI3K/AKT/NF-κB pathway. In addition, Brusatol (Xiang et al., 2017) induced apoptosis in PANC-1 and PATU-8988 cell lines, possibly through the JNK/p38 mitogen-activated protein kinases (p38 MAPK)/NF-KB/STAT3/b-cell lymphoma-2 (BCL-2) pathway. In colorectal cancer, Brusatol functions by facilitating the degradation process of hypoxia-inducible factor-1 (HIF-1α) (Oh et al., 2017), mediated by prolyl hydroxylase (PHD), while concurrently suppressing NRF2 (Evans et al., 2018). Another monomer metabolite with an important role in gastrointestinal tumors is BD. BD (Li et al., 2020) inhibited gastric cancer cell growth and proliferation through the LINC01667/miR-138-5p/Cyclin E1 pathway and induced cell cycle arrest in the s phase of gastric cancer cells. In pancreatic cancer, BD may cause an upregulation of caspase 3, 8, 9, and proapoptotic protein (BAK) protein levels, as well as a downregulation of BCL-2 expression. Furthermore, the p38-MAPK signaling pathway and mitochondrial pathway may also be involved in this process (Lau et al., 2009; Liu et al., 2012b). Furthermore, Bruceine A holds significant relevance in the investigation of digestive tract cancers. Bruceine A (Lu et al., 2021) inhibited pancreatic cancer cell growth by interacting with p38a MAPK P-loop residues. Interestingly, the apparent affinity of P38a MAPK to Bruceine A was higher than that of Brusatol. In the study of preparations, BJOE (Yan et al., 2015) stimulated HCT116 cancer cell apoptosis, which may be related to the reduction of light-chain 3 II (LC3-II), LC3-I expression and inhibition of autophagy. BJEE upregulated BAX, caspase 3, and caspase 9, and activated caspase via extrinsic and intrinsic mitochondria pathways to induce apoptosis (Bagheri et al., 2018b; Bagheri et al., 2018a).

4.2.4 Cancer of the reproductive system

Research on the use of BJ for treating reproductive system cancers has primarily focused on breast cancer, ovarian cancer, and cervical cancer. In the field of research on reproductive system cancer, several metabolites, including Brusatol, Bruceine A, Bruceine B, Bruceantinol, and Brujavanol E, have been investigated for their potential impact on breast cancer. Additionally, Bruceines B, BD, and H have shown significant cytotoxic effects against ovarian cancer. The mechanism of action of BD in breast cancer is Preliminary research has been conducted, and compound preparations mainly involve BJOE.

Brusatol, Bruceine B, BD, Bruceantinol, Bruceine A, Bruceantarin, and Brujavanol E (Liu et al., 2011; Su et al., 2013a;

Ye et al., 2015; Chumkaew et al., 2019) demonstrated significant cytotoxicity activity against MCF-7 cell lines with IC50 values of 0.08–10 μmol/L. Brusatol, Bruceantinol, Bruceine A, and Bruceantarin (Ye et al., 2015) demonstrated antineoplastic activity in MDA-MB-231 cells with IC50 values in the ranges of 0.081–0.238 μmol/L. Among the monomers of BJ against breast cancer, Bruceantinol showed the strongest cytotoxic effect. In the extract of BJ, the warmed water extract of BJ (Lau et al., 2005) induced cell death in the MDA-MB231 breast cancer cell line with an IC50 of approximately 50 ug/mL. BJ ethanolic extract (Haryanti et al., 2019) had anti-migratory effects on 4T1 cells at lower doses and exhibited selective cytotoxicity and induction of apoptosis at 90 ug/mL. In ovarian cancer, Bruceosides D, E, and F (Ohnishi et al., 1995) showed selective cytotoxicity in ovarian cancer cell lines, with log GI50 values in the range of −4.44 to −5.72. Javanicolide H, Bruceine B, D, E, H, Dehydrobrusatol, and Javanicolide E (Liu et al., 2012a) exhibited cytotoxicity against SKVO3, having IC50 values in the range 0.12–2.5 μmol/L. In the study of cervical cancer, Quassilactone A and B (Su et al., 2020) exhibited cytotoxic activity against HeLa with IC50 values of 78.95 ± 0.11 and 92.57 ± 0.13 μmol/L.

In the investigation of the mechanism of action, it was observed that BD triggered apoptosis, migration, and invasion of breast cancer cells by suppressing epithelial-mesenchymal transition (EMT) and activating the PI3K/AKT, p38 MAPK, and JNK signaling pathways (Luo et al., 2020; Mohan et al., 2021). Research on the mechanism of BJ's chemical preparations includes Ethanol Extract of BJ, BJOE, and BJO. Ethanol extract of BJ (Chen et al., 2020a) inhibited autophagy in MDA-MB-231 cells, possibly by activating the PI3K/AKT/mTOR pathway, while being non-toxic in non-target organs. BJOE (Ye et al., 2020) inhibited the expression of oncogene E6 and caused apoptosis in SiHa cells in a way that depended on the dose. This may have happened through the ERK/MAPK and NF-KB signaling pathways. BJO (Su et al., 2021) inhibited tumor growth in MDA-MB-231 xenograft mice, modulated the amino acid profile of the host through metabolic changes in the gut microbiota, and then activated mTOR.

4.2.5 Other cancers

Apart from its demonstrated anti-tumor efficacy in the aforementioned tumor types, BJ has also been employed for the therapeutic management of diverse malignancies, including nasopharyngeal cancer, glioma, renal cancer, bladder cancer, and osteosarcoma. Buceoside C, Bruceanol D, E, and F (Fukamiya et al., 1992; Imamura et al., 1993) demonstrated cytotoxic activity against human epidermoid carcinoma of the nasopharynx with ED50 < 0.15 μmol/L. Bruceanol D, E, and F (Imamura et al., 1993) exhibited cytotoxicity against TE-671 medulloblastoma with ED50 values of 0.14–0.22 μmol/L, respectively. Buceoside C, Bruceanol D, E, and F (Fukamiya et al., 1992; Imamura et al., 1993) have shown substantial cytotoxicity against melanoma with ED50 < 0.15 μmol/L. Meanwhile, Bruceoside D, E, and F (Ohnishi et al., 1995) demonstrated selective cytotoxicity in melanoma, with log GI50 values ranging from −4.25 to −4.64. Furthermore, Bruceoside D, E, F (Ohnishi et al., 1995) showed selective cytotoxicity in renal cancer cells with log GI50 values of −4.43 to −4.97. Bruceanol D exhibits the lowest ED50 value, which is measured at 0.14 μmol/L, suggesting a high level of safety.

In the study of anti-tumor mechanisms, Brusatol has been shown to induce apoptosis in head and neck tumors, nasopharyngeal carcinoma, and glioblastoma. Brusatol regulated the expression of key molecular targets (Bcl-xl, Bcl-2, Caspase-9, Caspase-7, Caspase-3, etc.) and proteins, and induced apoptosis through the STAT3 signaling pathway and the Akt/mTOR pathway (Lee et al., 2019; Guo et al., 2020; Dai et al., 2021). Additionally, Brusatol also exerted its inhibitory effect by downregulating the expression of extracellular matrix protein 1 (ECM1) in xenograft tumors of mice (Dai et al., 2021). Another significant metabolite is BD, which has been shown to potentially impede the proliferation and migration of osteosarcoma cells through the inhibition of the STAT3 signaling pathway. It effectively reduces STAT3 phosphorylation in mouse xenograft tumors (Wang et al., 2019). In the study of compound preparations, BJO (Lou et al., 2010) induced apoptosis of T24 bladder cancer cells, possibly by upregulating caspase-3 and caspase-9 expression by activating the caspase pathway and inhibiting the NF-KB and Cyclooxygenase-2 (COX-2).

Figure 10 presents an overview of the anti-tumor mechanism of BJ.

4.3 Other advances in pharmacological research of BJ

Besides the pharmacological effects listed above, BJ has been shown to have anti-tuberculosis, anti-microbial, anti-muscle atrophy, and lipolytic properties.

DeHydrobrustine, a quassinoid of BJ, has demonstrated inhibitory properties against *mycobacterium tuberculosis* strain H37Rv (Rahman et al., 1997). Research findings indicate that endophytic fungi derived from BJ's can impede the proliferation of various bacteria, such as *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus* (Zi-ning et al., 2014). One of the identified peptides, Brucin, which was isolated from BJ, exhibited a notable inhibitory impact on the growth of *Streptococcus* (Sornwatana et al., 2013). Additionally, the BJ extract showed antifungal properties against oral *candida in vitro*, suggesting that it might be helpful as a treatment for fungal infections (Nordin et al., 2013). Brucein A, Brucein B, Brucein C, 3′-hydroxybrucein A, Brusatol, and Bruceantinol (Lahrita et al., 2019) have been reported in the literature to exhibit lipolytic activity. Although the specific mechanism of action is still unclear, these metabolites have shown promising potential in the prevention and treatment of obesity. Moreover, BJ extract and BD can effectively correct splicing errors in surviving motor neuron 2 and alleviate symptoms related to spinal muscular atrophy in mice (Baek et al., 2019).

5 Clinical applications of BJ

5.1 Progress in preparation of BJ

BJ encompasses a diverse range of metabolites and various formulations. BJ has been commercially available in many forms, such as BJOE injection, BJO oral emulsion, and BJO soft capsules.

BJO preparations have been widely used clinically. However, reports of adverse reactions occur during clinical application,

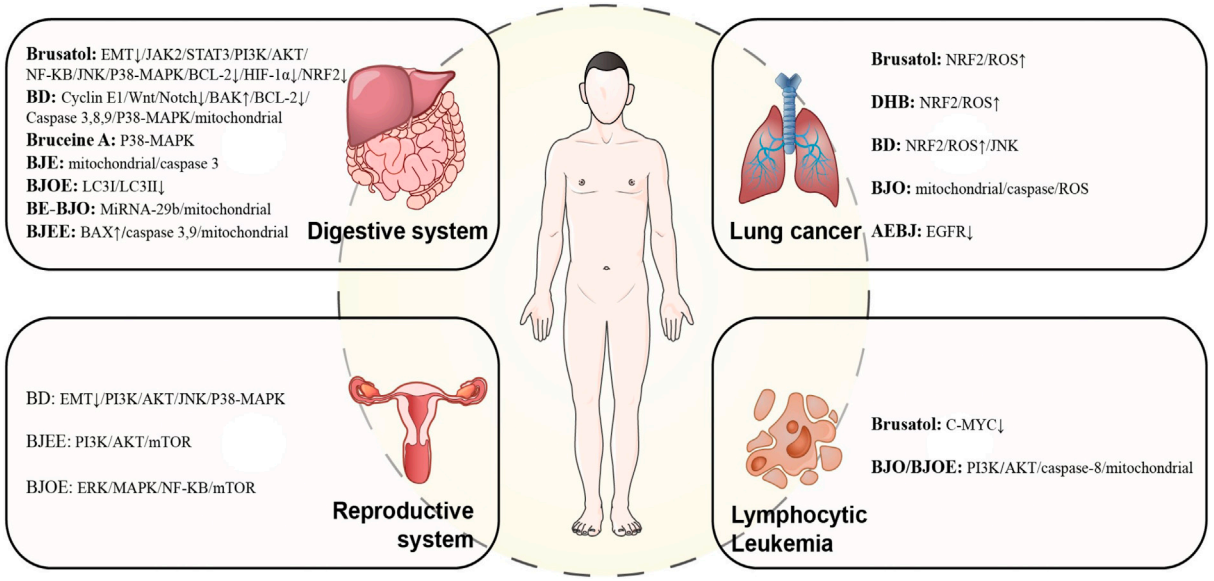


FIGURE 10
The pharmacological mechanism of BJ's anti-tumor.

including nausea, vomiting, liver damage, other digestive system damage, itching, rash, skin damage, and nervous system damage such as dizziness and headache (Dan et al., 2017). The adverse reaction to BJO preparation is attributed to the toxicity of its water soluble quassinoids (Rong and Qian, 2010). On the other hand, the primary constituent of BJO injection is oleic acid, which can interact with solvents, compromising its stability and leading to adverse reactions (Lina et al., 2020). Researchers conducted a study on novel formulations to enhance BJO's stability and mitigate its adverse effects. Through literature review, the preparations of BJ in research include BJ oil-loaded liposomes (Cui et al., 2010), BJO microemulsion (Yang et al., 2014), BJO cationic nanoemulsions (Liu et al., 2016), Luteinizing hormone releasing hormone receptor-targeted BJO liposomes (Ye et al., 2016), brusatol self-microemulsifying drug delivery system (Zhou et al., 2017), Self-assembled stable sponge-type nano-drug delivery system with BJO (Zou et al., 2017), self-nanoemulsifying drug delivery system of BD (Dou et al., 2018), self-microemulsifying drug delivery system (S-SMEDDS) of BJO (Huang et al., 2018), Disulfide-linked Pluronic-linoleic acid composed of redox-sensitive micelles of brusatol (Zhang et al., 2018a), BJO gastroretentive floating bead (Zhang et al., 2018b), Glycosaminoglycan-placental chondroitin sulfate-modified nanoparticle delivery brusatol system (Chen et al., 2020b), 3-β-homoalanine conjugate of brusatol (Hwang et al., 2020). These new preparations showed higher anti-tumor activity, better bioavailability, and lower toxicity to animals in the study.

5.2 Traditional clinical application of BJ

The existing body of literature predominantly centers around the traditional clinical application of BJ in treating dysentery, cutaneous warts, and corn. For more comprehensive information regarding the pertinent clinical studies, please consult Table 9.

5.3 Antitumor clinical application of BJ

5.3.1 BJ combined with radiotherapy and chemotherapy

Chemotherapy and radiation, as traditional cancer treatment methods, often come with severe side effects, including myelosuppression, mucositis, and hair loss (Cao et al., 2014). The advancement of molecular biotechnology has brought about targeted drugs that have proven to be beneficial for cancer patients. However, these drugs still face significant challenges, such as drug resistance and side effects, limiting their clinical application (Wu et al., 2022b). The combined use of BJ with radiotherapy and chemotherapy has been shown to enhance antitumor activity while minimizing cytotoxicity and toxic side effects. BJ is presently employed as adjunctive therapy for neoplasms and is anticipated to assume a more prominent function in the domain of anti-neoplastic interventions.

Cancers of the lungs, liver, stomach, pancreas, esophageal, and colon are the main types of cancer that use the BJ and chemotherapy medication combination. Out of these choices, combining BJOE with chemotherapy drugs has been the subject of a lot of research. It has shown impressive therapeutic benefits, such as better quality of life, more effective treatment, and fewer adverse events. The complex composition of BJOE poses a significant challenge to studying its mechanism of action in combination with chemotherapy drugs for malignancies. Therefore, using monomeric metabolites in BJ in combination with chemotherapy drugs for anti-tumor use has become a promising breakthrough. In lung cancer, dehydrobruceine B sensitizes A549 cells to cisplatin by regulating mitochondrial apoptosis, thereby reducing their resistance to cisplatin (Huang et al., 2017b). Canthin-6-one, when combined with cisplatin, induces cytotoxicity and has the potential for anti-drug resistance (Samat et al., 2017). It is noteworthy that the combination of Brusatol and chemotherapy

TABLE 9 Traditional clinical application of BJ.

Clinical application	Dosage form	Number of subjects	Result	Ref.
Dysentery	BJ capsule	12 cases	Effective rate: 91.7%	Qiang (1999)
Genital warts	BJ compound lotion	72 cases	Effective rate: 93.1%	Caiyong et al. (2019)
	BJ extract	34 cases	Effective rate: 94.1%	Jianmin et al. (2010)
	BJ extract	68 cases	Effective rate: 97.0%	Liwen and Wenlei (2016)
			HPV clearance rate: 55.9%	
	BJ compound lotion	63 cases	Effectiverate: 100%	Caiyong et al. (2009)
	BJ Cream	165 cases	Effective rate: 100%	Jumin (2006)
	BJ extract	60 cases	Effective rate: 92.3% (Warts < 2 mm)	Gangsheng et al. (2003)
			72.3% (2–5 mm)	
Flat wart	BJ tincture	260 cases	Effective rate: 93.4%	Ge (2001)
	BJ extract	46 cases	Effective rate: 82.6%	Hong (2007)
Plantar warts	BJ patch	60 cases	Effective rate: 88.3%	Fanxiu (2014)
	BJ Cream	60 cases	Effective rate: 90.8%	Hengan et al. (2008)
Corn	BJ extract	120 cases	Effective rate: 77.5%	Jianming (2004)
	BJ poultice	36 cases	Effective rate: 91.7%	Jianjun et al. (2000)

has shown a significant synergistic anti-tumor effect in colorectal cancer, pancreatic cancer, and pituitary tumors (Lu et al., 2017; Chen et al., 2018a; Wu et al., 2021). At the same time, research has found that brusatol can overcome the chemical resistance of a wide range of cancer types (Harder et al., 2017; Chen et al., 2018a). Based on the findings above, investigating the potential of combining brusatol with chemotherapeutic medications holds promise as a prospective avenue for future research. The integration of this combination has the potential to enhance therapeutic efficacy and present a novel therapeutic approach for individuals with drug-resistant tumors. Specific information on clinical studies is shown in Table 10.

5.3.2 BJ combined with other drugs

Several studies have shown that the co-administration of BJ with paclitaxel, cantharidin, baicalin, and other traditional Chinese medicines exhibits a synergistic anti-tumor effect (Ma et al., 2013; Ji et al., 2014; Chen et al., 2015). Furthermore, combining BJ with targeted drugs for cancer treatment has also shown promising results in synergistic anti-tumor effects and combating drug resistance in tumor cells (Peng et al., 2021; Wu et al., 2022a). In addition to the anti-tumor field, research has shown the efficacy of BJ combined with psoralen in treating Pneumocystis carinii infection (Qin et al., 2006; Dai et al., 2007). Clinical studies on BJ combined with other drugs to treat diseases are shown in Table 11.

It is noteworthy that the concurrent administration of BJ and other medications has demonstrated favorable clinical outcomes in various clinical settings. However, the reference value of these clinical studies is limited due to several factors, including small sample sizes, utilization of retrospective data, and inconsistent evaluation of clinical indicators. Another crucial aspect to consider is the inclusion of BJ in clinical studies alongside other medications, which frequently yields favorable clinical outcomes. These outcomes encompass the reduction of toxic side effects and the enhancement of drug efficacy. However, the potential adverse impacts in clinical practice are seldom demonstrated in clinical studies. Therefore, it is imperative to identify and select valuable combination regimens through rigorous clinical studies. Designing high-quality, large-sample, and authoritative clinical studies incorporating comprehensive evaluation indicators is crucial.

6 Discussion

In the research on the traditional pharmacological effects of BJ, preliminary studies have been conducted to investigate its pharmacological action mechanism in relation to anti-dysentery and treatment of skin warts and corns. BJ resists dysentery through the NF-κB and Nrf2 signaling pathways (Li et al., 2018; Zhou et al., 2018; Zheng et al., 2021)[25] and is used to treat genital warts by regulating the expression of TLR4 receptors (Weiqi et al., 2023). The research progress on the traditional pharmacological mechanism of BJ has not been addressed in other review literature. This article summarizes and elaborates on the traditional pharmacological mechanism of BJ to provide reference information for further research in this field. BJ's current research primarily focuses on the anti-tumor properties. In China, BJO and BJOE have been approved for the treatment of lung cancer, brain metastasis of lung cancer, and cancers affecting the digestive tract. The studies conducted by BJO and BJOE have demonstrated cytotoxic properties against various types of tumors (Zhang et al., 2011; Shi et al., 2015; Yan et al., 2015; Wang et al., 2016a). In the

TABLE 10 BJ combined with radiotherapy and chemotherapy.

Metabolites of BJ	Combination	Type of cancer	Mechanism/Advantage	Ref.
BJO	Radiotherapy	Esophageal cancer/ECA109 cells	Hypoxia-inducible factors 1α ↓	Pan et al. (2018)
BJOE	Radiotherapy	Esophageal cancer/EC109 cells	Cyclin D1-CDK4/6 axis	Qiu et al. (2019)
BJOE	Radiotherapy	Placental villous Carcinoma/JAR cells	Cyclin D1-CDK4/6 axis	Qiu et al. (2019)
BJO	Radiotherapy	Esophageal cancer	CRR ↑ RR ↑	Shan et al. (2011)
		80 patients	Karnofsky score ↑	
BJO	Radiotherapy	Esophageal cancer	CRR ↑ PRR ↑	Wang et al. (2017)
		1,269 patients	Quality of life ↑	
BJOE	Radiotherapy	Esophageal cancer	HIF-1α ↓	Pan et al. (2018)
		/20 patients	Apoptotic rate ↑	
BJO	Gemcitabine	Pancreatic cancer/mouse model	Survival rate ↑	Yang et al. (2020)
			Tumor growth rate ↓	
BJOE	Manganese	Endometrial cancer/RL95-2 cells	Proliferation ↓	Hu et al. (2021)
			Apoptosis ↑	
BJOE	Transarterial chemoembolization (TACE)	Liver cancer/64 patients	RR ↑	JIN et al. (2015)
BJOE	Oxaliplatin, Irinotecan, Paclitaxel, Docetaxel, Fluorouracil, etc.	Gastric cancer/75 patients	Karnofsky score ↑	Liu et al. (2013)
			Quality of life ↑	
BJ emulsion	FOLFOX4	Gastric cancer/150 patients	Vascular Endothelial Growth Factor ↓	Chen et al. (2018b)
			Recurrence rate ↓	
BJOE	Oxaliplatin, Cisplatin paclitaxel, etc.	Gastric cancer/912 patients	RR ↑ PS ↑	Wu et al. (2018)
			ADR ↓	
BJOE	CapeOX chemotherapy	Rectal cancer/80 patients	Relieve nausea	Meng et al. (2021)
BJOE	PP chemotherapy	Lung cancer/58 patients	Quality of life ↑ ORR ↑	Lu et al. (2013)
			Incidence of leukopenia ↓	
BJO	Doxorubicin	Breast cancer/MCF-7 cells	Apoptosis ↑	Li et al. (2019b)
			Resistance ↓	
Dehydrobruceine B	Cisplatin	Non-small-cell Lung carcinoma/ A549 cells	Regulation of mitochonDrial apoptosis pathway	Huang et al. (2017b)
			Resistance ↓	
Canthin-6-one	Cisplatin	Prostate/PC3 cells	Block G 2/M cancer cells	Samat et al. (2017)
		Cervical cancer/HeLa cells	Resistance ↓	
Brusatol	Cisplatin	Colon cancer/CT-26 cells	Procaspase-3/9 ↓	Chen et al. (2018a)
			Cytochrome c/BCL-2 ↑	
Brusatol	Gemcitabine/5-fluorouracil	Pancreatic cancer	Inhibit the activation of NF-κB	Lu et al. (2017)
		/PANC-1 cells		
Brusatol	Gemcitabine/5-fluorouracil	Pancreatic cancer/mouse model	E-cadherin expression ↑	Lu et al. (2017)
			Resistance ↓	

(Continued on following page)

TABLE 10 (*Continued*) BJ combined with radiotherapy and chemotherapy.

Metabolites of BJ	Combination	Type of cancer	Mechanism/Advantage	Ref.
Brusatol	Cabergoline	Pituitary adenoma/GH3/MMQ cells mouse model	ROS ↑	Wu et al. (2021)
			Inhibit the mTORC1 signaling pathway	
Brusatol	Metformin	Endometrial cancer cells	Nrf2/AKR1C1 ↓	Wang et al. (2016b)
			Progesterone resistance ↓	
Brusatol	Cisplatin/Desferal	Ovarian cancer cells	Nrf2 ↓ SLC40A1 ↑	Wu et al. (2017)
			Resistance ↓	
Brusatol	Oxaliplatin	Colorectal cancer LS174T cells	Nrf2 ↓ P-glycoprotei ↑	Mostafazadeh et al. (2022)
			Resistance ↓	

CRR, Complete Response rate; PRR, partial response; RR, Response rate; PS, performance status; ADR, Adverse reactions; ORR, overall response rate; ↑: rise ↓: decline.

TABLE 11 BJ combined with botanical drug and targeted drug.

Metabolites of BJ	Combination	Type	The advantage of combination	Ref.
BJO	Docetaxel	Rat Model	AUC ↑ T1/2 ↑	Ma et al. (2013)
BJ	Cantharidin	Clinical study	Quality of life ↑	Ji et al. (2014)
BJO	Baicalin	Cell experiment/A549 cells	Apoptosis ↑	Chen et al. (2015)
BJO	Anlotinib	Mouse Model/Small-cell lung cancer	Liver metastases ↓	Peng et al. (2021)
			Angiogenesis ↓ weight loss ↓	
Bruceine H	Gefitinib	Xenografted tumors in nude mice	PC-9/GR ↓	Wu et al. (2022a)
			NOTCH3 ↓	
Brusatol	Paclitaxel	Breast cancer/MCF-7 cells	Nrf2 ↓ ROS ↑	Wu et al. (2015)
		MDA-MB-231 cells	Resistance ↓	
BD	Sorafenib	Hepatocellular carcinoma/Huh7, Hep3B cells/ Mice	Apoptosis ↑	Cheng et al. (2017)
			Tumor growth ↓	
			Jagged1 expression ↓	
BJOE	Psoralea corylifolia	Pneumocystis carinii disease rats	Immunomodulation ↑	Qin et al. (2006)
			Pneumocystis carinii ↓	
BJOE	Psoralea corylifolia	Pneumocystis carinii disease rats	IL-2 ↑ NK cell activity ↑	Dai et al. (2007)
			Immunomodulation ↑	

AUC, Area under the curve.

study of the mechanism of action, BJ mainly plays a role in lymphocytic leukemia through the PI3K/Akt signaling pathway (Zhang et al., 2011; Zhang et al., 2021), lung cancer through the NRF2 (Zhao et al., 2016; Xie et al., 2021) and JNK (Yang et al., 2013) signaling pathways. The Wnt/Notch (Cheng et al., 2017) and PI3K/Akt/mTOR (Ye et al., 2018) signaling pathways are involved in liver cancer. It also plays a role in laryngeal cancer through the JAK2/STAT3 signaling pathway (Zhou et al., 2021). The p38 MAPK signaling pathway (Lau et al., 2009; Liu et al., 2012b; Xiang et al., 2017) has been found to play a role in lymphoma and gastrointestinal cancer. A role in breast cancer is played by the PI3K/AKT, p38 MAPK, and JNK signaling pathways (Luo et al., 2020; Mohan et al., 2021). Existing studies have shown that BJ exerts pharmacological effects through multiple signaling pathways. It should be noted that the current research only extensively explores the signaling pathways of pharmacological effects and does not indicate which signaling pathways are primarily involved in different pharmacological effects. At the same time, the effects of BJ on genes and proteins associated with the upstream and downstream signaling pathways have not been thoroughly

studied. Research on the pharmacological mechanism of BJ lacks a complete signaling pathway system as theoretical support.

BJO and BJOE have been widely used in clinical practice. Nevertheless, numerous instances of adverse responses have been observed during the clinical implementation, encompassing manifestations such as rash, vomiting, dizziness, palpitations, and various other untoward effects (Dan et al., 2017). About medicinal aspects, BJ possesses a diverse array of metabolites and exhibits inherent toxicity attributed to its water-soluble quassinoid chemicals. At the same time, excipients such as soy lecithin and glycerol are added, and the purity and stability of BJO and BJOE are easily changed during the production, storage, and use processes, leading to adverse drug reactions (ADR). Due to the complexity of BJO and BJOE formulas and the occurrence of adverse reactions, the widespread application of BJ worldwide is limited. Another issue that requires attention with BJO and BJOE is the addition of multiple excipients, making it challenging to identify adulteration of the preparations. Huang et al. used direct injection-electrospray ionization ultra-high resolution mass spectrometry technology to identify 69 metabolites in BJO for adulteration identification of BJO, providing theoretical support for the quality standards of BJO (Yan et al., 2023). Limited by the aforementioned factors, the clinical application of BJO and BJOE has not been able to progress towards broader implementation. At present, there have been many studies on the improvement of preparations (Cui et al., 2010; Yang et al., 2014; Liu et al., 2016). However, these new preparations are limited by factors such as clinical efficacy and drug economy. They still require a significant number of high-quality studies to be conducted to verify their suitability for clinical applications.

It is precisely because of the complexity of the metabolites of BJO and BJOE that studying the pharmacological mechanism of action becomes difficult. Studying the pharmacological mechanism of the metabolites of BJ monomer may lead to future breakthroughs. Among the monomer metabolites of BJ, we screened out 15 metabolites with research potential. In traditional pharmacological research of BJ, five monomer metabolites exhibit strong pharmacological activity. The monomeric metabolites include Brusatol, Bruceine A, and Bruceantinol, which have anti-dysenteric effects (Subeki et al., 2007; Zhou et al., 2018). Bruceantin has shown the most potent anti-malarial effect (Guo et al., 2005). BD and brusatol are potentially effective in treating warts and corn (Jingli et al., 2017; Weiqi et al., 2023), and the hypoglycemic effect of BD and Bruceine E warrants further research (NoorShahida et al., 2009). Modern research on BJ mainly focuses on its pharmacological effects in treating tumors. *In vitro* cell line studies, 14 monomeric metabolites exhibited significant cytotoxic effects. Bruceanol D, Brusatol, and Bruceantin exhibit strong cytotoxic effects in lymphocyte cell line studies (Imamura et al., 1993; Mata-Greenwood et al., 2002; Liu et al., 2011). Brusatol, Bruceloside C, and BD have demonstrated potent anti-tumor effects in lung cancer cell line studies (Fukamiya et al., 1992; Liu et al., 2011; Tan et al., 2019). Brusatol, BD, and Bruceine B exhibit significant cytotoxic effects on liver cancer cell lines (Liu et al., 2011; Liu et al., 2012a). Bruceanol G exhibits potent cytotoxicity in oral cancer (Imamura et al., 1995), while Brusatol is effective against laryngeal cancer (Zhou et al., 2021). BD and Javanicolide H exhibit potent cytotoxic effects in gastric cancer (Chen et al., 2011; Liu et al., 2012a), while BD, busatol and Bruceine H demonstrate efficacy in colorectal cancer (Chen et al., 2011; Liu et al., 2011). Bruceine A and brusatol showed the strongest cytotoxic effect in pancreatic cancer (Zhao et al., 2011a; Lu et al., 2021). Brusatol, Bruceine A, Bruceine B, Bruceantinol, and Brujavanol E exhibit cytotoxic solid effects against breast cancer (Okano et al., 1985; Su et al., 2013a; Ye et al., 2015; Chumkaew et al., 2019). Bruceine B, BD, and Bruceine H exhibit strong cytotoxic effects on ovarian cancer (Liu et al., 2012a). Bruceanol D, E, and F exhibit strong cytotoxic effects on nasopharyngeal squamous cell carcinoma, melanoma, and medulloblastoma (Lee et al., 1977; Fukamiya et al., 1992; Imamura et al., 1993). Through the study of BJ monomer metabolites, researchers screen out metabolites with research potential and explore the possibility of applying these compounds in clinical practice in the future.

The different metabolites of BJ possess diverse pharmacological properties. However, both the traditional pharmacological effects of BJ and the related mechanisms of modern pharmacological applications are still in the preliminary exploration stage. A comprehensive gene signaling pathway has not yet been established to elucidate BJ's pharmacological effects fully. Secondly, BJO and BJOE have been utilized in clinical applications. However, it is essential to note that adverse reactions may occur due to drug and preparation factors during clinical application. Finally, BJO and BJOE have different metabolites, making it quite difficult to explore their exact pharmacological mechanisms of action. Exploring the monomer metabolites for clinical practice could be a significant breakthrough.

7 Conclusion

In summary, the existing evidence regarding the safety and effectiveness of BJO and BJOE in clinical applications is confined mainly to clinical observational studies and needs robust evidence-based support. In terms of researching the mechanism of action, it is very challenging to study the mechanism of action of BJO and BJOE due to their multiple ingredients. There have been reports of adverse reactions in the clinical use of BJO and BJOE. The factors above constrain the global promotion of BJ. We identified 15 metabolites with research potential in BJ and aim to investigate further the potential application of these metabolites in clinical practice in the future. We suggest that future research should focus on three main areas: integrating the pharmacological mechanisms of BJ, establishing a comprehensive gene signaling pathway system, and providing theoretical support for clinical applications. The second approach is to improve the preparations of BJ by selecting safer and more soluble excipients, enhancing the stability of BJO and BJOE, and reducing the occurrence of adverse reactions. The third step is to screen monomer metabolites with research potential for further investigation and to explore their potential for clinical application.

Although BJ currently faces many challenges in more comprehensive clinical implementation, the continuous deepening of research in this field and the application of new technologies such as molecular docking technology, high-throughput screening technology, and cell membrane chromatography technology will undoubtedly accelerate the development of BJ. BJ will have broader clinical applications worldwide.

Author contributions

JC: Investigation, Visualization, Writing–original draft. DY: Conceptualization, Writing–review and editing. XL: Investigation, Methodology, Writing–original draft. QD: Investigation, Methodology, Writing–original draft. HY: Visualization, Writing–original draft. LC: Supervision, Writing–review and editing. LB: Writing–review and editing.

Funding

The authors declare financial support was received for the research, authorship, and/or publication of this article. This work was supported by Rongsheng Tong The 4th Sichuan Top Ten Famous Chinese Medicine Studio Construction Project (2023), the Sichuan Science and Technology Program (2023NSFSC0685), the Scientific research project of Sichuan Medical Association (S22084), the Open Research Fund of State Key Laboratory of Southwestern Chinese Medicine Resources (SKLTCM2022019); and the Science and Technology Research Project of Sichuan Administration of Traditional Chinese Medicine (2023MS222).

Conflict of interest

Author HY was employed by Power China Chengdu Engineering Corporation Limited.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The handling editor RS declared a parent affiliation with the author(s) at the time of review.

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Glossary

TCM	Traditional Chinese Medicine
CBD	Chinese botanical drugs
BJ	Brucea javanica
NRF2	nuclear factor erythroid-2 related factor 2
JNK	Jun n-terminal kinase
STAT3	signal transducer and activator of transcription 3
NF-κB	nuclear factor kappa-B
RhoA/ ROCK	member A/rho-associated kinase
BJO	Brucea javanica oil
BJOE	Brucea javanica oil emulsion
TLR4	toll-like receptor 4
HPV	human papillomavirus
DNA	Deoxyribonucleic acid
BD	Bruceine D
EAF	ethyl acetate fraction
BJE	Ethyl acetate Extract of BJ
ILS	the ratio of mean murine life span in the experimental group to murine in the control group
c-MYC	cellular-myelocytomatosis viral oncogene
PI3K	Phosphoinositide 3-kinase
Akt	Protein kinase B
EGFR	epidermal growth factor receptor
DHB	Dehydrobruceine B
ROS	Reactive Oxygen Species
NSCLC	non-small-cell lung carcinoma
AE-BJ	Aqueous BJ extract
HCC	Hepatocellular -carcinoma
mTOR	mammalian target of rapamycin
EMT	epithelial-mesenchymal transition
JAK2	janus kinase 2
p38 MAPK	p38 mitogen-activated protein kinases
BCL-2	b-cell lymphoma-2
PHD	prolyl hydroxylases
HIF-1α	hypoxia-inducible factor-1
BAK	proapoptotic protein
ERK	extracellular regulated protein kinases
MAPK	mitogen-activated protein kinase
ECM1	extracellular matrix protein 1
LC90	Lethal Concentration 90
MIC	minimum inhibitory concentration
LC3-II	light-chain 3 II
BJEE	Brucea javanica ethanolic extract
COX-2	Cyclooxygenase-2
S-SMEDDS	self-micro emulsifying drug delivery system
CRR	Complete Response rate
PRR	partial response
RR	Response rate
PS	performance status
ADR	Adverse reactions
ORR	overall response rate
AKR1C1	Aldo-keto reductase family 1 member C1
SLC40A1	Solute Carrier Family 40 Member 1

frontiers | Frontiers in Pharmacology

TYPE Systematic Review
PUBLISHED 14 February 2024
DOI 10.3389/fphar.2024.1366852

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RECEIVED 07 January 2024
ACCEPTED 02 February 2024
PUBLISHED 14 February 2024

CITATION
Cao S, Wei Y, Yue Y, Chen Y, Liao S, Li A, Liu P, Xiong A and Zeng H (2024), Targeting ferroptosis unveils a new era for traditional Chinese medicine: a scientific metrology study. *Front. Pharmacol.* 15:1366852. doi: 10.3389/fphar.2024.1366852

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Targeting ferroptosis unveils a new era for traditional Chinese medicine: a scientific metrology study

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In the past 11 years, there has been a surge in studies exploring the regulatory effect of Traditional Chinese Medicine (TCM) on ferroptosis. However, a significant gap persists in comprehensive scientometric analysis and scientific mapping research, especially in tracking the evolution, primary contributors, and emerging research focal points. This study aims to comprehensively update the advancements in targeting ferroptosis with various TCMs during the previous 11 years. The data, covering the period from 1 January 2012, to 30 November 2023, were retrieved from the Web of Science database. For in-depth scientometric and visualized analyses, a series of advanced analytical instruments were employed. The findings highlight China's predominant role, accounting for 71.99% of total publications and significantly shaping research in this domain. Noteworthy productivity was observed at various institutions, including Guangzhou University of Chinese Medicine, Chengdu University of Traditional Chinese Medicine, and Zhejiang University. Thomas Efferth emerged as the foremost author within this field, while *Frontiers in Pharmacology* boasted the highest publication count. This study pinpointed hepatocellular carcinoma, chemical and drug-induced liver injury, mitochondrial diseases, acute kidney injury, and liver failure as the most critical disorders addressed in this research realm. The research offers a comprehensive bibliometric evaluation, enhancing our understanding of the present status of TCM therapy in managing ferroptosis-related diseases. Consequently, it aids both seasoned researchers and newcomers by accelerating access to vital information and fostering innovative concept extraction within this specialized field.

KEYWORDS

global scientific frontiers, ferroptosis, Traditional Chinese Medicine, bibliometrics, visualized analysis

1 Introduction

Ferroptosis represents a novel form of programmed cell death (PCD), characterized by elevated intracellular iron levels and the accumulation of lipid reactive oxygen species within the cell (Dixon et al., 2012). Over the last 11 years, a global accumulation of evidence has emerged, highlighting the potential crucial role of ferroptosis in various biological processes such as tumor suppression and immunity (Sun et al., 2023). This underscores the significance of ferroptosis in health maintenance, achieved through the regulation of metabolism and redox homeostasis. Recent research has unveiled ferroptosis's critical involvement in various pathophysiological processes, including ischemic organ injury, stroke, cardiac myopathy, and neurodegenerative diseases. Moreover, ferroptosis has been implicated in various oncogenic pathways, suggesting its potential as an innovative target for cancer therapeutics (Friedmann Angeli et al., 2019; Wang X. et al., 2023). The annual publication of studies on ferroptosis is currently experiencing exponential growth (Stockwell, 2022). By the end of 2023, more than 10,000 papers worldwide had been published on this topic (https://www.ncbi.nlm.nih.gov), emphasizing its significance in maintaining health through the regulation of iron/lipid metabolism and glutathione (GSH)-dependent redox homeostasis (Stockwell, 2022; Wang X. et al., 2023; Sun et al., 2023). Considering the potential therapeutic relevance of ferroptosis regulatory mechanisms, the refinement of ferroptosis-mediated therapeutics holds profound clinical significance for treating a spectrum of diseases (Sun et al., 2023).

Traditional Chinese Medicine (TCM) stands as an extensive reservoir of practical clinical knowledge amassed over millennia, significantly contributing to human health (Pitschmann et al., 2013; Gao et al., 2022). It finds its roots in Chinese philosophy, emphasizing the harmonious equilibrium between humanity and the natural world. TCM encompasses fundamental theories including Yin and Yang, the Five Elements, zang-fu, channels-collaterals, qi, blood, body fluid, diagnostic methods, and the differentiation of symptom-complexes (Hao and Keji, 2003). It distinguishes itself through holistic treatment strategies, emphasizing syndrome differentiation and utilizing natural products guided by taste, Yin-Yang qualities, and active pharmaceutical ingredients (Chan, 1995; Dixon et al., 2012; Cragg and Newman, 2013). With its diverse components, objectives, connections, and pathways, TCM presents distinct clinical advantages in both disease prevention and treatment (Wang et al., 2020; Shi et al., 2023). In 2018, a noteworthy achievement was reached when the World Health Organization, for the inaugural time, incorporated TCM into its influential worldwide medical compendium (Wu et al., 2021). In the realm of oncology, particular active pharmaceutical ingredients in TCM act as ferroptosis inducers, inducing PCD and eliminating malignant cells (Wang et al., 2023b). Conversely, specific components of TCM serve as ferroptosis inhibitors, reducing cytotoxic damage in healthy cells like chondrocytes, neurons, hepatocytes, and cardiomyocytes due to irregularities in iron/GSH/lipid metabolism (Wang et al., 2020; Wu et al., 2021; Wu et al., 2022; Zhao et al., 2022; Cao et al., 2023a). This characteristic is in alignment with the TCM philosophical concept of Yin and Yang, which encompasses unity, opposition, and mutual transformation.

Although several reviews on "ferroptosis-TCM" have been published (Wang et al., 2020; Wu et al., 2021; Gao et al., 2022; Liu et al., 2022; Wu et al., 2022; Zhao et al., 2022; Zhang X. et al., 2023; Wang et al., 2023b; Ding et al., 2023; Shi et al., 2023; Xie et al., 2023), there remains a significant need for comprehensive scientometric analyses and detailed summaries encompassing developmental trends, principal institutions/authors, and central research themes. Therefore, this study aims to comprehensively update the advancements in targeting ferroptosis with various TCMs during the previous 11 years. Aiming to be a significant resource for both established experts and novices, this study offers thorough evaluations, identifies emerging research trends, and informs future research strategies. Adopting a visualization-focused approach significantly enhances research productivity and effectiveness in this domain. The current study, as far as we know, provides the first comprehensive analysis of this topic from a scientometric viewpoint.

2 Methods

2.1 Methodology for data retrieval

Widely embraced for its standardized, high-quality academic publication data, the Web of Science Core Collection (WoSCC) (https://www.webofscience.com/wos/) is a renowned resource in bibliometric analysis for tracking the evolution of scientific frontiers (Boudry et al., 2018; Zhang XL. et al., 2020; Pei et al., 2022; Ling et al., 2023). It provides bibliometric software with a source of general statistics (Pei et al., 2022), and the accuracy of document type labeling in the WoSCC has been demonstrated to be superior to that of other databases (Yeung, 2019). This study involved a comprehensive online search within the WoSCC, with a focus on original researches and reviews pertaining to "ferroptosis-TCM". The search covered publications from 1 January 2012, to 30 November 2023, using both Medical Subject Heading terms and free words for data extraction. The search strategy was refined through multiple iterations by three researchers (SYC, AKL, and SL) to fortify its sensitivity and precision. The Supplementary Materials contain an extensive exposition of the search methodology.

2.2 Inclusion and exclusion criteria

This study strictly adhered to well-defined eligibility criteria. The scope encompassed research pertaining to "ferroptosis-TCM", with specific attention given to original investigations and reviews issued in scientific journals using the English language. Excluded were dissertations, letters, commentaries, editorials, conference abstracts, and works with the same or similar titles published in different journals. The inclusion and exclusion criteria were finalized through discussions among team members and peer groups.

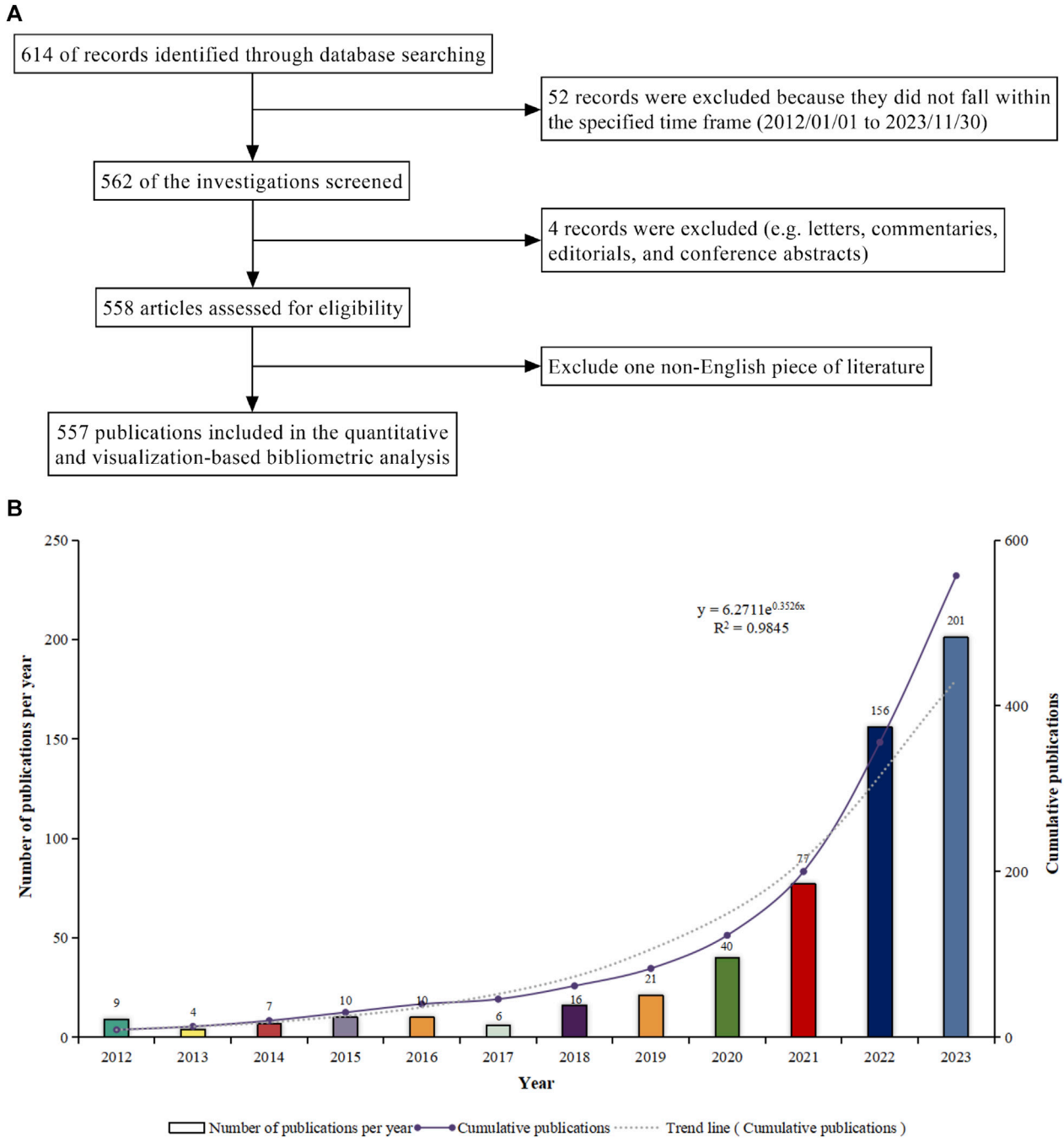


FIGURE 1
(A) Schematic representation of the literature search and selection process. **(B)** Trend analysis of research on "ferroptosis-traditional Chinese medicine" from 2012 to 2023.

2.3 Statistical analysis

Research data were gathered from the WoSCC and processed using specific software tools: WPS Office 12.1.0 (Kingsoft Office, China) for data organization, VOSviewer 1.6.18 (Leiden University, Netherlands) and Pajek 64 5.16 (University of Ljubljana, Slovenia) for co-occurrence analysis, Citespace version 6.2.6R (developed by Chaomei Chen, China) for visual mapping, Scimago Graphica version 1.0.35 (https://www.graphica.app/, USA) for graphical analysis. Additionally, various R packages (R Studio, version 4.2.0) including chorddiag, enrichplot, ggplot2, and clusterprofiler were utilized for generating specialized graphics.

The chorddiag R package and VOSviewer were employed to generate maps depicting national/regional collaboration and publication analysis charts. For co-occurrence analysis involving institutions, authors, journal publications, keywords, and diseases, a combination of VOSviewer, Scimago Graphica, and Pajek was utilized. Citespace was used for visualized analysis and mapping

of data related to countries/regions, institutions, authors, journals, co-citations, and keywords. In addition, we employed the clusterProfiler, enrichplot, and ggplot2 R packages to carry out enrichment analyses for Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways.

Data regarding disease information were obtained from the Citexs Data Analysis Platform (https://www.citexs.com). This platform facilitates the generation of relevant visual graphs, enabling an in-depth analysis of the current state, key focus areas, and emerging trends within this field of study.

3 Results and discussion

Staying abreast of industry developments and comprehending the latest research findings in the current era of information proliferation pose escalating challenges. With the purpose of disseminating the contemporary state of research pertaining to "ferroptosis-TCM" on a global scale, encompassing data from 2012 to 2023, we have applied bibliometric analysis. This methodology provides a pioneering approach for the management and elucidation of knowledge frameworks within specific research domains (Wang C. et al., 2021; Chen et al., 2022; Han et al., 2022).

3.1 Annual output and trends

Figure 1A depicts the procedure of data retrieval and collection. The quantity of scientific reports produced by a study within a given timeframe indicates its research progress (Koo, 2021; Li Z. et al., 2022; Sun G. et al., 2022). From 2012 to 2023, we compiled 557 relevant scientific reports on "ferroptosis-TCM", comprising 458 original articles and 99 reviews, with an annual average of 50.64 publications. In the past 11 years, there has been a continuous increase in academic production within this domain. Commencing from 2021, the annual tally of pertinent publications surpassed 50, culminating at 201 in 2023. This surge stands for a more than 22-fold increase since 2012, with a yearly growth rate of 48.61%. This trend underscores the continuous rise in research activity and the significant importance of this field. An exponential equation ($y = 6.2711e^{0.3526x}$, where x is the year and y the yearly publication count, $R^2 = 0.9845$) was employed to accurately depict the annual pattern, resulting in a well-fitted curve (Figure 1B). This growth reflects a dynamic research landscape, contributing extensive knowledge that enriches both present and future studies. Consequently, we anticipate substantial advancements in "ferroptosis-TCM" research in the foreseeable future.

The rise in academic publications signifies a noteworthy inclination towards investigating the regulatory impacts of TCM on ferroptosis. This pattern underscores the swift expansion of this domain, drawing in a broader spectrum of researchers and accentuating its escalating prominence within the academic sphere. This anticipated expansion could also engender increased financial support, expertise, and attention from diverse stakeholders. Potential breakthroughs in understanding ferroptosis and its integration with TCM could have profound implications, such as the development of innovative treatments, diagnostic tools, or therapeutic strategies. Industry practitioners, including pharmaceutical companies and healthcare providers, should take note of the rising research activity in this area.

3.2 Global research landscape

Global research on "ferroptosis-TCM" involves 57 countries/regions. To create maps of national collaboration networks, a minimum threshold of two publications per country/region was employed, as depicted in Figures 2A, B. Notably, China leads with 401 publications, constituting 71.99% of the total research output in this field. This highlights China's significant role in advancing knowledge in this area. Following China, the USA and Germany contribute 7.18% (40 publications) and 3.77% (21 publications), respectively, to global research on "ferroptosis-TCM", demonstrating the worldwide interest and involvement in this research.

The chord diagram utilizes peripheral curve segments to represent different countries and regions, with each segment's length indicating the corresponding publication volume (Cao et al., 2023b). Moreover, the extent of connectivity among nations reflects the degree of their collaborative engagement. Notably, significant international collaboration was observed during the study period. The most frequent academic collaborations were observed between China and the USA, exhibiting a notable link strength of 13 (Figure 2B). The number of publications serves as an indicator of a nation or region's prominence within this domain (Joshi, 2014). China and the USA, as leaders in publications related to "ferroptosis-TCM", underscore their substantial academic influence. This robust collaboration has the potential to propel theoretical progress and tackle ongoing field-specific challenges (Dara et al., 2017). Such collaboration can facilitate the interchange of ideas, resources, and expertise, potentially accelerating theoretical advancements and addressing challenges in the field. Researchers and practitioners from different countries should consider leveraging these collaborations for mutual benefit.

Citation bursts play a vital role in the identification of publications that have witnessed notable surges in citations over a defined time frame (Cao et al., 2023b). These bursts shed light on the dynamic trends and directions within research fields. Analyzing publications experiencing rapid citation increases aids in identifying emerging trends within areas of high scholarly interest. Figure 2C illustrates the citation bursts for the leading 10 nations, with the red line denoting the burst magnitude for each country. During the period between 2013 and 2019, Iran witnessed a significant surge in publication citations (strength = 4.55), closely followed by England (strength = 3.64), indicating emerging trends and areas of high scholarly interest. Researchers and industry practitioners should pay attention to these countries and their specific research directions, as they may provide valuable insights and opportunities for further exploration and collaboration. Researchers and industry professionals can use this information to identify potential partners and track the evolution of research collaborations. This can help them gain a better understanding of the global distribution of expertise in the field.

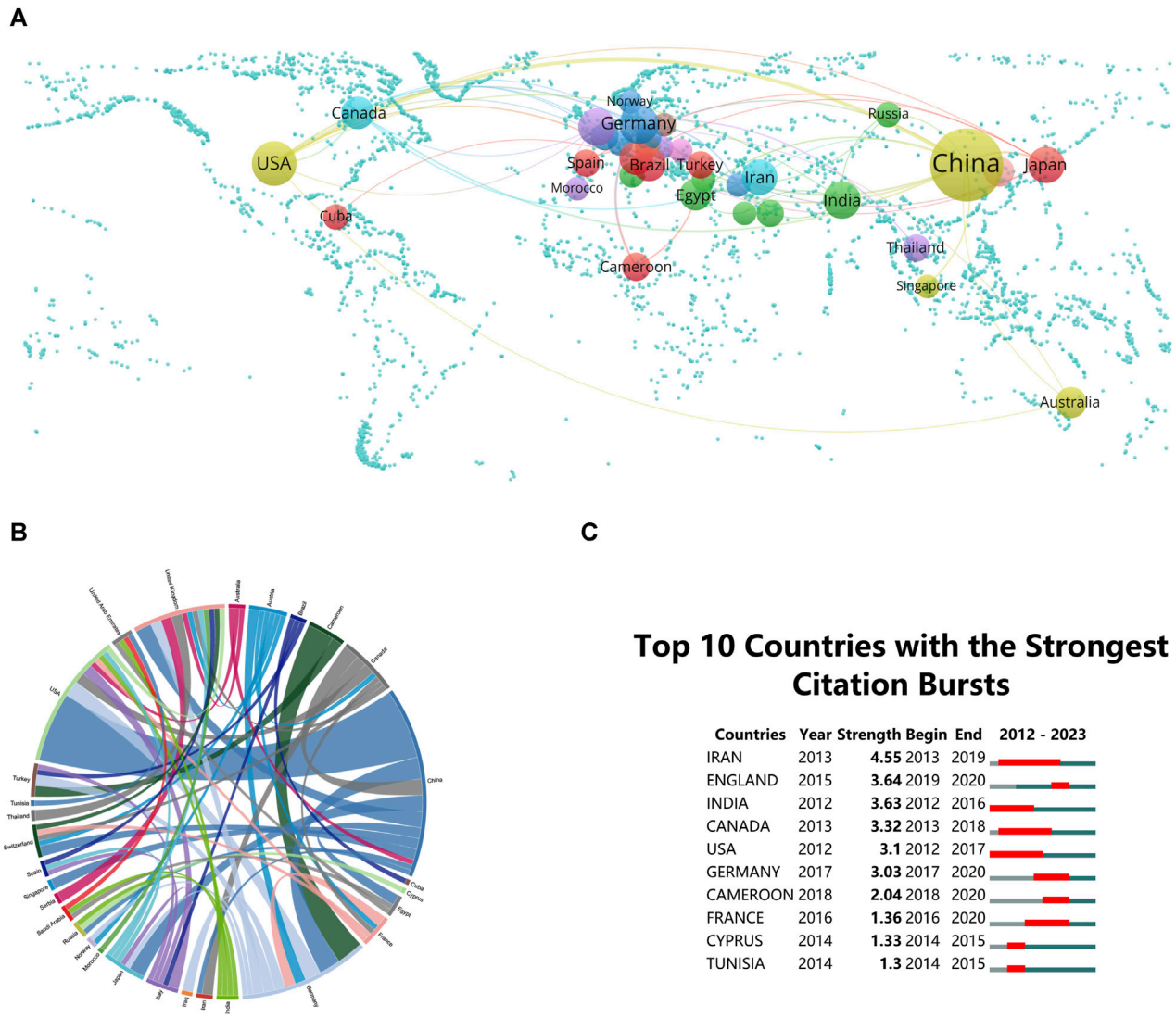
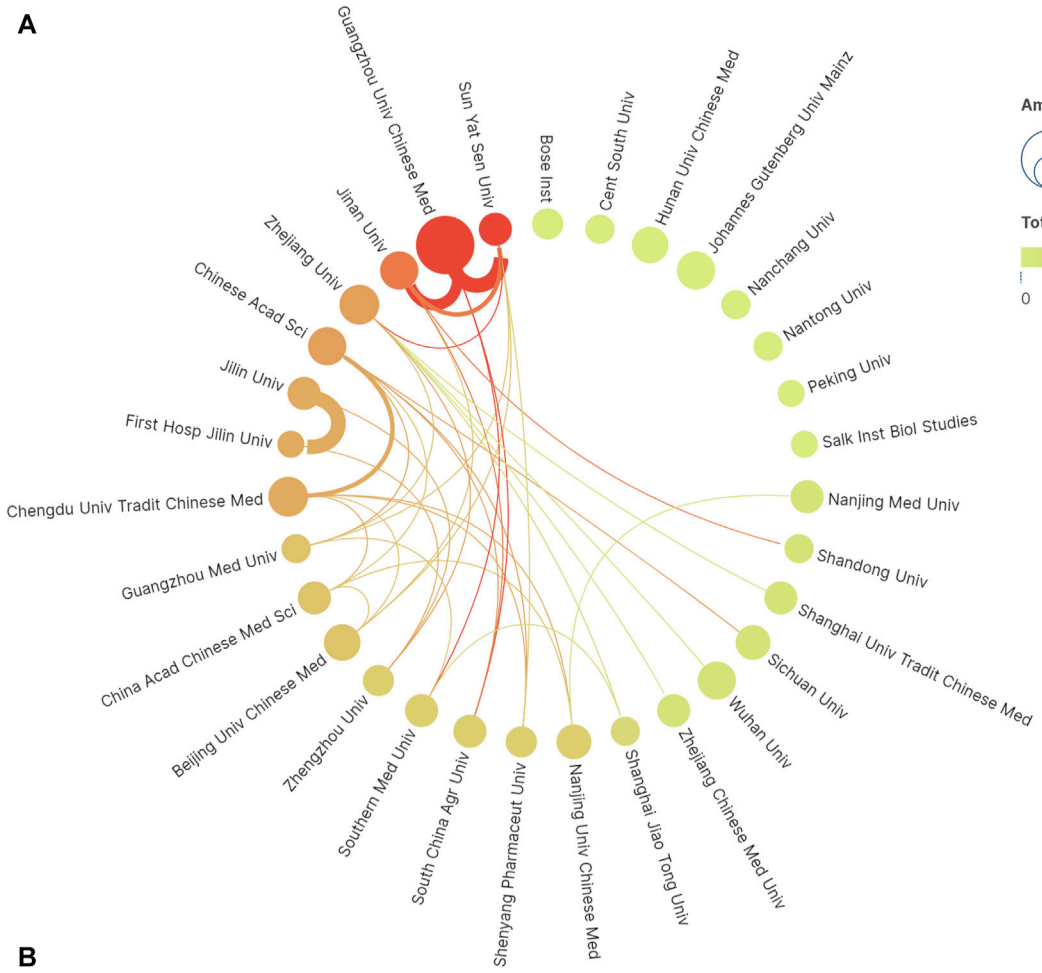


FIGURE 2
(A) Global distribution of "ferroptosis-traditional Chinese medicine (TCM)" research. **(B)** Chord diagrams illustrating international collaborations. **(C)** Research output on "Ferroptosis-TCM" from the top 10 countries (highlighted in red signifying increases in document production).

Fundamentally, the worldwide dispersion, collaborative structures, and emerging directions within "ferroptosis-TCM" investigation imply a substantial shift towards comprehensive worldwide involvement. These discoveries underscore the necessity for expanded partnerships, pinpoint emerging research focal points, and signify prospects for researchers and industry stakeholders alike to leverage varied proficiency in promoting remedies for human ailments through TCM.

3.3 Institutional performance

Identifying top-performing institutions and analyzing their citation bursts provides valuable insights for researchers seeking collaborators and funding opportunities. The dynamic collaborative network among institutions signifies a vibrant research ecosystem. Over the past 11 years, global research on "ferroptosis-TCM" has made significant strides, involving over 760 entities. A collaboration network, illustrated in Figure 3A, was established among these institutions, requiring a minimum of six publications for inclusion. In this network, circles and texts represent each institution; connecting lines denote collaborative occurrences; line thickness signifies collaboration strength; gradient colors represent the total collaboration strength between an institution and others; circle size correlates with the institution's publication count. Guangzhou University of Chinese Medicine emerges as the leading contributor, accounting for approximately 5.02% (28 publications) of the total research output. Following closely, Chengdu University of Traditional Chinese Medicine and Zhejiang University contribute 2.33% (13 publications) respectively. These institutions have demonstrated a substantial contribution to the field, making them potential collaborators for future research projects. Our analysis of interinstitutional collaboration revealed remarkable enthusiasm from Guangzhou University of Chinese



B

Top 10 Institutions with the Strongest Citation Bursts

Institutions	Year	Strength	Begin	End	2012 - 2023
Chengdu University of Traditional Chinese Medicine	2021	**3.82**	2021	2023	
Bose Institute	2012	**3.58**	2012	2018	
Department of Science & Technology (India)	2012	**3.58**	2012	2018	
Johannes Gutenberg University of Mainz	2017	**3.31**	2017	2021	
Salk Institute	2019	**2.3**	2019	2020	
Tehran University of Medical Sciences	2014	**2.19**	2014	2018	
Chinese Academy of Medical Sciences - Peking Union Medical College	2021	**1.35**	2021	2023	
Buddhist Tzu Chi General Hospital	2020	**1.34**	2020	2023	
University of Waterloo	2013	**1.32**	2013	2014	
Isfahan University Medical Science	2013	**1.32**	2013	2014	

FIGURE 3
(A) Diagram illustrating the intensity of institutional cooperation. **(B)** Top 10 Institutions with Citation Bursts (Red Bars Represent Periods of Increased Citations).

Medicine and Sun Yat-sen University in engaging with partnering institutions. It is worth mentioning that the vast majority of these organizations prioritize domestic cooperation rather than international collaboration. The dynamic collaborative network among institutions indicates a vibrant research ecosystem in the "ferroptosis-TCM" field, which can foster innovation and knowledge exchange. This ecosystem provides fertile ground for sharing ideas, resources, and expertise. Researchers can tap into this ecosystem to stay updated on the latest developments, access shared resources, and enhance the overall quality of research.

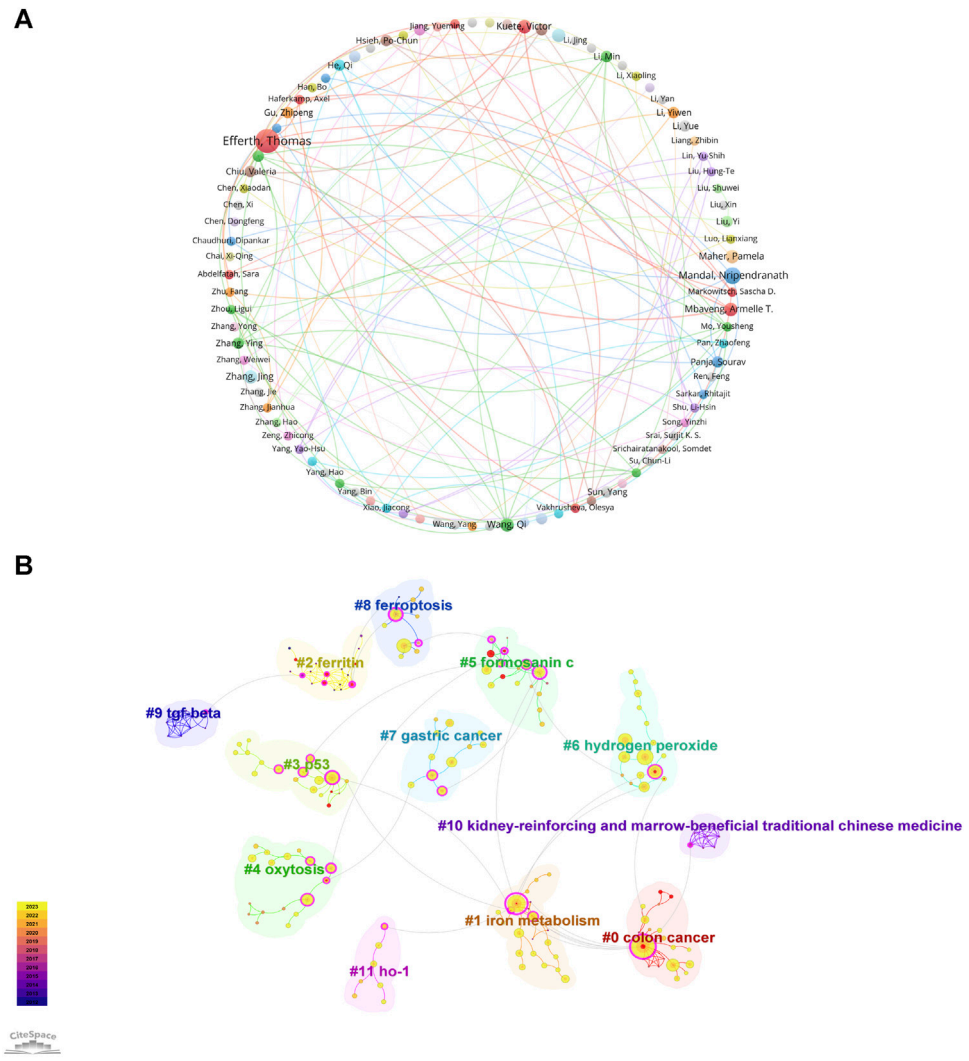


FIGURE 4
(A) Author Co-occurrence map. **(B)** Author cluster analysis. **(C)** Top 10 authors with significant citation bursts in "ferroptosis-traditional Chinese medicine" publications.

Additionally, understanding which institutions are experiencing citation bursts can help researchers identify institutions with active and impactful research programs that may offer collaboration opportunities or funding prospects. When contemplating collaborations, one should evaluate not only the quantity of publications but also the enduring impact and flexibility of research endeavors over an extended duration (Cao et al., 2024). Using CiteSpace analysis (Figure 3B), this study identified institutions with significant citation bursts. Bose Institute and the Department of Science & Technology (India) experienced

remarkably long burst periods in citations from 2012 to 2018. However, this trend declined in the last 5 years. In contrast, Chengdu University of Traditional Chinese Medicine exhibited a delayed citation burst from 2021 to 2023. This pattern suggests a shift in focus and a delayed timeline for research output in this institution's field.

3.4 Author Contributions

Recognizing prolific authors and analyzing their collaboration networks can help researchers identify key contributors in the field. Authors with high citation rates and a consistent publication history offer valuable insights and can guide future research directions. Following a thorough examination of authorship within the domain of 'ferroptosis-based TCM', a total of 3,615 authors emerged as significant contributors. Among these, eight authors were particularly prolific, each having authored a minimum of five papers. These authors likely possess in-depth knowledge and expertise in the field, making their work essential for those seeking guidance in "ferroptosis-TCM" research. To conduct a detailed examination of co-authorship networks, we utilized VOSviewer software to generate visualization maps. These maps were created with a baseline criterion of a minimum of three publications per author. In these representations, the dimensions of the circles are proportional to the number of publications by each author. Various color coding denotes discrete author clusters. The thickness of interconnecting lines among these circles indicates the degree of collaborative interactions. Remarkably, 90 authors exceeded this publication threshold, Thomas Efferth, Victor Kuete, and Armelle T. Mbaveng demonstrated the most robust collaborative relationships, as illustrated in Figure 4A. Furthermore, Thomas Efferth and Nripendranath Mandal were recognized as high-yielding scholars in the research on "ferroptosis-TCM", emphasizing their essential contributions to this scientific field.

CiteSpace assesses network integrity and clustering clarity using Modularity (*Q* value) and Mean Silhouette (*S* value). A *Q* value above 0.3 indicates robust clustering, while an *S* value above 0.5 suggests clear and reasonable clustering (Wang B. et al., 2022). The study recorded a cluster modularity value (*Q*) of 0.8341 and a mean silhouette value (*S*) of 0.9492, indicating highly significant and clear delineation of keyword clusters. In Figure 4B, authors are categorized into 12 groups and labeled with keywords, including: #0 colon cancer, #1 iron metabolism, #2 ferritin, #3 p53, #4 oxytosis, #5 formosanin c, #6 hydrogen peroxide, #7 gastric cancer, #8 ferroptosis, #9 transforming growth factor-β (TGF-beta), #10 kidney-reinforcing and marrow-beneficial Traditional Chinese Medicine, #11 Heme Oxygenase-1 (HO-1).

Citation burst analysis serves as a critical metric, indicating the frequency of citations received by an author in a specific research domain during a defined timeframe (Jiang et al., 2022; Li and Du, 2022). Figure 4C presents the top ten authors with the highest citation counts in the realm of "ferroptosis-TCM". Nripendranath Mandal leads with a burst strength of 4.31, closely followed by Thomas Efferth and Sourav Panja. Researchers and industry practitioners can refer to their work as a reference framework for future research directions, ensuring that their research builds upon the foundation of influential and highly cited studies. These influential authors offer valuable guidance for researchers navigating this research domain. Moreover, their work can inform industry practitioners about key players and emerging trends, potentially influencing their strategic decisions and collaborations.

3.5 Analysis of high-contributing journals

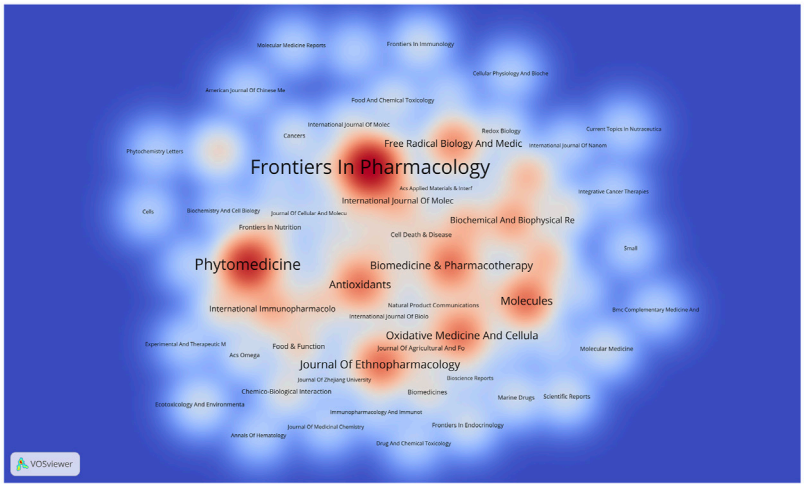
Drawing from visualized journal publication data, it becomes evident that 268 journals have disseminated articles related to "ferroptosis-TCM". To illustrate the dispersion of these documents among journals, a thermodynamic chart, as depicted in Figure 5A, has been constructed, employing a minimum criterion of two documents per journal. The intensity of color in the chart corresponds to the quantity of journal papers issued. The journal '*Frontiers in Pharmacology*' ranks highest in the number of published documents (n = 33, 5.92%), followed by '*Phytomedicine*' (n = 24, 4.31%), '*Journal of Ethnopharmacology*', and '*Molecules*' (n = 14, 2.51%). This provides researchers with a comprehensive understanding of the publication landscape in this field, helping them select appropriate journals for their own work and ensuring that their work reaches the relevant audience. Additionally, Figure 5B lists the top 20 journals with the strongest citation bursts for "ferroptosis-TCM" publications. Researchers can assess the significance of specific journals and prioritize them when citing or referring to previous research in their own work.

The dual-map overlay technique effectively presents the interdisciplinary dispersion of journals, the development of citation trajectories, and the relocation of scientific research centers (Yuan et al., 2023). Labels on the map depict the various subject areas covered by the journals. Journals referencing others are positioned on the left side, while those being referenced are situated on the right side (Zhang T. et al., 2022). Diverse colored lines visually illustrate the citation pathways, originating from the citation map and concluding there. The breadth of these connecting pathways is closely associated with the frequency of *z*-score-scaled citations. Figure 5C depicts the classification of research pertaining to "ferroptosis-TCM" into three key domains: molecular biology, immunology, and genetics. Such interdisciplinary insights can inspire collaboration among researchers from diverse backgrounds, fostering the exchange of ideas and innovative approaches. The findings can assist industry professionals, including pharmaceutical companies and biotechnology firms, in pinpointing potential investment areas and opportunities for product development in the "ferroptosis-TCM" domain. Understanding the interdisciplinary nature of research can guide them in recruiting experts from relevant domains and developing innovative therapies or products related to ferroptosis and TCM.

3.6 Co-cited references

Understanding which works have garnered the most attention and citations is essential for researchers as it helps them build upon existing knowledge and concepts, contributing to the advancement

A



B

Top 20 Cited Journals with the Strongest Citation Bursts

Cited Journals	Year	Strength	Begin	End	2012 - 2023
J BIOL CHEM	2012	**14.51**	2012	2020	
BLOOD	2012	**13.03**	2012	2019	
PLOS ONE	2014	**12.23**	2014	2020	
J INORG BIOCHEM	2012	**10.3**	2012	2019	
TOXICOLOGY	2012	**9.48**	2012	2021	
ANN NY ACAD SCI	2012	**8.33**	2012	2019	
MOL PHARMACOL	2018	**7.84**	2018	2021	
J MED CHEM	2015	**7.47**	2015	2020	
BIOCHEM PHARMACOL	2012	**7.46**	2012	2020	
J AM CHEM SOC	2016	**6.62**	2016	2021	
NEW ENGL J MED	2012	**6.6**	2012	2021	
HEPATOLOGY	2012	**6.43**	2012	2021	
LANCET	2012	**6.4**	2012	2018	
J HEPATOL	2012	**6.38**	2012	2016	
BBA-GEN SUBJECTS	2016	**6.18**	2016	2021	
GASTROENTEROLOGY	2012	**6.12**	2012	2020	
NATURE	2016	**6.12**	2016	2020	
BIOCHEM J	2012	**6.08**	2012	2019	
ARCH BIOCHEM BIOPHYS	2012	**6.07**	2012	2016	
FRONT NEUROSCI-SWITZ	2019	**5.76**	2019	2021	

C

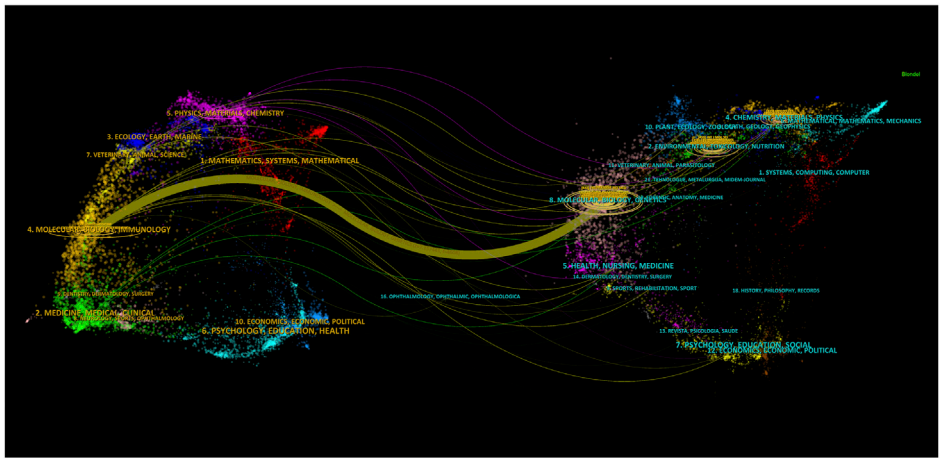
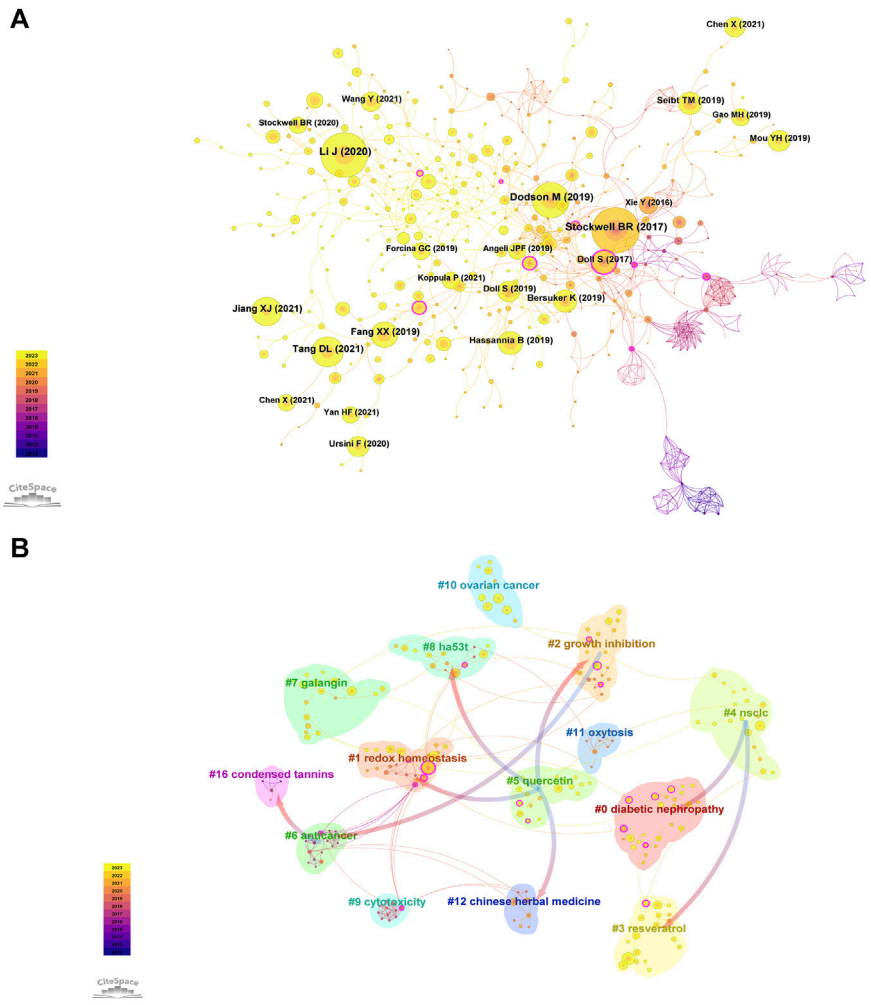


FIGURE 5
(A) Density visualization map of Journal citations. **(B)** Top 20 journals with significant citation bursts. **(C)** Dual-map overlay of journals in "ferroptosis-traditional Chinese medicine" research.

of their respective fields. Figure 6A displays a co-citation network diagram, delineating the co-citation network of literature related to "ferroptosis-TCM" from 1 January 2012, to 30 November 2023, as analyzed using CiteSpace. In this representation, the sizes of the spheres, aggregating their sizes across annual rings, are proportionate to their co-citation frequencies. The color scheme spans from purple, indicating earlier citation instances, to yellow, representing more recent citations. Spheres with overlapping colors



C **Top 20 References with the Strongest Citation Bursts**

References	Year	Strength	Begin	End	2012 - 2023
Xie Y, 2016, CELL DEATH DIFFER, V23, P369, DOI 10.1038/cdd.2015.158, DOI	2016	**11.26**	2019	2021	
Stockwell BR, 2017, CELL, V171, P273, DOI 10.1016/j.cell.2017.09.021, DOI	2017	**9.88**	2018	2023	
Yang WS, 2016, TRENDS CELL BIOL, V26, P165, DOI 10.1016/j.tcb.2015.10.014, DOI	2016	**7.8**	2019	2021	
Yang WS, 2014, CELL, V156, P317, DOI 10.1016/j.cell.2013.12.010, DOI	2014	**6.16**	2016	2019	
Lewerenz J, 2018, FRONT NEUROSCI-SWITZ, V12, P0, DOI 10.3389/fnins.2018.00214, DOI	2018	**5.35**	2019	2021	
Doll S, 2017, NAT CHEM BIOL, V13, P91, DOI 10.1038/NCHEMBIO.2239, DOI	2017	**5.11**	2020	2023	
Latunde-Dada GO, 2017, BBA-GEN SUBJECTS, V1861, P1893, DOI 10.1016/j.bbagen.2017.05.019, DOI	2017	**4.85**	2019	2020	
Sun XF, 2016, HEPATOLOGY, V63, P173, DOI 10.1002/hep.28251, DOI	2016	**4.37**	2019	2021	
Ooko E, 2015, PHYTOMEDICINE, V22, P1045, DOI 10.1016/j.phymed.2015.08.002, DOI	2015	**4.24**	2019	2020	
Cao JY, 2016, CELL MOL LIFE SCI, V73, P2195, DOI 10.1007/s00018-016-2194-1, DOI	2016	**4.09**	2017	2021	
Jiang L, 2015, NATURE, V520, P57, DOI 10.1038/nature14344, DOI	2015	**4.02**	2017	2020	
Hou W, 2016, AUTOPHAGY, V12, P1425, DOI 10.1080/15548627.2016.1187366, DOI	2016	**3.88**	2019	2021	
Skouta R, 2014, J AM CHEM SOC, V136, P4551, DOI 10.1021/ja411006a, DOI	2014	**3.69**	2016	2019	
Yang WS, 2016, P NATL ACAD SCI USA, V113, PE4966, DOI 10.1073/pnas.1603244113, DOI	2016	**3.65**	2020	2021	
Do Van B, 2016, NEUROBIOL DIS, V94, P169, DOI 10.1016/j.nbd.2016.05.011, DOI	2016	**3.4**	2019	2021	
Xie YC, 2016, BIOCHEM BIOPH RES CO, V473, P775, DOI 10.1016/j.bbrc.2016.03.052, DOI	2016	**3.4**	2019	2021	
Dixon SJ, 2012, CELL, V149, P1060, DOI 10.1016/j.cell.2012.03.042, DOI	2012	**3.34**	2016	2017	
Dixon SJ, 2014, NAT CHEM BIOL, V10, P9, DOI 10.1038/nchembio.1416, DOI	2014	**3.2**	2018	2019	
Zilka O, 2017, ACS CENTRAL SCI, V3, P232, DOI 10.1021/acscentsci.7b00028, DOI	2017	**3.13**	2020	2021	
Angeli JPF, 2014, NAT CELL BIOL, V16, P1180, DOI 10.1038/ncb3064, DOI	2014	**3.07**	2016	2019	

FIGURE 6
(A) Co-citation analysis chart for "ferroptosis-traditional Chinese medicine". **(B)** Co-cited literature network map. **(C)** Top 20 references with the highest citation bursts.

indicate sustained citation over the specified years. The interconnecting lines between spheres illustrate the co-citation relationships among various pieces of literature. Notably, nodes highlighted in magenta signify pivotal nodes in the network, characterized by a centrality exceeding 0.1. The review titled 'Ferroptosis: A Regulated Cell Death Nexus Linking Metabolism, Redox Biology, and Disease', authored by Brent Stockwell et al. and published in *Cell* in 2017, stands out among the most frequently cited works, with the highest co-citation count (n = 60) (Stockwell et al., 2017). The 2020 *Cell Death & Disease* paper 'Ferroptosis: past,

present and future' by Li Jie et al. followed with 59 co-citations (Li et al., 2020).

CiteSpace employs metrics such as Modularity (*Q* value) and Mean Silhouette (*S* value) to assess network structures and clustering clarity. A *Q* value above 0.3 indicates significant clustering, while an *S* value higher than 0.5 indicates clear and effective clustering. In the analysis conducted, the computed values were $Q = 0.8569$ and $S = 0.9535$, confirming the presence of robust and convincing clustering structures within the network. This result emphasizes the reliability of the employed citation clustering method. The analysis identified 13 distinct clusters, labeled as #0 diabetic nephropathy, #1 redox homeostasis, #2 growth inhibition, #3 resveratrol, #4 non-small cell lung cancer, #6 anticancer, #7 galangin, #8 ha53t, #9 cytotoxicity, #10 ovarian cancer, #11 oxytosis, #12 Chinese herbal medicine, #16 condensed tannins, as illustrated in Figure 6B. Researchers can use this information to ensure their work aligns with established research clusters, increasing the likelihood of their research being recognized and cited by peers and experts.

The analytical capabilities of CiteSpace were used to detect citation bursts, highlighting studies that have garnered significant scholarly attention in the field of "ferroptosis-TCM". As depicted in Figure 6C, an analysis of the top 20 references reveals their substantial impact, evidenced by significant citation bursts. The field experienced a notable surge in citations starting from 2016, with numerous co-citation references gaining substantial citations in the following years. This trend highlights the lasting significance of research in "ferroptosis-TCM". Notably, in 2019, 45% (9 out of 20) of these references exhibited citation bursts, marking it as the year with the highest frequency, followed by 2016 and 2020, accounting for 20% (4 out of 20) and 15% (3 out of 20) of the bursts, respectively. The study with the most prominent citation burst (strength = 11.26) was titled 'Ferroptosis: Process and Function', originally published in *Cell Death & Differentiation* (Xie et al., 2016). This was closely followed in impact by the works of Brent Stockwell et al. (Stockwell et al., 2017), and Wan Seok Yang et al. (Yang and Stockwell, 2016). Researchers can identify key studies that have gained significant scholarly attention and understand when these bursts of interest occurred. This information can help researchers stay updated on emerging trends and focus their efforts on areas of high impact. By analyzing co-citation references and identifying works that are frequently cited together, researchers can identify potential collaborators who share common research interests, enhancing their ability to conduct collaborative research projects. Collaborating with authors whose works are frequently cited alongside one's own research can enhance the visibility and impact of their work. Industry practitioners can use the information on influential works and research trends to inform their strategic decisions, such as identifying research partnerships or investment opportunities in areas aligned with the most cited and impactful studies.

3.7 Keyword analysis

Keyword co-occurrence clustering analysis was performed using VOSviewer software, with a minimum keyword occurrence threshold set at three instances. Keywords meeting these criteria were included in the graph. A total of 131 keywords were selected from the initial pool of 1,469 keywords (after removing duplicates) to create the visualized network. Figure 7A represents a word frequency-time graph, where circles and corresponding labels collectively constitute a node. The size of each circle corresponds to the frequency of keyword occurrence, while the thickness of connections between circles indicates the strength of relationships among keywords. The color of each circle, as indicated in the lower right corner, gradually changes to represent the average year of appearance. Blue indicates earlier appearances, while yellow represents later appearances of the keywords. Based on the data presented in Figure 7A, it is evident that ferritin, phytochemicals, and thalassemia were topics of earlier research, while metabolomics, neuroinflammation, and diabetic nephropathy have emerged as recent research hotspots and directions. This information is invaluable for researchers as it highlights areas of growing interest and potential for further exploration.

The identification of keyword bursts, particularly those with substantial increases in citations, indicates areas of intense scholarly attention (Figure 7B). Figure 7B illustrates that 30% of the keywords (3 out of 10) experienced their initial citation surge in 2020, followed closely by 2014, which accounted for 20% of the surges (2 out of 10). Notably, 40% of these keywords maintained elevated citation rates over the past 3 years, indicating a sustained and growing interest in specific research domains. The keyword 'iron overload' displayed the most significant burst, with a burstness value of 2.78, followed by 'iron chelation' and 'cell death', with burstness values of 1.64 and 1.45, respectively. These insights bear significant implications for researchers in their respective fields.

Co-occurrence analysis reveals the relationships between keywords and identifies key themes and concepts in the field. Figure 7C illustrates a co-occurrence analysis chart of keyword frequencies, utilizing CiteSpace to analyze the co-occurrence of "ferroptosis-TCM" from 1 January 2012, to 30 November 2023. The spheres' sizes indicate the cumulative size of spheres on yearly rings, in proportion to the frequency of keyword usage. Purple represents earlier keyword appearances, while yellow indicates later appearances, and overlapping colors indicate citations in corresponding years. Lines connecting spheres represent co-citation connections among literature, and nodes highlighted in magenta represent critical nodes with a centrality above 0.1. Figure 7C shows that the most frequently co-occurring keyword is 'oxidative stress', followed by 'iron overload' and 'traditional Chinese medicine', which are tied for second place.

Understanding the evolving trends and hotspots in "ferroptosis-TCM" research is crucial as it can guide investment and collaboration decisions, ensuring strategic alignment with current research interests. Figure 7D depicts a temporal analysis of keyword frequency clustering within hotspots. The diagram showcases circles of varied sizes, where the cumulative diameter on each annual ring corresponds to the keyword frequency. Interconnections among keywords signify their co-occurrence. The color scheme is informative: purple represents earlier keyword emergence, yellow denotes recent appearances, and overlapping colors indicate consistent keyword presence across years. Magenta nodes highlight keywords with significant centrality, underscoring their pivotal role as network hubs. Keywords within the same cluster are horizontally aligned. The timeline orientation positions the initial keyword occurrence at the top, progressing chronologically to the

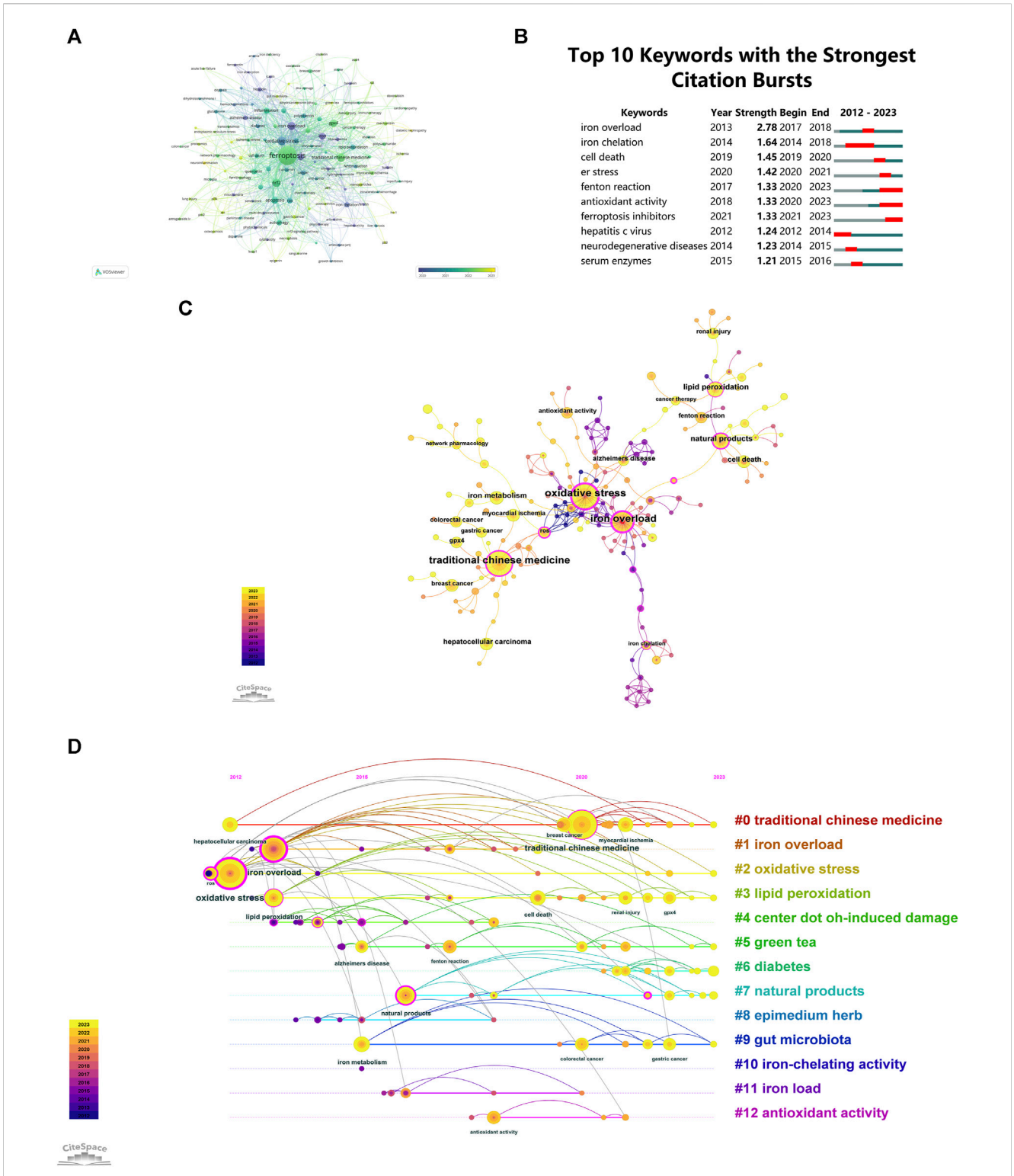


FIGURE 7
(A) Keywords intensity visualization timing overlay. **(B)** Top 10 keywords with significant citation bursts. **(C)** Co-occurrence analysis chart of Keyword frequencies. **(D)** temporal trends in Keyword Co-occurrence.

right. This visualization aids in understanding keyword distribution within each cluster, with a larger quantity signifying greater cluster relevance. Additionally, it outlines the temporal duration of keywords in each cluster. The keywords are categorized into 13 distinct groups: #0 Traditional Chinese Medicine, #1 Iron Overload, #2 Oxidative Stress, #3 Lipid Peroxidation, #4 •OH-induced Damage, #5 Green Tea, #6 Diabetes, #7 Natural Products, #8 Epimedium Herb, #9 Gut Microbiota, #10 Iron-Chelating Activity, #11 Iron Load, #12 Antioxidant Activity. These clusters encapsulate primary themes and hotspots in the

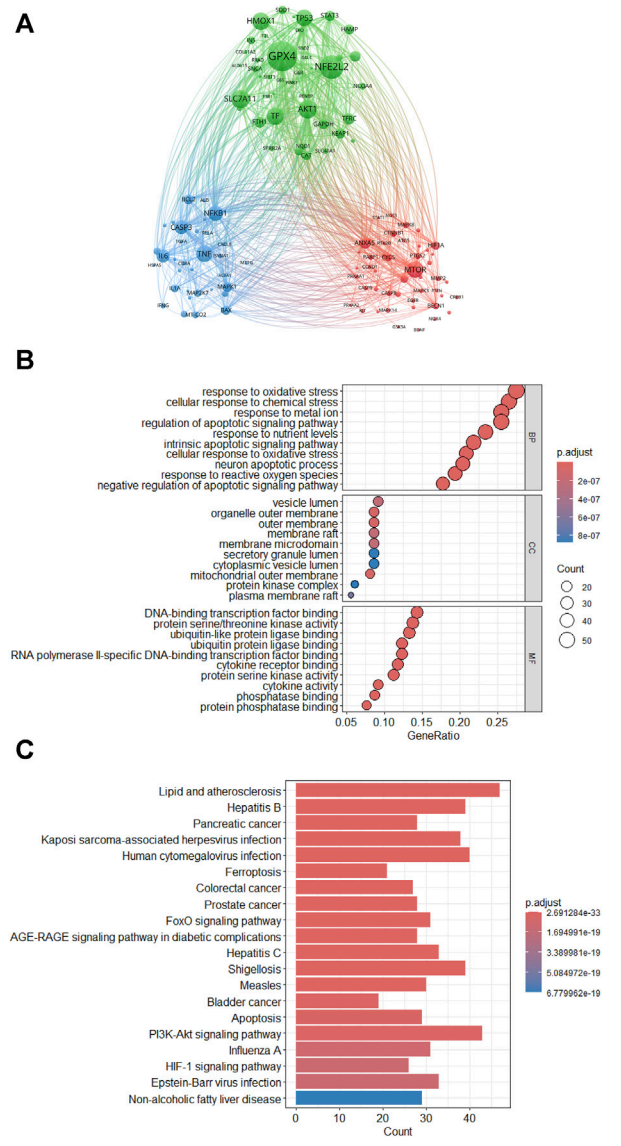


FIGURE 8
(A) VOSviewer critical gene clustering visualization. **(B)** Bubble Plots of gene Ontology enrichment analysis. **(C)** Kyoto Encyclopedia of genes and Genomes pathway enrichment analysis.

research domain, reflecting their significance in scientific advancements, therapeutic potential, or clinical applicability. Researchers and industry practitioners can use this information to navigate the various research directions and pinpoint areas of particular relevance, helping them focus their efforts more effectively.

It is not difficult to observe that within the multiple analyses of keywords above, 'iron overload' and 'oxidative stress' appear with the highest frequency. TCMs offer a plethora of natural antioxidant substances, encompassing polyphenols, alkaloids, flavonoids, terpenoids, etc., all of which exhibit excellent antioxidant properties. Therefore, future investigations should explore the potential of TCMs in modulating iron metabolism in the context of oxidative stress, aiming to identify potential targets for synergistic combination therapy, with the goal of achieving a "one TCM-multiple targets " approach for future interventions. To date, progress has already been made in this area with compounds such as baicalein, curcumin, and epigallocatechin gallate (Mandel et al., 2008; Perez et al., 2009; Dijiong et al., 2019; Guerrero-Hue et al., 2019; Kose et al., 2019; Rainey et al., 2019).

3.8 Key genes and pathways

Utilizing VOSviewer software, we conducted a co-occurrence clustering analysis of genes related to "ferroptosis-TCM". From a dataset of 557 articles obtained from the Citexs big data platform, we extracted a total of 1,109 genes, each appearing at least 6 times, which was then visualized in a map (Figure 8A). This visualization is categorized based on gene function similarity or their co-occurrence in research. Every node consists of a circle and a label, where the circle's size corresponds to the gene's frequency of occurrence, and the line thickness connecting circles indicates the strength of gene associations. Nodes of distinct colors form separate clusters, with each color symbolizing genes grouped within different domains, signifying particular biological or medical gene categories. These clusters represent specific biological or medical gene groups, enabling researchers to identify key genes and their functions within the context of ferroptosis and TCM.

Genes associated with "ferroptosis-TCM" form the core components of this research focus, effectively acting as dual indicators: one for recognizing shifting research patterns and the other for identifying changes in research orientation and the deepening of investigations. Illustrated in Figure 8A, the top three genes with the highest frequency were GPx4, NFE2L2, and SLC7A11. The sensitivity of ferroptosis correlates directly with the expression level of nuclear factor erythroid 2 like 2 (Nrf2), also known as nuclear factor erythroid 2-related factor 2 (NFE2L2). An increase in Nrf2 expression hinders ferroptosis, while a decrease in expression promotes it (Sun et al., 2016; Dodson et al., 2019). The mechanisms through which Nrf2 regulates ferroptosis encompass two primary aspects. Firstly, Nrf2 amplifies the antioxidant system by upregulating the expression of GSH and glutathione peroxidase 4 (GPx4), thereby enhancing antioxidant functionality. Secondly, Nrf2 stimulates the expression of ferritin and ferroportin, aiding in the storage or export of free iron. Consequently, this reduction in iron accumulation averts ferroptosis (Yang et al., 2017). Solute carrier family 7 member 11 (SLC7A11) functions as a cystine/glutamate antiporter (System Xc^-), playing a crucial role in importing cystine for GSH biosynthesis and antioxidant defense (Koppula et al., 2021). In response, employing TCMs to target the specified pivotal genes for modulating ferroptosis could potentially offer an innovative strategy for preventing and managing human illnesses. This encompasses, without restriction, enhancers of iron metabolism, controllers of Nrf2-associated routes, and substances that inhibit or activate system Xc^- and GPx4.

Moreover, we performed GO and KEGG enrichment assessments on genes linked to "ferroptosis-TCM", with a focus on genes referenced in the articles two or more times (Figures 8B,C). Researchers can use this information to pinpoint the specific

functions and pathways influenced by these genes, guiding their experimental design and hypothesis generation. The identification of key genes and pathways can also facilitate collaborations between researchers and industry professionals, as it allows for the targeted leveraging of academic expertise in specific genes and pathways, potentially accelerating the development of novel treatments or interventions. The bubble chart for GO enrichment analysis illustrates each bubble as a GO term. Bubble size corresponds to the quantity of genes linked to that function, while color indicates the level of enrichment. The X-axis displays the GeneRatio, signifying the proportion of genes associated with the GO term in relation to the total gene count in the background gene set. A higher GeneRatio suggests a greater number of genes associated with the GO term, potentially indicating greater significance. The Y-axis categorizes GO terms into various biological processes, molecular functions, or cellular components.

Figure 8B illustrates the GO functional enrichment results, covering biological processes (BP), molecular functions (MF), and cellular components (CC). In terms of BP, the genes display enrichment in functions associated with responses to oxidative stress and cellular responses to chemical stress. Regarding CC, the genes exhibit enrichment in functions related to the vesicle lumen, outer membrane, and organelle outer membrane. Concerning MF, the genes show enrichment in functions such as DNA-binding transcription factor binding and protein serine/threonine kinase activity. Identifying specific cellular components and molecular functions enriched in these genes can assist researchers in pinpointing the organelles and activities critical to ferroptosis, thus facilitating the development of targeted interventions or therapies.

Furthermore, we performed KEGG pathway enrichment analysis to identify the top 10 signaling pathways, which is presented as a histogram. The X-axis represents the number of genes significantly enriched in each pathway, while the Y-axis displays different signaling pathways. The height of each column in the histogram signifies the gene count within the pathway and its level of significance after enrichment. As shown in Figure 8C, this research area is predominantly linked to signaling pathways such as 'Lipid and Atherosclerosis' and 'PI3K/AKT signaling pathway'. This information can be of particular interest to industry practitioners, including pharmaceutical companies, as it suggests potential therapeutic targets or areas for drug development. Understanding the signaling pathways enriched in this context can guide industry professionals in designing interventions or treatments related to ferroptosis and its implications for various health conditions.

In relation to signaling pathways, as illustrated in Figure 8C, this subject is primarily associated with pathways such as lipid metabolism and atherosclerosis. It is noteworthy that various age-related diseases are frequently observed in the middle-aged and elderly population. Furthermore, these individuals are susceptible to concurrent chronic conditions, including cardiovascular and cerebrovascular diseases (atherosclerosis). To comprehensively grasp the implications of these changes in the context of a globally aging population, it is imperative to explore the capacity of TCMs in modulating ferroptosis and the aforementioned signaling pathways. The ultimate objective is to identify potential targets for a synergistic combination therapy.

3.9 Related diseases

In recent investigations, it has been discovered that the regulatory role of ferroptosis is pivotal in the initiation and advancement of various illnesses. This has positioned ferroptosis as a central and extensively researched area, aiming to improve the management and prognostication of associated conditions (Figure 9) (Li et al., 2020; Stockwell, 2022; Wang X. et al., 2023). Understanding the diseases most closely associated with "ferroptosis-TCM" research allows researchers to focus their efforts on specific medical conditions. This targeted approach can accelerate the development of drugs and therapies tailored to address these diseases, ultimately increasing the potential for more effective treatments. The Citexs Data Platform identified 765 diseases from 557 articles, with a minimum requirement of five articles mentioning each disease for inclusion. These diseases that met the criterion were visualized in a heatmap, created using VOSviewer, illustrating the frequency or relationships of diseases related to "ferroptosis-TCM" research (Figure 10A). The top five most frequently mentioned diseases include hepatocellular carcinoma, chemical and drug-induced liver injury, mitochondrial diseases, acute kidney injury, and liver failure. Additionally, a co-occurrence cluster analysis was performed, also requiring a minimum of five occurrences for each disease, using VOSviewer (Figure 10B). In this visualization, nodes—represented by circles and labels—are sized according to disease frequency. The thickness of lines connecting circles indicates the strength of relationships between diseases, and various colors represent distinct clusters corresponding to specific disease categories.

The identification of diseases associated with "ferroptosis-TCM" research can also stimulate efforts to discover biomarkers. Researchers can explore potential biomarkers associated with both ferroptosis and these specific diseases, which may have implications for diagnosis, prognosis, or therapy. In summary, these findings provide valuable insights into the diseases most closely linked to "ferroptosis-TCM" research, guiding researchers and industry practitioners toward targeted research, drug development, interdisciplinary collaborations, and potential clinical applications. Understanding the disease landscape in this context can pave the way for advancements in the treatment and management of related medical conditions.

3.10 TCM intervention of ferroptosis based on Yin-Yang theory

TCM modulates ferroptosis by promoting it in tumor cells (addressing excess) and inhibiting it in other diseases (addressing deficiencies), aligning with the treatment principle of balancing Yin and Yang.

3.10.1 Damaging the excess

Disruptions in the signaling pathways associated with PCD may result in uncontrolled cell proliferation and, ultimately, cancer (Refaat et al., 2014). This leads to abnormal cell proliferation (excess Yang) and a significant reduction in PCD (deficiency in Yin). The treatment approach for cancer involves promoting ferroptosis (nourishing Yin) in tumor cells to inhibit their

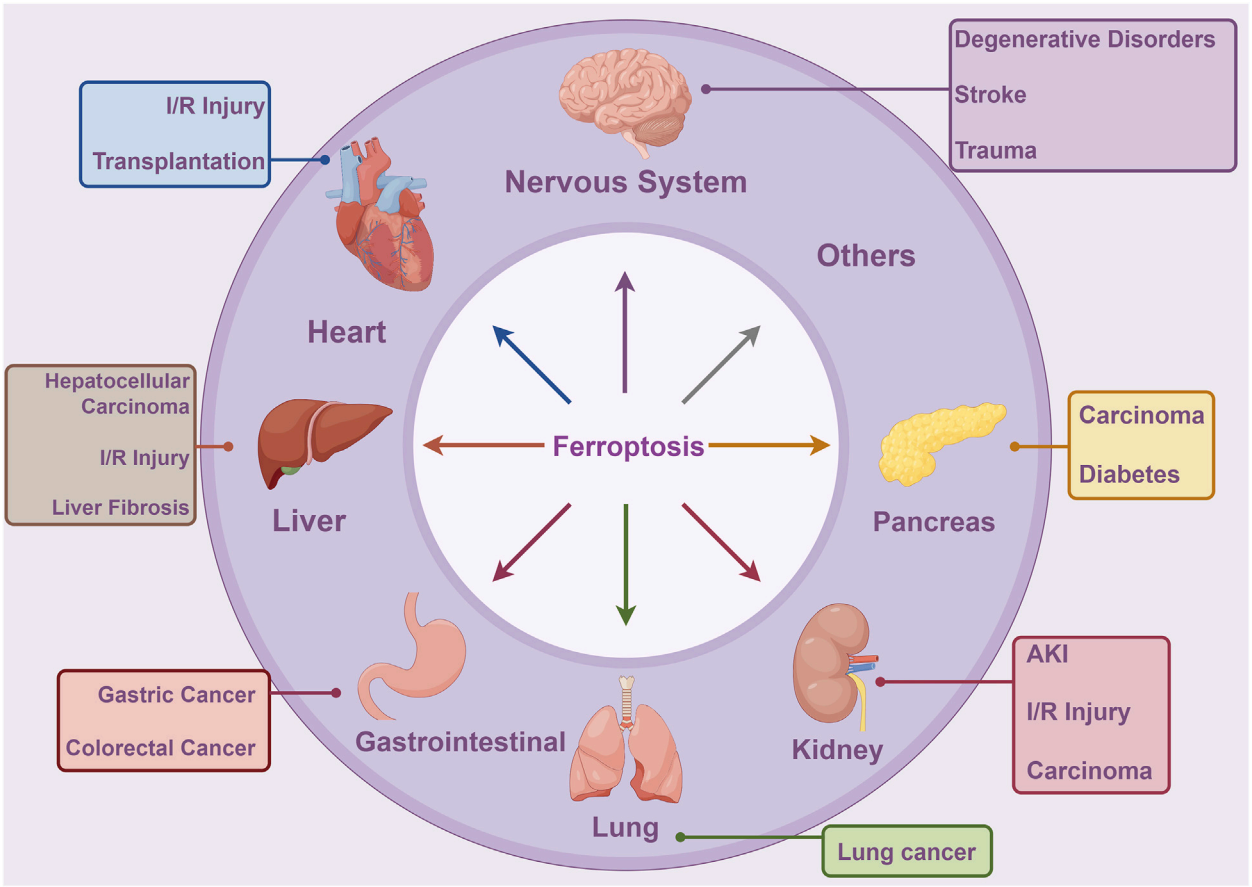


FIGURE 9
Ferroptosis plays significant roles in various systemic disorders, including conditions affecting the nervous system, cardiovascular diseases, hepatic disorders, gastrointestinal ailments, respiratory illnesses, renal conditions, pancreatic maladies, and others. This figure was created using Figdraw (https://www.figdraw.com/static/index.html#/). Abbreviations: AKI, acute kidney injury; I/R Injury, ischemia/reperfusion injury.

abnormal proliferation (restricting Yang) (Wang et al., 2023b), as stated in the Huang Di Nei Jing (Huangdi's Internal Classic): "When Yang is excessive, treat with Yin; when Yin is deficient, treat with Yang". TCM triggering ferroptosis primarily focuses on tumor-related research, encompassing various cancers such as lung (Han et al., 2023), breast (Li et al., 2023), stomach (Huang et al., 2024), liver (Wu et al., 2022), and ovarian (Chan et al., 2020) cancer, etc. The intervention mechanisms include Chinese herbal compounds, monomers, with key pathways involving the GPx4/GSH pathway, iron metabolism pathway, lipid metabolism pathway, and others (Wang et al., 2023b).

3.10.2 Replenishing the deficiencies

In contrast to carcinomas, diseases such as acute kidney injury (Shi et al., 2023), type 2 diabetes mellitus (Miao et al., 2023), non-alcoholic fatty liver disease (Wang S. et al., 2022), and age-related orthopaedic diseases (Ru et al., 2023) often involve a decline in the function of tissues and organs. This is characterized by reduced normal cell proliferation (Yang deficiency) and an increase in PCD processes such as aging, apoptosis, and necrosis (excessive Yin). The approach to addressing these diseases includes replenishing deficiencies by inhibiting ferroptosis in normal cells (restricting Yin) to maintain both their quantity and functional integrity (nourishing Yang), as prescribed in the Huang Di Nei Jing (Huangdi's Internal Classic): "When Yin is excessive, treat with Yang; when Yang is deficient, treat with Yin". TCM inhibiting ferroptosis can intervene in various diseases or pathological conditions such as cerebral infarction (Hui et al., 2022), atherosclerosis (Zhang J. et al., 2023), Parkinson's Disease (Wu et al., 2021), among others. Chinese medicine suppresses cell ferroptosis through herbal compounds, monomers, and external treatments (acupuncture and moxibustion) (Chen et al., 2024).

3.11 Challenges and future vistas

Despite significant advancements in "ferroptosis-TCM" research, ongoing critical challenges demand immediate attention.

3.11.1 Achilles' heel of present ferroptosis-related research

As of now, the majority of *in vivo* investigations pertaining to ferroptosis have been dependent upon preclinical animal models (Sun et al., 2023). However, there are several obstacles that hinder their transition into clinical use. Research and clinical trials focused on the molecular facets of ferroptosis have barely delved beneath the

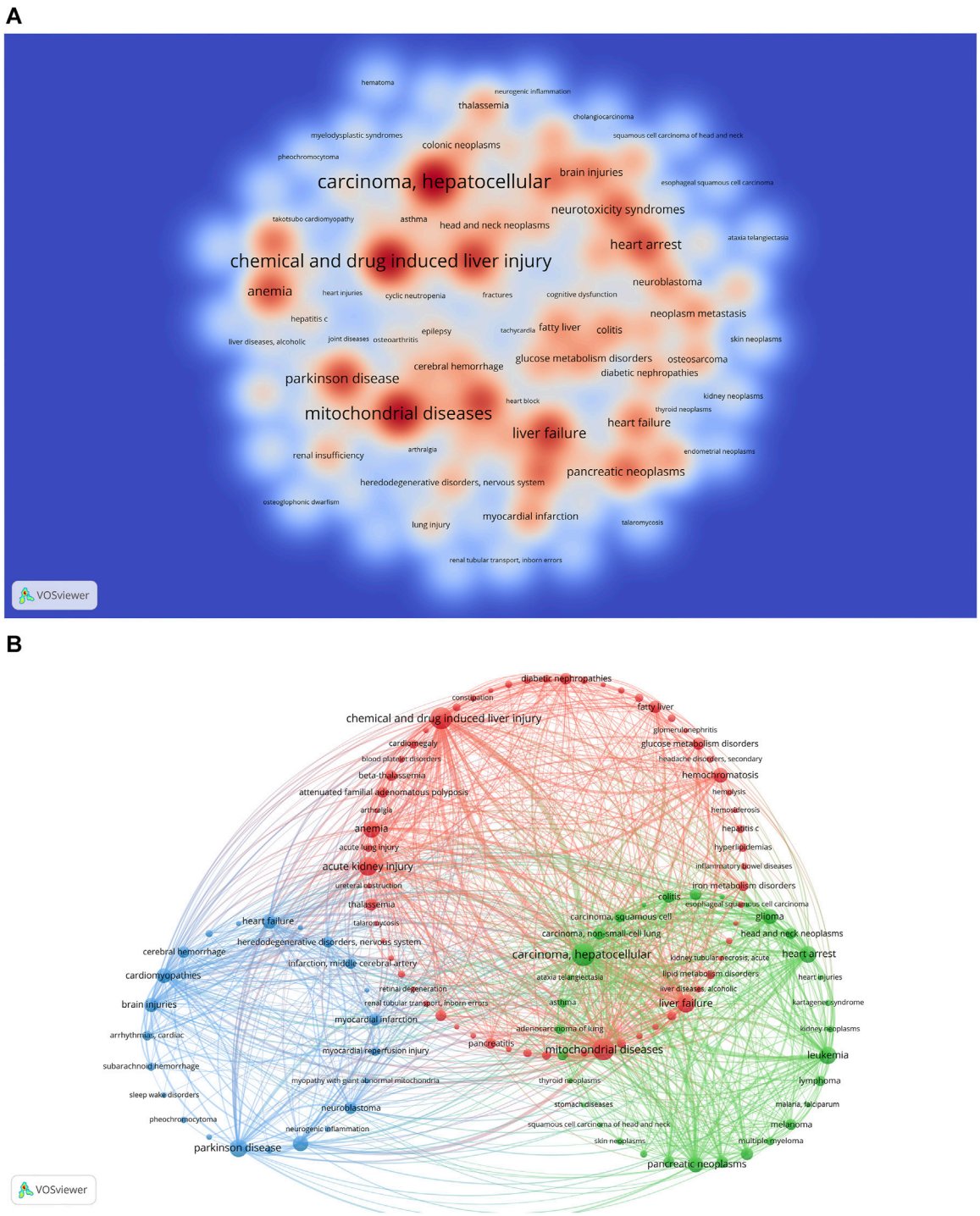


FIGURE 10
(A) Density visualization map of related diseases. **(B)** Disease clustering analysis chart.

surface of this PCD pathway. In fundamental research, the central inquiry revolves around identifying the ultimate executor responsible for triggering ferroptosis after lipid peroxidation. Such a discovery may also reveal additional distinguishing characteristics of ferroptosis, setting it apart from other forms of PCD. While ferroptosis possesses unique features that differentiate it from other PCD modalities, recent studies have raised questions about its distinctiveness. These studies suggest interactions between ferroptotic factors and components associated with other PCD pathways, such as autophagy and pyroptosis (Ru et al., 2023). A comprehensive understanding of the interplay within the ferroptosis system, rather than focusing solely on individual regulators, is crucial for gaining deeper insights into the mechanisms of ferroptosis. As exploration delves deeper, this crosstalk may yield

novel insights and offer opportunities for synergistic combination therapy aimed at achieving "one TCM-multiple targets" for future interventions in diseases related to ferroptosis.

Regarding clinical applications, there is a lack of information about potential biomarkers specifically applicable for diagnosing ferroptosis in clinical settings (Wang X. et al., 2023). Distinct non-invasive biomarkers are crucial not only for identifying ferroptosis in pathological conditions but also for assessing the pharmacodynamics of innovative anti-ferroptosis therapies and monitoring treatment progress. It would be more meaningful to search for ferroptosis biomarkers that can indicate disease severity. Furthermore, there is limited exploration regarding the parameters that dictate the utilization of ferroptosis inducers or inhibitors. These parameters encompass application conditions, initiation time, dosage, administration form, and duration. Current research on the regulatory influence of TCM on ferroptosis has predominantly focused on animal models and specific cell types, with minimal evaluation of clinical safety and efficacy. Comprehensive preclinical and clinical trials are crucial to validate ferroptosis's involvement in human physiology and provide the groundwork for TCM advancements in treating ferroptosis-related ailments.

3.11.2 Emerging technologies-assisted TCM

3.11.2.1 Artificial intelligence (AI)-Assisted drug screening and design in TCM

AI, originally termed by John McCarthy in 1956 as the 'science and engineering of developing intelligent machines', primarily encompasses the fields of computer science, mathematics, and neuroscience. However, it has also found utility in the realm of drug discovery, as indicated by studies (Yang et al., 2019; Zhu Y. et al., 2022; Lin et al., 2022). Given the time-consuming and labor-intensive nature of conventional drug discovery, there is widespread acknowledgment that AI, especially machine learning, has the potential to enhance predictive accuracy and expedite the drug discovery process. Deep learning, a fusion of machine learning and AI, offers a cost-effective platform for drug discovery by enabling rapid, machine-driven decision-making through artificial neural networks. Currently, AI-driven technologies play a pivotal role in various phases of the drug discovery journey, including target identification, drug formulation, screening, synthesis, and anticipating drug characteristics and mechanisms of action (Yang et al., 2019; Gupta et al., 2021). Furthermore, AI proves invaluable in swiftly identifying lead compounds derived from plants and microorganisms (Li G. et al., 2022). Notably, AI has made substantial contributions to cancer research and precision medicine (Bhinder et al., 2021). Within the framework of ferroptosis, Wu et al. employed a blend of bioinformatics and AI to posit that ferroptosis and the TGF-β signaling pathway potentially underpin the protective attributes of celastrol against type 2 diabetes (Wu and Zhang, 2022). In October 2023, Liu et al. demonstrated the potential of deep-learning-assisted phenotypic screening to identify promising lead compounds for alleviating doxorubicin-induced cardiomyopathy by preventing ferroptosis (Liu C. et al., 2023). Their research introduces innovative viewpoints regarding drug discovery in the epoch of AI. As a result, it is plausible to foresee that AI will notably hasten the exploration of innovative TCMs aimed at modulating ferroptosis.

3.11.2.2 Multi-omics

Unlike modern pharmaceuticals, TCM formulations adhere to the TCM theory and the "monarch, minister, assistant, envoy " principle. They constitute comprehensive systems with the capacity for "multiple ingredients, multiple targets, and multiple pathways" (Yi and Chang, 2004; Wang and Zhang, 2017). This enables them to exert synergistic effects in preventing and treating diseases. The complexity of TCM lies in its components, including structurally similar ones, metabolites, and bioactivities. The intricacy of these processes has presented obstacles in the exploration of TCM's therapeutic mechanisms across diverse diseases. The advent of high-throughput sequencing technology has effectively addressed the requirements of TCM research. Systems biology-driven omics approaches have emerged as viable tools for decoding intricate components, targets, and drug-disease interactions (Subramanian et al., 2020). Multi-omics approaches include various high-throughput analytical methods used in contemporary biological research systems, including genomics, transcriptomics, metagenomics, metabolomics, epigenomics, and proteomics (Olivier et al., 2019; Subramanian et al., 2020). From an omics standpoint, TCM can be regarded as a blend of small molecules. Consequently, a single TCM concoction has the potential to address numerous ailments by leveraging the small molecules and their corresponding gene/protein targets. This shift has significantly advanced the digitization and globalization of TCM (Zhu X et al., 2022).

Network pharmacology analysis stands out as one of the most successful applications in omics investigations (Yang H-Y. et al., 2022; Zhu Y. et al., 2022; Zhu X. et al., 2022; Zhao et al., 2023). TCM formulations usually comprise a variety of components and target multiple biological pathways, with the aim of achieving synergistic effects in treating diseases. This aligns with the fundamental principle of network pharmacology analysis (Zhang et al., 2019; Yang P. et al., 2022), which is dedicated to elucidating the specific compounds within TCM formulations responsible for therapeutic effects and identifying potential targets for drug development (Boezio et al., 2017; Wu et al., 2018; Zhang et al., 2019; Gan et al., 2023). Consequently, network pharmacology analysis has evolved into a powerful approach, leveraging the intricate network of interactions among ingredients, compounds, proteins/genes, and diseases to advance TCM research (Zhang et al., 2019; Yang P. et al., 2022). It offers an effective means to explore the complex interactions between TCM and diseases. To date, several pioneering researchers have investigated the regulatory mechanism of TCM formulations on ferroptosis using omics methodologies. This has marked a transition in TCM research from the traditional "one-target, one-drug" model to a "multi-target, multi-component" framework (Ding et al., 2021; Liu et al., 2021; Zhang Q. et al., 2022; Chen J. et al., 2023; Liu S. et al., 2023; Wang Y. et al., 2023; Wang et al., 2023d; Gu et al., 2023; Qin et al., 2023; Wu et al., 2023; Bai et al., 2024; Shi et al., 2024).

However, applying the multi-omics approach to TCM comes with several limitations (Zhu X. et al., 2022). Network analyses, for instance, are still in their early stages and can sometimes be misleading. These limitations encompass various aspects. First, the vast amount of heterogeneous data, characterized by high dimensionality and complexity, poses a formidable challenge for TCM analysis. Second, a scarcity of data resources hinders a comprehensive comprehension of TCM formulations. This encompasses the identification of herbal components and their

bioactive compounds, as well as the elucidation of interactions among small molecules within herbal formulas and human genes and proteins. The complex chemical composition of TCM further complicates both the characterization of their bioactive compounds and the study of their systemic effects in humans. Thirdly, a constrained comprehension of biochemistry has impeded a more profound insight into the pharmacological and toxicological impacts of TCM. Furthermore, despite the extensive utilization of multi-omics in TCM research and clinical contexts, augmenting our understanding of the connections between TCM and diseases could be achieved through the integration of supplementary digital data, encompassing protein structural information, biological visuals, and electronic health records. Finally, while omics approaches have been extensively employed in new drug discovery for TCM, the pharmacodynamic effects of these new drugs should be rigorously evaluated through clinical trials. In future studies, it is imperative that bioinformatics experts with strong clinical backgrounds serve as intermediaries, facilitating the integration of multi-omics methodologies and datasets into established clinical practices to enhance clinical diagnostics. We are confident that these efforts will prove beneficial in advancing the modernization, global integration, and digitization of TCM to address a myriad of complex medical conditions.

3.11.2.3 Nano-TCM

While TCM and its natural components show potential in treating various diseases, their clinical application faces challenges such as limited administration methods, low solubility, instability, brief biological half-life, limited targeting, facile metabolism, and swift elimination (Thanki et al., 2013; Wei et al., 2022). Therefore, the concept of nano-TCM has been introduced as an innovative strategy to enhance the clinical applicability of TCM. Nanotechnology confers substantial benefits in surmounting these impediments (Zhang C. et al., 2020; Shan et al., 2020; Wong et al., 2020; Wang J. et al., 2021; Zheng et al., 2021; Zhang S. et al., 2022). Nanotechnology can enhance the bioavailability and specificity of TCM while concurrently reducing adverse reactions through nano-processing (Huang et al., 2023).

First, nano drug delivery systems (NDDSs) improve the permeability of TCMs, facilitating their transport across physiological barriers such as the skin, mucosa, and blood-brain barrier (Arvanitis et al., 2020; Hakim et al., 2020; Paiva-Santos et al., 2021). This improved transport occurs through transcellular, paracellular, and carrier-mediated pathways (Khan et al., 2015). Secondly, NDDSs facilitate the prolonged or regulated release of TCMs (Moradi Kashkooli et al., 2020). Prolonged release extends the duration of therapeutic effects, ensuring consistent release rates, lessening the need for frequent dosing, and mitigating side effects. Controlled release, accomplished via external means (such as ultrasound, near-infrared light, or microwaves) or internal factors (such as the microenvironment conditions at the lesion site, including pH and specific enzymes), reduces toxicity in healthy tissues while optimizing therapeutic efficacy (Hossen et al., 2019; Jiang and Zhang, 2023). Thirdly, NDDSs upgrade the bioavailability of hydrophobic TCM ingredients. These NDDSs greatly augment the bioavailability of TCM components by enhancing their aqueous solubility and biocompatibility (Bilia et al., 2019). Lastly, NDDSs enable the co-delivery of different TCM ingredients, harnessing synergistic effects and allowing for lower and safer doses. Various NDDSs, including polymer-based, mesoporous silica, and lipid-based carriers, have been utilized for this purpose (Gurunathan et al., 2018; Cano et al., 2020; Paris and Vallet-Regí, 2020).

In the field of "ferroptosis-TCM", a biomimetic NDDS was designed by a team of researchers from Nanjing Medical University. This system involved the creation of nanoparticles (NPs) loaded with resveratrol and coated with erythrocyte membrane. The objective was to facilitate the evasion of macrophage phagocytosis, leading to an extended circulation period and ultimately enhancing ferroptosis-induced anticancer effects (Zhang Z. et al., 2022). In the same year, researchers from Shanghai University of Traditional Chinese Medicine developed camptothecin (CPT) NPs (Su et al., 2022). These NPs were designed as a NDDS consisting of CPT-loaded polydopamine, coated with manganese dioxide. This innovative approach enabled both diagnosis and therapy, utilizing magnetic resonance imaging and chemo-photothermal therapy, respectively.

However, nano-TCM is still in its infancy. Nano-TCM is currently characterized by a lack of guidance from TCM theory and inadequate basic research. Nanotechnology has altered or controlled certain physical and chemical reactions among TCM ingredients, introducing uncertainty regarding the active components of TCM. This poses a challenge to TCM theory. Therefore, it is crucial to gain a deeper understanding of metabolic pathways to ensure the clinical safety of nanocarriers and guide their systematic design (Wei et al., 2022). Secondly, although nanotechnology's application in TCM research has expanded the possibilities for TCM modernization, there remains a pressing need to bolster fundamental and exploratory research. Urgent attention should be given to addressing the deficiencies in nanotechnology preparation techniques, the insufficiencies in evaluating pharmacological efficacy, and the discrepancies between the prerequisites for encapsulating pharmacodynamic elements of TCM and nanoparticles NPs. Furthermore, the clinical efficacy of nanodrugs raises thought-provoking concerns (Cabral et al., 2023). Over the past 3 decades, nanodrugs have proliferated, with increasingly complex designs. However, only ten nanodrugs have received approval from the US Food and Drug Administration, and only 14% of these medications have demonstrated enhanced clinical efficacy (He et al., 2019). Finally, the safety of NPs is a critical issue that requires thorough investigation (Zhang et al., 2021; Chen Y. et al., 2023; Lu et al., 2023). Although the majority of safety assessments for nanocarriers are conducted in diverse cell lines and animal models, human biological responses can exhibit variability, which constrains the applicability of safety evaluations solely reliant on animal research.

To advance TCM, it is essential to promote interdisciplinary cooperation and facilitate the fusion of TCM with modern scientific and technological methods. While adhering to the core principles of TCM theory, it is crucial to foster creativity and advancements to revitalize its practical applications. NDDSs present a chance to investigate the utilization of TCM, and the amalgamation of TCM with nanotechnology can provide inventive approaches and viewpoints to propel the modernization of TCM, consequently bolstering its sustained growth (Sun R. et al., 2022; Wei et al., 2022; Huang et al., 2023).

3.11.3 Future development prospects

TCM has shown significant potential in the regulation and treatment of human diseases; however, further exploration in this research area is needed. Currently, "ferroptosis-TCM" research faces three major challenges. Firstly, studies on TCM are mostly limited to the treatment of active ingredients or herbal monomers, with few investigations into TCM compound prescriptions, proprietary Chinese medicines, and external treatment methods (Xie et al., 2023; Chen et al., 2024). Secondly, there are four broad mechanisms have been identified for inducing ferroptosis (Dixon et al., 2012): inhibition of the system Xc^- (Sun et al., 2023), inhibition/degradation/inactivation of GPx4 (Wang X. et al., 2023), depletion of reduced coenzyme Q_{10}, and (Friedmann Angeli et al., 2019) induction of lipid peroxidation through peroxides, iron, or polyunsaturated fatty acid overload. A great deal of TCMs induce tumor ferroptosis via different mechanisms (Wang et al., 2023b). However, these investigations often focus on a single regulatory pathway mentioned above, neglecting the cross-dialogue within the overall regulatory networks of ferroptosis. A similar issue is observed in the domain of anti-ferroptotic TCMs. Thirdly, different diseases have distinct therapeutic targets. Promoting ferroptosis in tumor cells aids in inhibiting and killing tumors, but normal cells like cardiomyocytes, pancreatic β-cells, and neurons are also sensitive to ferroptosis. Addressing the selective regulation of ferroptosis is a major issue that requires extensive future research.

In the future, efforts should integrate clinical TCM practice with basic research. Exploring the molecular mechanisms of TCM in regulating ferroptosis from multiple channels and perspectives is essential. Additionally, there is a need to increase the study of ferroptosis-related mechanisms in TCM compound prescriptions, proprietary Chinese medicines, and external treatments with remarkable clinical effects. This exploration aims to understand the specific mechanisms of preventing and treating human diseases by targeting ferroptosis, contributing to the development of new theoretical foundations and potential therapeutic strategies for clinical treatment.

3.12 Strengths and limitations

In contrast to previous investigations primarily limited to systematic or narrative reviews, the combination of scientometric and visualized analysis provides readers with a clearer representation of research focal points and trends across various dimensions (Nguyen et al., 2021; Zhang J. et al., 2022). In its role as the inaugural bibliometric analysis endeavoring to chart the knowledge landscape of "ferroptosis-TCM" during the last 11 years. This research provides a relatively exhaustive and unbiased point of reference, notwithstanding inherent limitations.

This study has quite a few limitations: 1) The use of CiteSpace, which is limited to WoSCC publications, may introduce selection bias due to the software's inherent limitations (Ling et al., 2023). 2) Citation bursts, influenced by factors like publication date and journal quality, may not accurately reflect a paper's influence. 3) The inability to comprehensively review and analyze all papers and their subfields necessitated equal attention to both high and low-quality publications, potentially impacting the study's credibility. 4) Scientometric methods, reliant on natural language processing, may manifest biases, as evidenced in previous studies (Zhang et al., 2018; Yan et al., 2021; Zhang J. et al., 2022; Cao et al., 2023b; Cao et al., 2024). 5) Limiting the research to English-language documents may introduce publication bias. 6) Incomplete retrieval of recent literature and keywords may impact the results due to gaps in literature collection. 7) VOSviewer automatically extracts author names, which may not always be extracted accurately. Some authors may use different name spellings or multiple names, potentially leading to inaccuracies in research results for these authors (Peng et al., 2022).

4 Conclusion

The present study conducts a trend analysis of "ferroptosis-TCM" within the biomedical field, spanning the years 2012–2023 and utilizing scientometric methodologies. The analysis specifically focuses on international collaboration, publication trends, and research hotspots. These findings serve to empower the scientific community by identifying emerging ideas and frontiers that will shape the future of ferroptosis research for TCM. For researchers, keeping abreast of these trends and utilizing existing knowledge to propel progress in this field is paramount. However, "ferroptosis-TCM" faces numerous pressing issues, including, but not limited to (Dixon et al., 2012): the elusive primary factor responsible for triggering ferroptosis following lipid peroxidation (Sun et al., 2023); the inadequate exploration of TCM, encompassing both ferroptosis inducers and inhibitors, with regard to conditions of application, initiation time point, dose, administration form, and duration (Wang X. et al., 2023); the current understanding of "ferroptosis-TCM" relies primarily on data from animal or cellular studies, with limited evaluation of its clinical safety and efficacy; and (Friedmann Angeli et al., 2019) crosstalk exists between ferroptosis and other types of PCD. The targeting of ferroptosis heralds a new era for TCM, with the potential to revolutionize healthcare despite the existing challenges. To overcome these challenges and fully leverage the potential of "ferroptosis-TCM" within this field, increased efforts and enhanced collaboration are imperative across the fields of pharmacology, biology, basic science, and clinical medicine.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Author contributions

SC: Conceptualization, Formal Analysis, Writing–original draft. YW: Conceptualization, Formal Analysis, Writing–original draft. YY: Data curation, Investigation, Writing–review and editing. YC: Data curation, Investigation, Writing–review and editing. SL: Methodology, Software, Writing–review and editing. AL: Methodology, Software, Writing–review and editing. PL: Funding acquisition, Project administration, Supervision, Writing–review and editing. AX: Funding acquisition, Project administration, Supervision, Writing–review and editing. HZ: Funding acquisition, Project administration, Supervision, Writing–review and editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This study received funding from various grants: the National Natural Science Foundation of China (Nos 82172432, 82102568, and 82001319); the National & Local Joint Engineering Research Centre of Orthopaedic Biomaterials (No. XMHT20190204007); the Guangdong Basic and Applied Basic Research Foundation (Nos. 2021A1515012586, 2021A1515220054, 2022B1515120046, 2022A1515220038, 2022A1515220111, 2021A1515220037, and 2022A1515220165); the Shenzhen High-Level Hospital Construction Fund; the Shenzhen Key Laboratory of Orthopaedic Diseases and Biomaterials Research (No. ZDSYS20220606100602005); the Shenzhen Key Medical Discipline Construction Fund (No. SZXK023); the Sanming Project of Medicine in Shenzhen (No. SZSM202211038); the Research and Development Projects of Shenzhen (Nos. JCYJ20210324110214040, JCYJ20220531094214032, JCYJ20190809152409606, JCYJ20210324105806016, and JCYJ20220531094406015); the Shenzhen Sustainable Development Project (No. KCXFZ20201221173411031); and the Scientific Research Foundation of Peking University Shenzhen Hospital (No. KYQD2021099).

Acknowledgments

We express our heartfelt gratitude to the researchers whose significant contributions have driven progress in this field. We also acknowledge the valuable language assistance provided by Home for Researchers (https://www.home-for-researchers.com) and the unwavering support from the funding organizations that facilitated this work. Figure 9 were generated using Figdraw (https://www.figdraw.com/static/index.html#/).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2024.1366852/full#supplementary-material

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frontiers | Frontiers in Pharmacology

TYPE Systematic Review
PUBLISHED 13 March 2024
DOI 10.3389/fphar.2024.1373663

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OPEN ACCESS

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RECEIVED 20 January 2024
ACCEPTED 29 February 2024
PUBLISHED 13 March 2024

CITATION
Wang Z, Wu Z, Miao Y, Hao A, Chen H, Zhao S, Luo M, Guo S, Liu Y and Lu Y (2024), The protective effects of ligustrazine on ischemic stroke: a systematic review and meta-analysis of preclinical evidence and possible mechanisms.
Front. Pharmacol. 15:1373663.
doi: 10.3389/fphar.2024.1373663

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The protective effects of ligustrazine on ischemic stroke: a systematic review and meta-analysis of preclinical evidence and possible mechanisms

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Introduction: The objective of this study is to systematically evaluate the effect of ligustrazine on animal models of ischemic stroke and investigate its mechanism of action.

Materials and Methods: The intervention of ligustrazine in ischemic diseases research on stroke model animals was searched in the Chinese National Knowledge Infrastructure (CNKI), Wanfang Database (Wanfang), VIP Database (VIP), Chinese Biomedical Literature Database (CBM), Cochrane Library, PubMed, Web of Science, and Embase databases. The quality of the included literature was evaluated using the Cochrane risk of bias tool. The evaluation included measures such as neurological deficit score (NDS), percentage of cerebral infarction volume, brain water content, inflammation-related factors, oxidative stress-related indicators, apoptosis indicators (caspase-3), and blood-brain barrier (BBB) permeability (Claudin-5).

Results: A total of 32 studies were included in the analysis. The results indicated that ligustrazine significantly improved the neurological function scores of ischemic stroke animals compared to the control group (SMD = −1.84, 95% CI −2.14 to −1.55, $P < 0.00001$). It also reduced the percentage of cerebral infarction (SMD = −2.97, 95% CI −3.58 to −2.36, $P < 0.00001$) and brain water content (SMD = −2.37, 95% CI −3.63 to −1.12, $P = 0.0002$). In addition, ligustrazine can significantly improve various inflammatory factors such as TNF-α (SMD = −7.53, 95% CI −11.34 to −3.72, $P = 0.0001$), IL-1β (SMD = −2.65, 95% CI −3.87 to −1.44, $P < 0.0001$), and IL-6 (SMD = −5.55, 95% CI −9.32 to −1.78, $P = 0.004$). It also positively affects oxidative stress-related indicators including SOD

Abbreviations: APC, 3K3A-activating protein C; BBB, blood-brain barrier; CBM, Chinese Biomedical Literature Database; CI, confidence interval; CIS, cerebral ischemic stroke; CNKI, China National Knowledge Infrastructure; HPLC, high-performance liquid chromatography; HUK, human urinary kininogenase; IL, interleukin; INOS, inducible NOS; MCAO, middle cerebral artery occlusion; MDA, malondialdehyde; MMP-9, matrix metalloproteinase-9; NDS, neurological deficit score; NO, nitric oxide; NOS, nitric oxide synthase; RR, relative risk; SMD, standardized mean difference; SOD, superoxide dismutase; TNF, tumor necrosis facto; tPA, tissue plasminogen activator; VIP, VIP Database.

(SMD = 4.60, 95% CI 2.10 to 7.10, $P = 0.0003$), NOS (SMD = −1.52, 95% CI −2.98 to −0.06, $P = 0.04$), MDA (SMD = −5.31, 95% CI −8.48 to −2.14, $P = 0.001$), and NO (SMD = −5.33, 95% CI −8.82 to −1.84, $P = 0.003$). Furthermore, it shows positive effects on the apoptosis indicator caspase-3 (SMD = −5.21, 95% CI −7.47 to −2.94, $P < 0.00001$) and the expression level of the sex-related protein Claudin-5, which influences BBB permeability (SMD = 7.38, 95% CI 3.95 to 10.82, $P < 0.0001$).

Conclusion: Ligustrazine has been shown to have a protective effect in animal models of cerebral ischemic injury. Its mechanism of action is believed to be associated with the reduction of inflammation and oxidative stress, the inhibition of apoptosis, and the repair of BBB permeability. However, further high-quality animal experiments are required to validate these findings.

KEYWORDS

ligustrazine, ischemic stroke, inflammation, oxidative stress, apoptosis, meta-analysis

1 Background

Stroke is the second leading cause of death and the third leading cause of disability in the world (Lozano et al., 2012; Murray et al., 2012). In 2016, there were 13.7 million new stroke events worldwide, with 87% of them being ischemic strokes. During the same year, 2.7 million people died from stroke. Cerebral ischemic stroke (CIS) imposes a significant health and economic burden on the world, particularly on low- and middle-income countries (Collaborators, 2019; Saini et al., 2021). CIS is typically caused by embolism or thrombotic artery occlusion, resulting in reduced cerebral blood flow and various forms of brain damage, including tissue and structural damage, as well as neuronal death and defects (Xing and Bai, 2020; Zhao et al., 2022). Recombinant tissue plasminogen activator (tPA) is currently the only CIS therapy approved by the U.S. Food and Drug Administration. However, this therapy has a limited time window of 4.5 h and carries the potential risk of hemorrhagic transformation, resulting in only 10% of stroke patients benefiting from it (Bansal et al., 2013; Tao et al., 2020). Recent advancements in the research of stroke neuroprotective drugs have identified promising candidates such as human urinary kininogenase (HUK), statins, edaravone, 3K3A-activating protein C (APC), and vinpocetine. These drugs target multiple pathophysiological pathways involved in stroke-related brain injury, with a primary focus on inflammation and oxidative stress. (Paul and Candelario-Jalil, 2021). While these studies provide optimism for CIS treatment, further research is needed to fully address the complexities of this condition. Moreover, there is an urgent need to enhance the current treatment's effectiveness in addressing motor dysfunction and neurological damage caused by CIS. Therefore, it is crucial to explore new complementary and alternative therapies to overcome the limitations of existing treatments in stroke management.

Ligustrazine, an alkaloid monomer derived from the dried rhizome of *Ligusticum striatum DC* [Apiaceae] (Figure 1). *Ligusticum striatum DC* was first documented in *Shennong's Materia Medica*, a compilation of Chinese herbal medicine information dating back to 2800 BC (Li et al., 2021). According to traditional Chinese medicine, CIS is attributed to wind, fire, phlegm, blood stasis, and deficiency, with *L. striatum DC* to improve clinical symptoms of CIS by enhancing qi and blood circulation, activating blood flow, and resolving blood stasis. Modern scientific studies have shown that *L. striatum DC* can be effective in stroke treatment, with its mechanism of action linked to the regulation of the TNF/IL-17 signaling pathway (Zhang et al., 2023). Ligustrazine, an active compound in *L. striatum DC*, plays a key role in these therapeutic effects. Preclinical studies have demonstrated that ligustrazine has the potential to mitigate CIS injury through various mechanisms, including alleviating inflammatory response, resisting

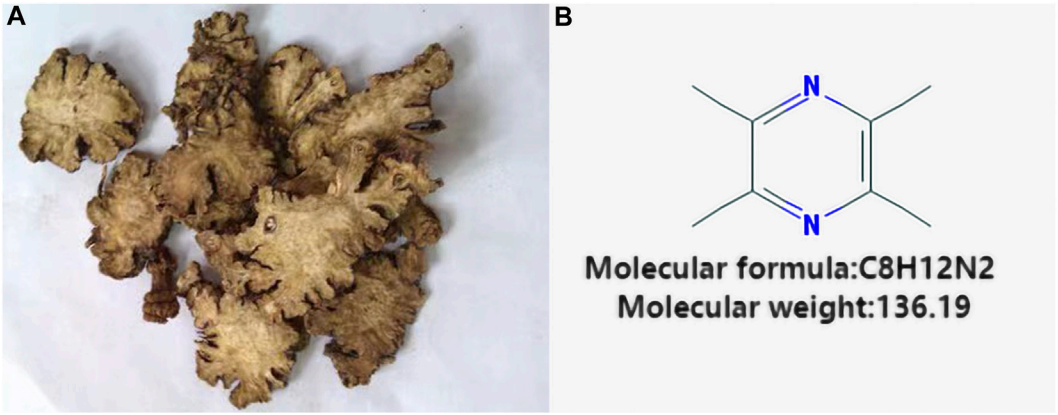

FIGURE 1
Vegetal products of *Ligusticum striatum DC* and chemical structure of Ligustrazine. **(A)** Vegetal products of *Ligusticum striatum DC*. **(B)** Chemical structure of Ligustrazine.

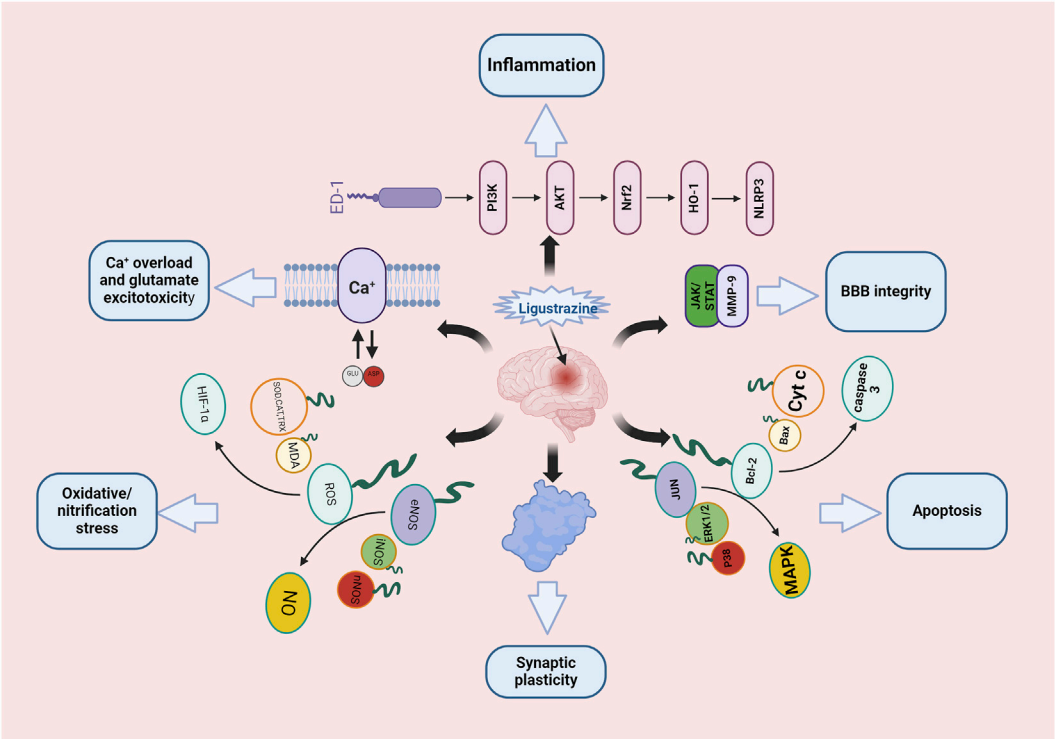

FIGURE 2
Mechanism diagram of ligustrazine intervention in CIS.

apoptosis, protecting the BBB, combating oxidative stress, inhibiting calcium overload and glutamate excitotoxicity, and enhancing synaptic plasticity (Figure 2). Specifically, ligustrazine has been shown to regulate inflammation-related factors by inhibiting the immunoreactivity of ED-1, DNA fragmentation, caspase-3 activation, and Cyt c release. Additionally, it inhibits the activation of the JAK/STAT signaling pathway and matrix metalloproteinase-9 (MMP-9) expression to reduce BBB permeability. Ligustrazine also reduces the concentration of ROS and malondialdehyde (MDA) while upregulating the activity of superoxide to relieve oxidative stress. Furthermore, it intervenes in the course of ischemia-reperfusion by reducing $Ca2^{+}$ overload and trough amino acid excitotoxicity, as well as improving synaptic ultrastructure to enhance synaptic plasticity (Liu et al., 2022).

While numerous studies have delved into the neuroprotective effects and potential mechanisms of ligustrazine in CIS models, most have primarily focused on specific pathways or a limited set of efficacy indicators. A comprehensive and quantitative analysis of the various mechanisms of action of ligustrazine in CIS has yet to be reported. Although some studies have conducted systematic reviews and meta-analyses of clinical trials on ligustrazine for treating CIS (Shao et al., 2021), it remains essential to quantify and evaluate its mechanism of action through preclinical meta-analysis. This study aims to systematically evaluate and quantify the intervention effect of ligustrazine on CIS across multiple mechanisms and efficacy indicators. By synthesizing existing data, the study seeks to uncover the therapeutic potential and multifaceted action mechanism of ligustrazine in CIS, offering insights for future clinical research.

2 Materials and methods

2.1 Search strategy

Two researchers conducted independent searches on several databases including CNKI, Wanfang, VIP, CBM, Cochrane Library, PubMed, Web of Science, and Embase. The studies focused on the intervention of ligustrazine in animal models of CIS. The search period ranged from the establishment of the databases to January 2024. Additionally, the researchers traced the references of included articles and visited the International Clinical Trials Registry of the National Institutes of Health and the Chinese Clinical Trials Registry to supplement the acquisition of relevant literature. Any disagreements were resolved by involving a third researcher. The search terms used were: “brain infarction” or “brain ischemia” or “cerebral ischemia reperfusion injury” or “cerebral thrombosis” or “cerebral embolism” or “ischemic stroke” or “middle cerebral artery occlusion” or “MCAO” and “tetramethylpyrazine” or “ligustrazine".

2.2 Inclusion criteria and outcome indicators

2.2.1 Outcome indicators

1) **Primary outcome measures:** Neurological deficit score (NDS).
2) **Secondary outcome measures**:
   a. Percentage of infarct volume
   b. Brain water content

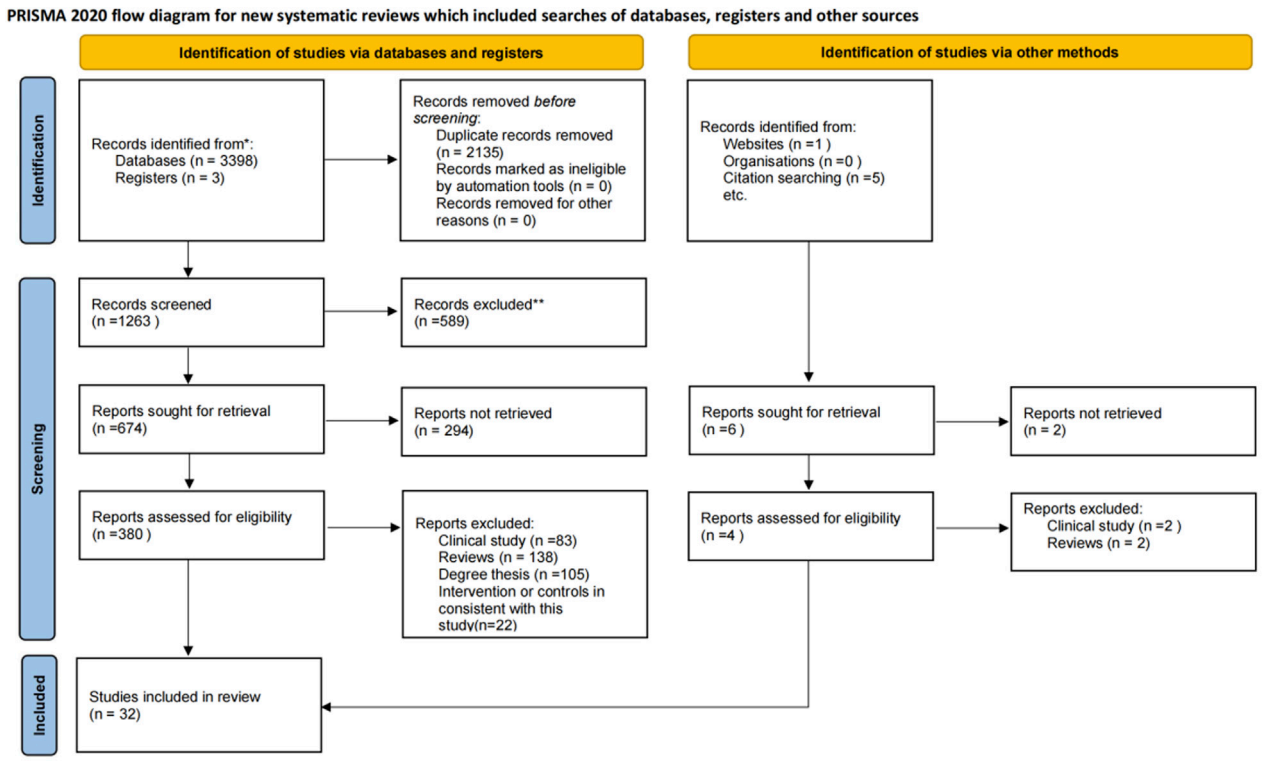

FIGURE 3
PRISMA flow chart for literature screening.

c. Inflammation related factors, including tumor necrosis factor-α (TNF-α), interleukin -1β (IL-1β) and interleukin −6 (IL-6).
d. Oxidative stress-related indicators, including Superoxide Dismutase (SOD), Nitric Oxide Synthase (NOS), MDA and Nitric Oxide (NO).
e. Apoptosis marker caspase-3.
f. BBB -related protein Claudin-5.

2.2.2 Inclusion criteria

1)Study Type: This study focuses on animal experiments conducted on rats or mice using permanent CIS or ischemic stroke/reperfusion models. 2) Interventional measures: experimental group was treated with ligustrazine without restriction, control group was treated with placebo only, placebo included saline, PBS, etc., 3) Language: There are no language restrictions for this study.

2.2.3 Exclusion criteria

1) Animal experiments in permanent CIS or ischemic stroke/reperfusion models other than rats or mice. 2) Drug combination therapy. 3) Review, conference, case report, dissertation, clinical trial research. 4) Duplicated studies are excluded according to time. 5) Failure to reach the result index or to extract effective indicators. 6) Clinical studies or *in vitro* experiments.

2.3 Literature screening and data extraction

Two reviewers independently screened the literature, extracted data, and cross-checked. In the event of disagreements, a third party was consulted for assistance in making a judgment. If any data were missing, the authors were contacted to provide additional information. During the literature screening process, the title and abstract were initially read, and after excluding obviously irrelevant literature, the full text was further examined to determine its inclusion. The data extraction process mainly involved capturing the basic characteristics of the study, such as the study name, publication date, animal species, animal weight, and animal model. It also included specific details such as intervention measures, control measures, intervention time, drug dosage, and other relevant information used in the study. Key elements for evaluating the risk of bias were also considered, along with the outcome indicators involved in the study. For studies reporting data only in the form of images, we used the GetData Graph Digitizer software to extract the data from the images.

2.4 Quality evaluation of the included studies

In this study, two researchers conducted a systematic review of the included studies using the Cochrane risk of bias assessment tool

(Cumpston et al., 2019). The evaluation focused on several key aspects, including selection bias (whether the random sequence generation was adequately described and whether allocation concealment was implemented), implementation bias (whether the subjects and experimenters were blinded), measurement bias (whether the outcome assessors were blinded), follow-up bias (whether the outcome data was complete), reporting bias, and other potential biases.

2.5 Statistical methods

Statistical analysis of the data was conducted using Review Manager 5.4 software, and sensitivity analysis was performed using Stata16. This study utilizes relative risk (RR) and 95% confidence interval (CI) as indicators for dichotomous variables, while Standardized mean difference (SMD) and 95% CI are used for continuous variables. Heterogeneity between studies was analyzed using the χ^2 test. If $I^2 \leq 50\%$, it indicates small heterogeneity between studies, and the fixed effects model is applied. On the other hand, if $I^2 \geq 50\%$, it suggests large heterogeneity between studies, and a random effects model is utilized. Subgroup analysis or sensitivity analysis is employed when necessary. The presence of publication bias was assessed by analyzing outcome indicators from studies with a minimum of 10. A statistically significant difference is considered when $p < 0.05$.

2.6 Publication bias

Publication bias was assessed using a funnel plot and calculated with STATA 16 using Begg's test.

3 Results

3.1 Literature screening process and results

A total of 3,398 relevant documents were retrieved. After applying the inclusion and exclusion criteria, 32 studies (Liao et al., 2004; Kao et al., 2006; Chang et al., 2007; Li et al., 2008; Qi et al., 2008; Ren et al., 2008; Zhang et al., 2008; Jia et al., 2009; Zhu et al., 2009; Hu et al., 2010a; Hu et al., 2010b; Chen et al., 2010; Fang et al., 2010; Ma and Chen, 2010; Xiao et al., 2010; Yang and Ren, 2011; Zhang et al., 2011; Kao et al., 2013; Li et al., 2013; Han et al., 2014; Tian et al., 2014; Tan et al., 2015; Liu, 2016; Li et al., 2017; Gong et al., 2019; Zhu et al., 2020; Ge et al., 2021; Li et al., 2022; Liang et al., 2022; Chang et al., 2023; Feng et al., 2023; Gao et al., 2023) were included. The detailed literature search and screening process is illustrated in Figure 3.

3.2 Basic characteristics of included literature

A total of 32 studies were included in this study. Among these, three studies used Wistar rats (Chang et al., 2007; Li et al., 2008; Zhang et al., 2011) weighing 250–320 g, two studies used Kunming mice (Hu et al., 2010a; Hu et al., 2010b) weighing 30–40 g, and one study used C57BL/6 mice (Li et al., 2022), but the weight of the mice was not reported. The remaining 26 studies utilized SD rats weighing 220–350 g. Five studies employed the MCAO model, while 27 studies used the MCAO/reperfusion model. Intraperitoneal injection was used in 29 studies, tail vein injection in two studies (Ma and Chen, 2010; Zhu et al., 2020), and oral gavage in one study (Qi et al., 2008). The basic characteristics of the 32 included documents are presented in Table 1, and information on the preparations used in the study can be found in supplementary documents.

3.3 Risk of bias

A total of 12 studies did not provide a description of random random-sequence generation, indicating a high risk of selection bias. None of the studies mentioned allocation concealment, resulting in unclear judgments for both performance bias and detection bias. Since the animal's baseline characteristics and data have been fully reported, both attrition bias and reporting bias are considered to have low risks. The authors of four studies (Hu et al., 2010a; Hu et al., 2010b; Ma and Chen, 2010; Li et al., 2013) had published similar papers during the same period, indicating a high risk. However, due to multiple factors influencing the risk of bias, the remaining 28 studies were judged to have an unclear risk. The specific bias situation is illustrated in Figure 4.

3.4 Analysis results

3.4.1 NDS

A total of 18 studies (Li et al., 2008; Qi et al., 2008; Ren et al., 2008; Jia et al., 2009; Zhu et al., 2009; Fang et al., 2010; Yang and Ren, 2011; Zhang et al., 2011; Kao et al., 2013; Han et al., 2014; Tian et al., 2014; Tan et al., 2015; Liu, 2016; Li et al., 2017; Gong et al., 2019; Li et al., 2022; Liang et al., 2022; Gao et al., 2023) reported NDS. The heterogeneity test showed a significance of $p = 0.02$, with an I^2 value of 44%. Therefore, a fixed effects model was adopted. The results indicated that ligustrazine could reduce the NDS after CIS (SMD = −1.84, 95% CI -2.14 to −1.55, $p < 0.00001$, Figure 5A). To enhance the reliability of the results, a subgroup analysis was conducted based on the animal modeling method and animal species, which demonstrated the robustness of the findings (Figures 5B, C).

3.4.2 Percentage of infarct volume

A total of nine studies (Kao et al., 2006; Fang et al., 2010; Kao et al., 2013; Tan et al., 2015; Liu, 2016; Li et al., 2017; Gong et al., 2019; Li et al., 2022; Chang et al., 2023) reported the percentage of infarct volume. The heterogeneity test showed $p = 0.09$ and $I^2 = 41\%$. Therefore, a fixed effects model was adopted. The results demonstrated that ligustrazine significantly reduced the percentage of cerebral infarct volume in CIS (SMD = −2.97, 95% CI -3.58 to −2.36, $p < 0.00001$, Figure 6A). To enhance the reliability of the findings, subgroup analysis was conducted based on the animal modeling method and animal species, which confirmed the robustness of the results (Figures 6B, C).

TABLE 1 Basic characteristics of included studies.

Characteristics of studies included in the meta-analysis								
Study ID	Animal characteristics	Animal model	Ligustrazine dosage (mg/kg)	Method of administration	Interventions		Course	Outcome
					T	S		
Chang (2007)	Male Wistar rats (250–300 g)	MCAO/reperfusion model	20	Intraperitoneal injection	Ligustrazine	Vehicle	20 min before animal modeling	d, k
Chang (2023)	Male SD rats	MCAO model	20	Intraperitoneal injection	Ligustrazine	Vehicle	Administration 30 min before and 60 min after animal modeling	b, e
Chen (2010)	Male SD rats (280–320 g)	MCAO/reperfusion model	NA	Intraperitoneal injection	Ligustrazine	Vehicle	NA	g, i
Fang (2010)	Male SD rats (280–320 g)	MCAO/reperfusion model	80	Intraperitoneal injection	Ligustrazine	Vehicle	After animal modeling	b
Feng (2023)	Male SD rats (300 ± 20 g)	MCAO model	20	Intraperitoneal injection	Ligustrazine	Vehicle	4 h after MCAO	f
Gao (2023)	Male SD rats (220–280 g)	MCAO/reperfusion model	50	Intraperitoneal injection	Ligustrazine	Vehicle	Injection immediately after ischemia reperfusion	a
Ge (2021)	Male SD rats (250–300 g)	MCAO/reperfusion model	50	Intraperitoneal injection	Ligustrazine	Vehicle	Injection 24 h after molding	d, f, g, i, j
Gong (2019)	SD rats (240–280 g)	MCAO/reperfusion model	40	Intraperitoneal injection	Ligustrazine	Vehicle	12 h after animal modeling	a, b, c
Han (2014)	Male SD rats (290 ± 10 g)	MCAO/reperfusion model	50	Intraperitoneal injection	Ligustrazine	Vehicle	Injection immediately after reperfusion	b
Hu (2010a)	Kunming mice (30–40 g)	MCAO/reperfusion model	40	Intraperitoneal injection	Ligustrazine	Vehicle	After 3 days of continuous injections	h, j
Hu (2010b)	Kunming mice (30–40 g)	MCAO/reperfusion model	40	Intraperitoneal injection	Ligustrazine	Vehicle	After 3 days of continuous injections	h, j
Jia (2009)	Male SD rats (300–350 g)	MCAO/reperfusion model	20	Intraperitoneal injection	Ligustrazine	Vehicle	Intervention administered 1, 2, 4 and 6 h after reperfusion	a
Kao (2013)	Male SD rats (300–350 g)	MCAO model	20	Intraperitoneal injection	Ligustrazine	Vehicle	Intervention 30 min before and 60 min after MCAO	a, b
Kao (2006)	Male SD rats (300–350 g)	MCAO model	40	Intraperitoneal injection	Ligustrazine	Vehicle	Intervention 30 min before and 60 min after MCAO	b
Li (2008)	Wistar rats (250–320 g)	MCAO/reperfusion model	50	Intraperitoneal injection	Ligustrazine	Vehicle	30 min after reperfusion	a
Li (2013)	Male SD rats (250–300 g)	MCAO/reperfusion model	40	Intraperitoneal injection	Ligustrazine	Vehicle	2 h after surgery administration intervention	l
Li (2017)	Male SD rats (280 ± 20 g)	MCAO/reperfusion model	20	Intraperitoneal injection	Ligustrazine	Vehicle	Post-reperfusion drug administration intervention	a, b, c, d, e, f

(Continued on following page)

TABLE 1 (*Continued*) Basic characteristics of included studies.

Characteristics of studies included in the meta-analysis								
Study ID	Animal characteristics	Animal model	Ligustrazine dosage (mg/kg)	Method of administration	Interventions		Course	Outcome
					T	S		
Li (2022)	Male C57BL/6	MCAO/reperfusion model	20	Intraperitoneal injection	Ligustrazine	Vehicle	15 min administration intervention	a, b, e
Liang (2022)	Male SD rats (200–250 g)	MCAO/reperfusion model	72	Intraperitoneal injection	Ligustrazine	Vehicle	7 days continuous administration intervention	a, k
Liao (2004)	Male SD rats (300–350 g)	MCAO/reperfusion model	40	Intraperitoneal injection	Ligustrazine	Vehicle	60 min before MCAO administration intervention	c
Liu (2016)	Male SD rats (240–270 g)	MCAO/reperfusion model	30	Intraperitoneal injection	Ligustrazine	Vehicle	Two days before molding, once a day. 0.5 h before molding, 4h and 8 h after molding	a, b, g, i
Ma (2010)	Male SD rats (325 ± 25 g)	MCAO/reperfusion model	120	Tail vein injection	Ligustrazine	Vehicle	10 min before modeling intervention	e
Qi (2008)	Male SD rats (250–300 g)	MCAO/reperfusion model	40	Oral gavage	Ligustrazine	Vehicle	After successful animal modeling	a
Ren (2008)	Male SD rats (280 ± 20 g)	MCAO/reperfusion model	40	Intraperitoneal injection	Ligustrazine	Vehicle	12 h before and 12 h after animal modeling	a
Tan (2015)	Male SD rats (250–270 g)	MCAO/reperfusion model	20	Intraperitoneal injection	Ligustrazine	Vehicle	15 min before animal modeling	a, b, c
Tian (2014)	Male SD rats (220–250 g)	MCAO/reperfusion model	30	Intraperitoneal injection	Ligustrazine	Vehicle	Once a day for 7 days	a
Xiao (2010)	Male SD rats (180–200 g)	MCAO model	40	Intraperitoneal injection	Ligustrazine	Vehicle	MCAO 2 h later	c
Yang (2011)	Male SD rats (280 ± 20 g)	MCAO/reperfusion model	35	Intraperitoneal injection	Ligustrazine	Vehicle	Before and 12 h after surgery	a
Zhang (2008)	Male SD rats (280 ± 20 g)	MCAO/reperfusion model	80	Intraperitoneal injection	Ligustrazine	Vehicle	Once daily for 3 days before surgery and daily after surgery	g, h
Zhang (2011)	Wistar (280–320 g)	MCAO/reperfusion model	10	Intraperitoneal injection	Ligustrazine	Vehicle	After successful animal modeling	a, g
Zhu (2009)	Male SD rats (300–350 g)	MCAO/reperfusion model	20	Intraperitoneal injection	Ligustrazine	Vehicle	1, 2, 4, 6 h after reperfusion	a
Zhu (2020)	Male SD rats (220–250 g)	MCAO/reperfusion model	10	Tail vein injection	Ligustrazine	Vehicle	30 min before animal modeling	c, d

T, treatment group; C, control group; NA, not available; a: NDS; b: percentage of infarct volume; c: brain water content; d: TNF - α; e: IL-1β: f: IL-6; g: SOD; h: NOS; i: MDA; j: NO; k: caspase-3; l: Claudin-5.

3.4.3 Brain water content

A total of six studies (Liao et al., 2004; Xiao et al., 2010; Tan et al., 2015; Li et al., 2017; Gong et al., 2019; Zhu et al., 2020) examined brain water content. To account for high heterogeneity (p = 0.003, I^2 = 73%), a random effects model was applied. The findings indicate that ligustrazine can reduce brain water content in patients with CIS (SMD = −2.37, 95% CI -3.63 to −1.12, p = 0.0002, Figure 7A). To enhance the reliability of the results and identify the source of

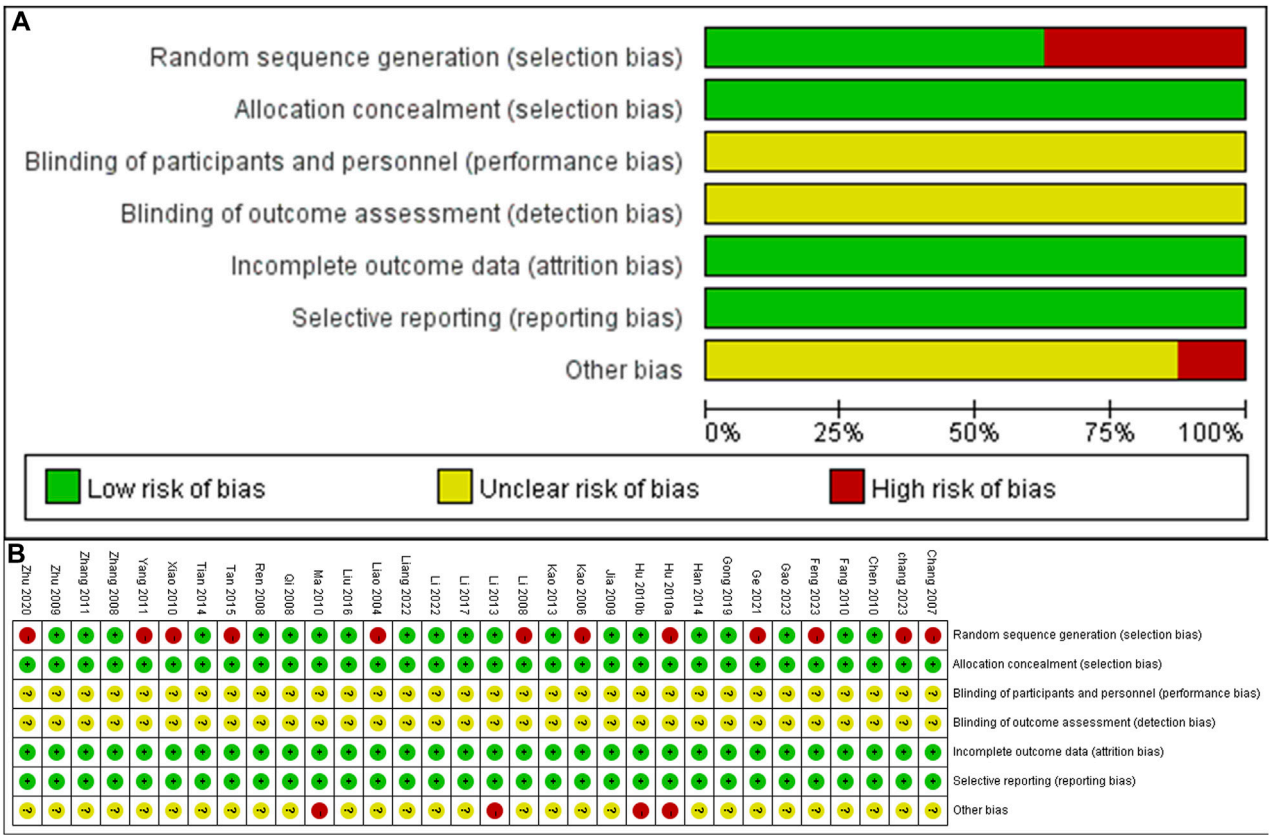

FIGURE 4
Risk of bias summary and Risk of bias graph **(A)** Risk of bias graph **(B)** Risk of bias summary.

heterogeneity, we performed a subgroup analysis based on the animal modeling method. The results demonstrated the robustness of the findings (Figure 7B). However, the animal modeling method did not contribute to the observed heterogeneity according to the subgroup analysis (Figure 7B).

3.4.4 Inflammation related factors

A total of four studies (Chang et al., 2007; Li et al., 2017; Zhu et al., 2020; Ge et al., 2021) reported TNF-α levels. Due to high heterogeneity (p = 0.002, I^2 = 79%), a random effects model was used. The findings indicated that ligustrazine was effective in reducing TNF-α indicators in animals with CIS (SMD = −7.53, 95% CI -11.34 to −3.72, p = 0.0001, Figure 8A).

A total of four studies (Ma and Chen, 2010; Li et al., 2017; Liang et al., 2022; Chang et al., 2023) reported IL-1β levels. The heterogeneity test indicated a p-value of 0.07 and an I^2 value of 58%. Therefore, a random effects model was employed. The findings demonstrated that ligustrazine had a significant impact in reducing IL-1β indicators in animals with CIS (SMD = −2.65, 95% CI -3.87 to −1.44, p < 0.0001, Figure 8B).

A total of three studies (Li et al., 2017; Ge et al., 2021; Feng et al., 2023) reported IL-6 levels. Due to high heterogeneity (p = 0.01, I^2 = 78%), a random effects model was used. The findings indicated that ligustrazine could effectively reduce IL-6 indicators in animals with CIS (SMD = −5.55, 95% CI -9.32 to −1.78, p = 0.004, Figure 8C).

3.4.5 Oxidative stress-related indicators

Five studies (Zhang et al., 2008; Chen et al., 2010; Zhang et al., 2011; Liu, 2016; Ge et al., 2021) reported the measurement of SOD, and due to significant heterogeneity (p < 0.00001, I^2 = 87%), a random effects model was employed. The findings demonstrated that ligustrazine had the ability to decrease SOD levels in animals with CIS (SMD = 4.60, 95% CI 2.10 to 7.10, p = 0.0003, Figure 9A).

Three studies (Zhang et al., 2008; Hu et al., 2010a; Hu et al., 2010b) reported NOS. Due to high heterogeneity (p = 0.0002, I^2 = 88%), a random effects model was used. The results indicated that ligustrazine could reduce NOS indicators in animals with CIS (SMD = −1.52, 95% CI -2.98 to −0.06, p = 0.04, Figure 9B).

Three studies (Chen et al., 2010; Liu, 2016; Ge et al., 2021) reported MDA levels, and due to significant heterogeneity (p = 0.002, I^2 = 84%), a random effects model was employed. The findings indicated that ligustrazine demonstrated a reduction in MDA indicators among animals with CIS (SMD = −5.31, 95% CI -8.48 to −2.14, p = 0.001, Figure 9C).

Three studies (Hu et al., 2010a; Hu et al., 2010b; Ge et al., 2021) reported NO, and due to high heterogeneity (p < 0.00001, I^2 = 94%), a random effects model was employed. The findings indicated that ligustrazine had the potential to decrease NO indicators in animals with CIS (SMD = −5.33, 95% CI -8.82 to −1.84, p = 0.003, Figure 9D).

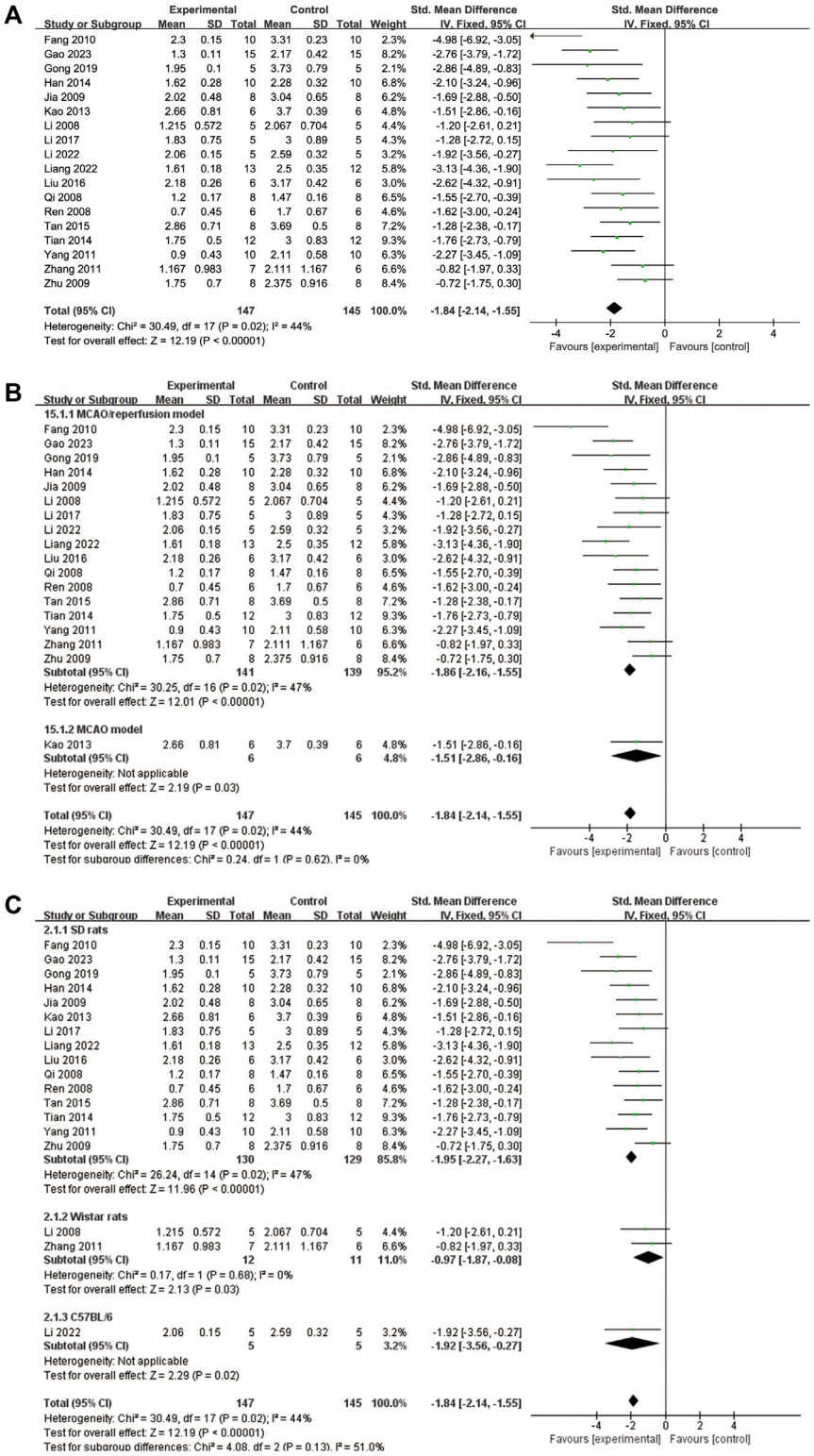

FIGURE 5
The forest plot of NDS. **(A)** The forest plot of NDS. **(B)** The forest plot of subgroup analysis based on the animal modeling method. **(C)** The forest plot of subgroup analysis based on the animal species.

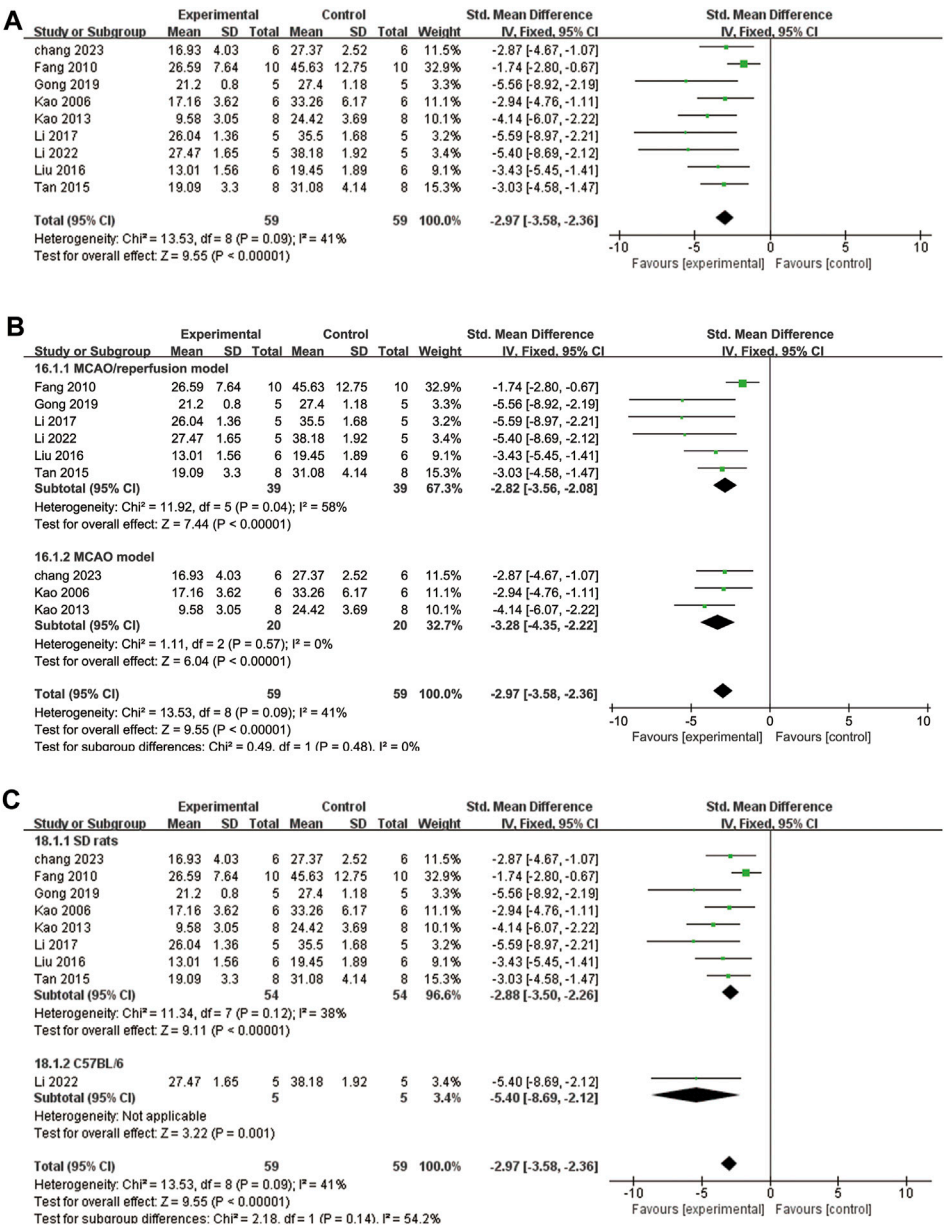

FIGURE 6
The forest plot of percentage of infarct volume. **(A)** The forest plot of percentage of infarct volume. **(B)** The forest plot of subgroup analysis based on the animal modeling method. **(C)** The forest plot of subgroup analysis based on the animal species.

3.4.6 Caspase-3

Two studies (Chang et al., 2007; Li et al., 2022) reported caspase-3 levels, and the heterogeneity test showed no significant variation (p = 0.67, I^2 = 0%). Therefore, a fixed effects model was used. The results indicated that ligustrazine had a significant effect in reducing caspase-3 levels in animals with CIS (SMD = −5.21, 95% CI -7.47 to −2.94, p < 0.00001, Figure 10).

3.4.7 Claudin-5

Three studies (Li et al., 2013; Tan et al., 2015; Gong et al., 2019) examined Claudin-5, and after conducting a heterogeneity test (p = 0.007, I^2 = 80%), a random effects model was applied. The findings revealed that ligustrazine had the potential to decrease the Claudin-5 index in animals with CIS (SMD = 7.38, 95% CI 3.95 to 10.82, p < 0.0001, Figure 11).

3.5 Sensitivity analysis

3.5.1 Brain water content

Due to the significant heterogeneity observed in this study, a sensitivity analysis was conducted. After excluding the study conducted by Zhu 2020 (Zhu et al., 2020), the heterogeneity decreased to 61%. These findings continue to support the notion that ligustrazine can effectively reduce the water content of brain tissue in animals with CIS. Further literature analysis revealed that

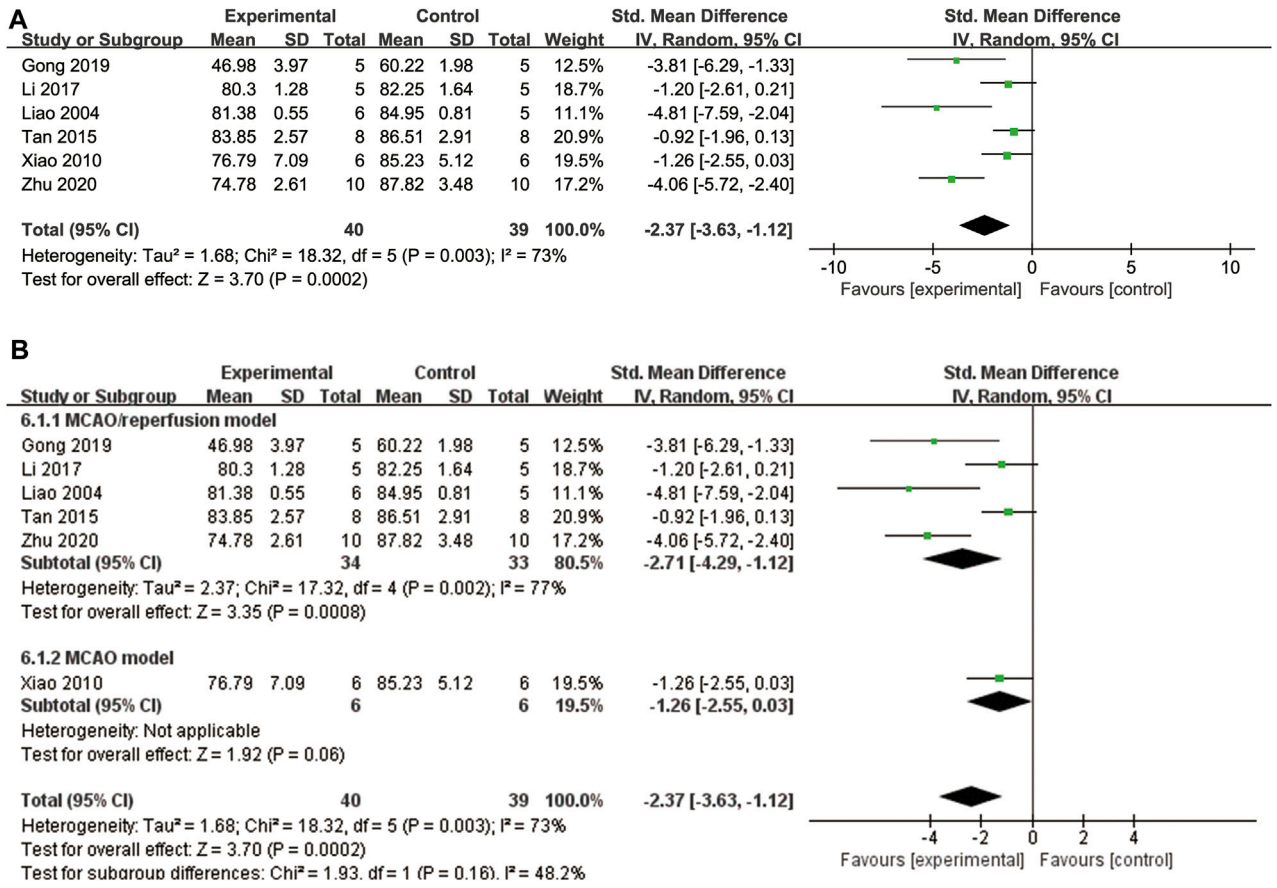

FIGURE 7
The forest plot of percentage of brain water content. **(A)** The forest plot of percentage of brain water content. **(B)** The forest plot of subgroup analysis based on the animal modeling method.

only Zhu 2020 administered the treatment via tail vein injection, whereas the remaining studies utilized intraperitoneal injection. This observed heterogeneity is likely attributed to the differences in the method of administration.

3.5.2 Inflammation related factors

TNF-α exhibits significant heterogeneity, prompting a sensitive analysis. Upon excluding Ge 2021 (Ge et al., 2021), the heterogeneity decreased to 50%. A comprehensive literature analysis identified a total of four studies contributing to this indicator. Notably, Ge 2021 utilized a dosage of 50 mg/kg, while the remaining three studies employed dosages of 20, 20, and 10 mg/kg respectively. Hence, it is speculated that the heterogeneity is attributable to the varying dosages of Ge 2021, resulting in divergent medication effects. As for IL-6, which also displays substantial heterogeneity, a heterogeneity analysis was conducted. After excluding Li 2017 (Li et al., 2017), the heterogeneity decreased to 0%. Literature analysis suggests that this heterogeneity may arise from differences in administration time and two other studies.

3.5.3 Oxidative stress-related indicators

SOD exhibited high heterogeneity, thus a sensitivity analysis was conducted. After excluding Zhang 2011 (Zhang et al., 2011), the heterogeneity decreased to 0%. Literature analysis revealed that this study employed Wistar rats, while the other four studies utilized SD rats. Hence, the heterogeneity of SOD indicators may be attributed to the different rat species.

NOS also demonstrated high heterogeneity, thus a sensitivity analysis was conducted. After excluding Hu 2020a (Hu et al., 2010a), the heterogeneity decreased to 55%. Since only three studies were included, it is speculated that the heterogeneity is linked to the limited number of experimental animals. However, the research results still support the notion that ligustrazine can regulate oxidative stress through NOS indicators, thereby protecting animals with CIS.

MDA displayed high heterogeneity, prompting a sensitivity analysis, which resulted in a heterogeneity decrease to 0% after excluding Ge 2021 (Ge et al., 2021). Literature analysis suggested that the heterogeneity may be due to varying dosages.

The heterogeneity of NO is also high, thus a sensitivity analysis was performed. Despite excluding studies one by one, the heterogeneity remained high. It is speculated that this is due to the small number of experimental animals. Nevertheless, the research results still support the idea that ligustrazine can regulate oxidative stress through NO indicators, effectively protecting animals with CIS.

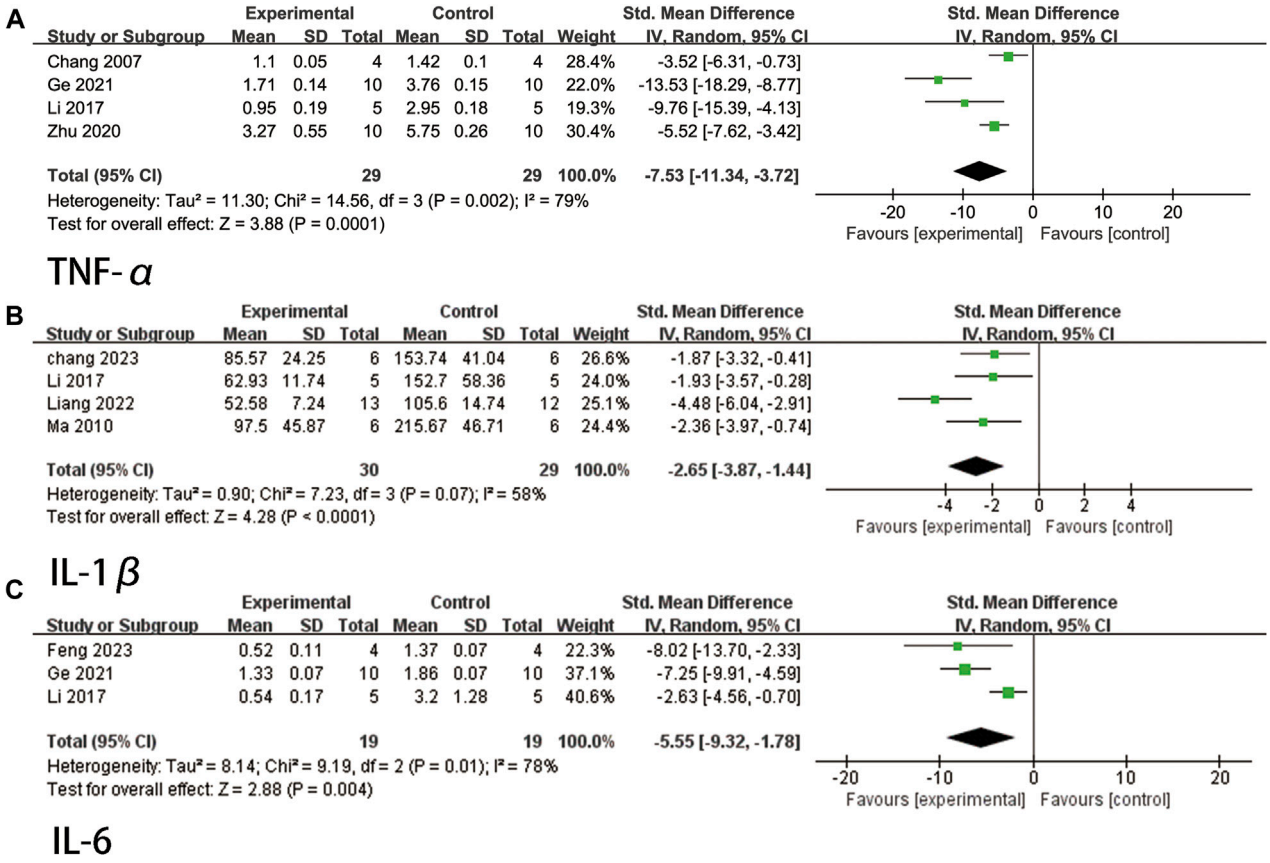

FIGURE 8
The forest plot of percentage of Inflammation related factors. **(A)** The forest plot of percentage of TNF-α. **(B)** The forest plot of percentage of IL-1β. **(C)** The forest plot of percentage of IL-6.

3.5.4 Claudin-5

Claudin-5 exhibits high heterogeneity, thus necessitating a sensitivity analysis. Upon excluding the study conducted by Li 2013 (Li et al., 2013), the heterogeneity decreased to 0%. Further examination of the literature revealed that this reduction could be attributed to the limited sample size of experimental animals. Nevertheless, the research findings continue to support the notion that Ligustrazine can safeguard animals with CIS by modulating oxidative stress through Claudin-5 indicators.

3.6 Publication bias

Given that the NDS had more than 10 articles among the outcome indicators, publication bias was evaluated. The findings indicated that there was no significant publication bias in the NDS ($z = -1.63$, $p = 0.103$, Figure 12); however, it is important to exercise caution when interpreting these results.

4 Discussion

CIS is a clinical syndrome caused by cerebral vascular disease, resulting in a disruption of cerebral blood supply, tissue necrosis due to brain tissue hypoxia and glucose deficiency, and neurological impairment (Chen et al., 2021). Current guidelines recommend thrombolysis and endovascular therapy (Kapil et al., 2017). However, thrombolytic treatment can result in rapid reoxygenation during reperfusion, leading to the generation of reactive oxygen species and subsequent oxidative stress reaction and inflammatory storm, which can be detrimental to the brain. Moreover, due to strict time constraints and various contraindications, many patients are unable to benefit from this treatment. As a result, complementary and alternative therapies are gaining increasing attention from clinical practitioners. Ligustrazine, a new calcium channel antagonist, has shown promise in the treatment of ischemic cerebrovascular disease and coronary atherosclerotic disease (Zhao et al., 2016).

Over the past 2 decades, there has been a growing body of evidence demonstrating the positive effects of ligustrazine on CIS in both *in vivo* and *in vitro* studies (Zhu et al., 2021). The mechanism of action of ligustrazine involves several beneficial processes, such as activating free radical scavenging (Yang et al., 2017; Zhang et al., 2018a), reducing BBB destruction (Tan et al., 2015; Gong et al., 2019), inhibiting inflammation (Zhou et al., 2019; Chang et al., 2023), maintaining mitochondrial function (Zhang et al., 2018b), preventing apoptosis (Chang et al., 2007), and promoting oligodendrogenesis and gliogenesis (Zhang et al., 2018a). To date,

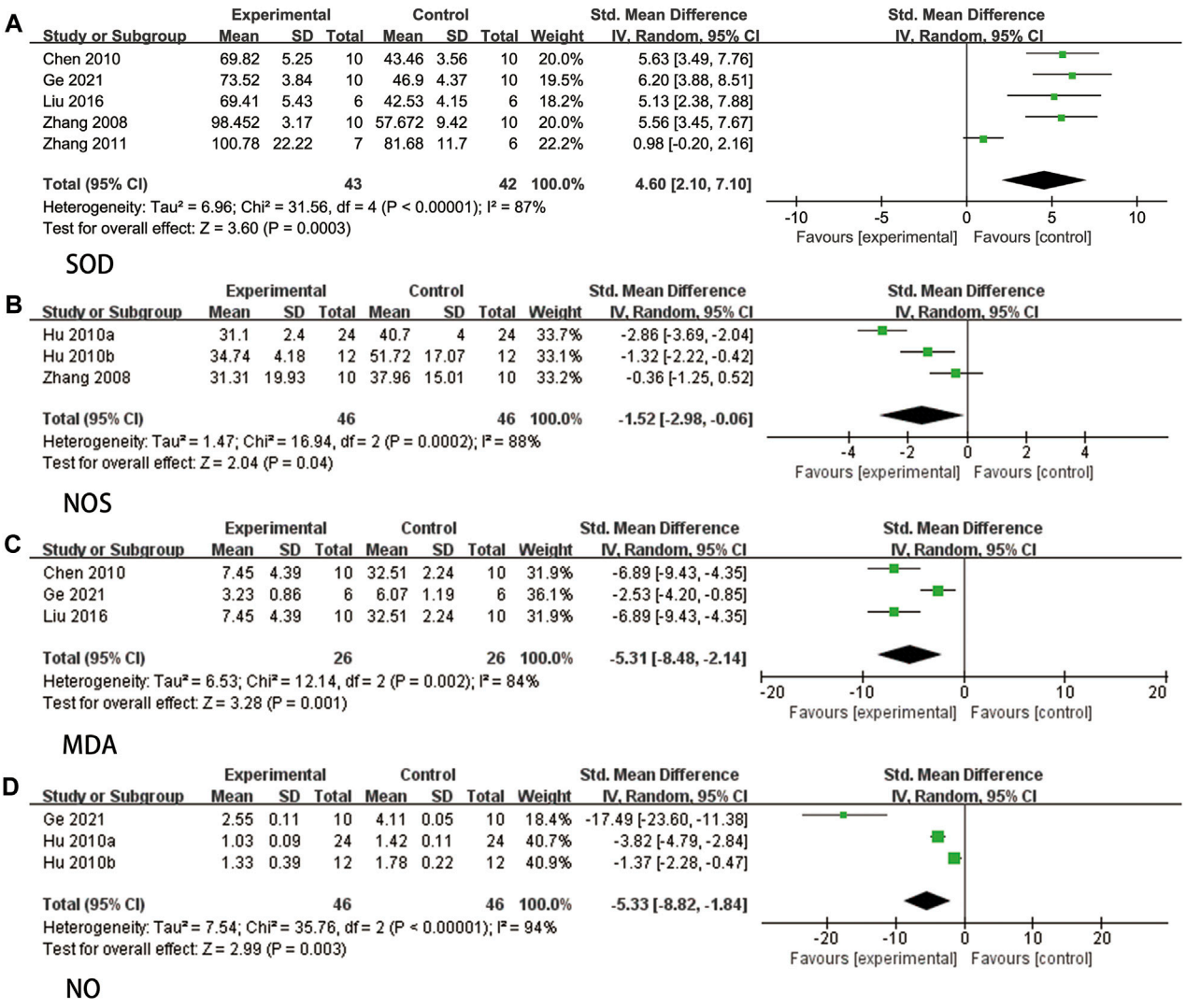

FIGURE 9
The forest plot of percentage of Oxidative stress-related indicators. **(A)** The forest plot of percentage of SOD. **(B)** The forest plot of percentage of NOS. **(C)** The forest plot of percentage of MDA. **(D)** The forest plot of percentage of NO.

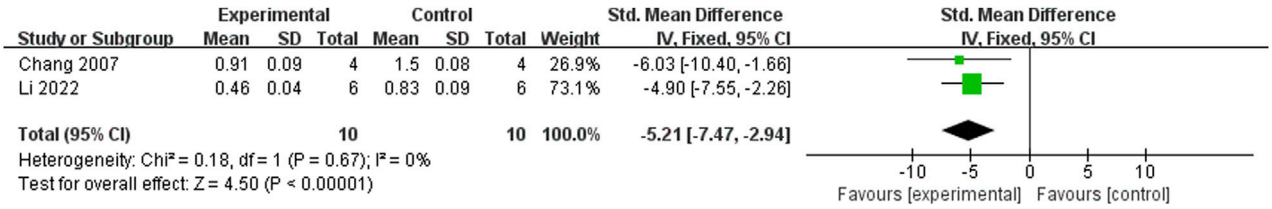

FIGURE 10
The forest plot of percentage of caspase-3.

no studies have conducted a systematic review and meta-analysis on the mechanism of action of ligustrazine. In this study, we aimed to provide a comprehensive evaluation of various studies on the treatment of CIS using ligustrazine. Ultimately, we included 32 studies to elucidate the effectiveness and mechanism of action of this treatment. Studies have demonstrated that following an ischemic stroke, there is a likelihood of significant brain edema and the occurrence of large-area infarcts. Additionally, neurons may undergo extensive necrosis and apoptosis, leading to severe damage to the cell structure (Jurcau and Simion, 2021; Fu et al., 2023). This

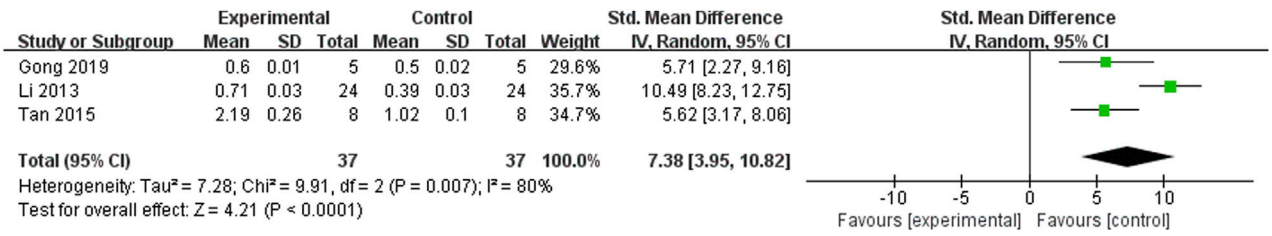

FIGURE 11
The forest plot of percentage of Claudin-5.

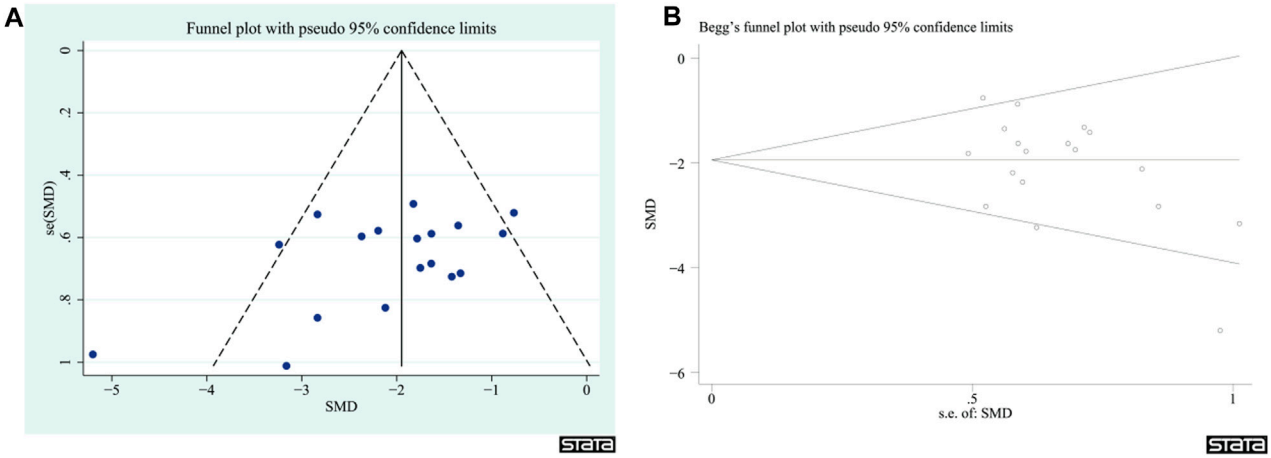

FIGURE 12
Publication bias plots. **(A)** Funnel plot of the NDS. **(B)** Begg's plot of the NDS.

study conducted a statistical analysis of the included studies and found that ligustrazine exhibits a significant improvement in cerebral edema, reduction in cerebral infarction area, and amelioration of neurological damage. Furthermore, the statistical analysis of outcome indicators in the included studies revealed that ligustrazine can effectively enhance inflammatory factor-related indicators (TNF-α, IL-1β, and IL-6), oxidative stress-related indicators (SOD, NOS, MDA, and NO), apoptosis indicator caspase-3, and BBB permeability-related protein Claudin-5.

Inflammation plays a crucial role in the pathophysiology of ischemic stroke. Specifically, inflammatory cytokines IL -1, IL -6, and TNF have been identified as key regulators of the immune response following ischemic stroke (Lambertsen et al., 2019; Clausen et al., 2020). In the hyperacute window after ischemia, resident microglia are recruited from the site of cellular injury due to the action of various cytokines such as TNF-α, IL-6, and IL-1β. This localized inflammation then triggers systemic inflammation, resulting in the breakdown of the BBB, brain edema, and neuronal death (Jin et al., 2010; Naik et al., 2023). The findings of this study demonstrate that ligustrazine can effectively modulate TNF - α, IL-6, and IL-1β levels in animals with ischemic stroke, thereby improving the damage caused by the stroke.

During ischemia reperfusion, the level of NO initially increased, then decreased, and then increased again. In pathological conditions, there was a significant increase in inducible NOS (INOS), which led to excessive production of NO. This excessive NO production caused damage to nerve tissue, resulting in a neurotoxic effect (Fan et al., 2022) Cerebral ischemia reperfusion injury leads to the production of a large amount of oxygen free radicals, which in turn cause peroxidation reactions and changes in the basic characteristics of cell membranes. This process results in the production of MDA in large quantities. SOD, as a main enzyme responsible for scavenging oxygen free radicals, plays a crucial role in protecting cells against oxidative damage (Korenić et al., 2014; Deng et al., 2022). In this study, mice in the ligustrazine intervention group showed a significant decrease in oxidative stress index levels of NO, NOS, and MDA, as well as a significant increase in the antioxidant index SOD level. These findings suggest that ligustrazine effectively regulates the oxidative balance in brain tissue injury, thereby reducing damage caused by oxygen free radicals and protecting nerve cells.

Apoptosis, a process of programmed cell death, is activated during cerebral ischemia (Love, 2003). Caspase-3, a member of the cysteine protease family, plays a crucial role in mediating apoptosis (Uzdensky, 2019). The destruction of the BBB is a significant pathophysiological process in acute ischemic stroke, which can result in destructive malignant brain edema and hemorrhagic transformation (Qiu et al., 2021). Previous research has demonstrated that ligustrazine has the ability to penetrate the BBB and distribute across various brain

regions (Tsai and Liang, 2001). However, its biological half-life when taken orally is limited to 0.5–2 h. Utilizing ligustrazine - loaded liposomes (Xia et al., 2016), transdermal administration drugs (Qi et al., 2002), intranasal administration (Feng et al., 2009), and intraocular administration (Mao et al., 2019) can enhance the efficiency of ligustrazine in crossing the BBB and reaching the brain. Moreover, ligustrazine exhibits protective effects against CIS damage by reducing BBB permeability (Tan et al., 2015; Jin et al., 2021), possibly through the inhibition of the JAK/STAT signaling pathway (Gong et al., 2019) and the reduction of MMP-9 levels (Jin et al., 2021). Claudin-5, a specific protein found in the tight junctions of brain microvascular endothelial cells, has been identified as an inducer of BBB formation (Ueno, 2007). The analysis conducted in this study reveals that ligustrazine can reduce the expression of Caspase-3 and increase Claudin-5 levels. These findings suggest that ligustrazine may protect against cerebral ischemic injury by reducing apoptosis and repairing the BBB.

We conducted a meta-analysis of animal studies investigating the effects of ligustrazine intervention in CIS for the first time. We summarized and classified the indicators included in the study, finding that the mechanism of ligustrazine is closely related to inflammatory response, oxidative stress, apoptosis, and BBB permeability. This provides valuable insights for future studies in this area. However, there are several limitations in our study that should be acknowledged. Firstly, most of the included articles did not mention blinding of participants and results, which increases the risk of bias. Secondly, the secondary outcome indicators in our study showed heterogeneity. Although we identified the source of heterogeneity through sensitivity analysis, the limited data available may have led to inaccurate results. Thirdly, some indicators had small sample sizes, which increases the risk of bias. Fourthly, the safety of ligustrazine could not be evaluated in our study as most included studies did not record adverse reactions in animal models. Lastly, the extraction of outcome measures from images using GetData Graph Digitizer software may introduce inaccuracies. These limitations should be considered when interpreting the findings of our study.

A meta-analysis of clinical trials examining the combination of ligustrazine injection with western medicine for acute cerebral infarction showed better efficacy when compared to using western medicine alone (Shao et al., 2021). These findings align with the results of our study. While ligustrazine may cause damage to the gastrointestinal, peripheral nervous, or central nervous systems, most adverse effects are self-limiting and the compound is generally considered safe (Lin et al., 2022). However, due to the limited depth of research on the toxicology and side effects of ligustrazine, it is crucial to strictly adhere to the recommended indications and consider the patient's drug allergy history. Researchers should prioritize investigating the toxicology and side effects of ligustrazine in patients with CIS to enhance its therapeutic potential for human use.

5 Conclusion

Our study suggests that ligustrazine has a protective effect on animal models of CIS. The mechanism behind this effect may be attributed to the reduction of inflammation and oxidative stress, inhibition of apoptosis, and repair of BBB permeability. However, discrepancies between animal models of CIS and the physiological and pathological processes in humans pose challenges in translating preclinical findings to clinical applications. Future preclinical investigations on ligustrazine for CIS treatment should adhere rigorously to predefined protocols to mitigate bias in subsequent studies.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

ZmW: Writing–review and editing, Writing–original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal Analysis, Data curation, Conceptualization. ZhW: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing–original draft, Writing–review and editing. YM: Writing–review and editing, Writing–original draft. AH: Writing–review and editing, Writing–original draft. HC: Writing–review and editing, Writing–original draft. SZ: Writing–original draft, Writing–review and editing. ML: Writing–review and editing, Writing–original draft. SG: Writing–review and editing, Writing–original draft. YiL: Writing–review and editing, Writing–original draft. YuL: Writing–review and editing, Writing–original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal Analysis, Data curation, Conceptualization.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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frontiers | Frontiers in Pharmacology

TYPE Systematic Review
PUBLISHED 15 March 2024
DOI 10.3389/fphar.2024.1321405

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RECEIVED 14 October 2023
ACCEPTED 21 February 2024
PUBLISHED 15 March 2024

CITATION
Cui L, Liu X, Li Y, Jing T, Liu D, Ren C, Yin T, Wang Y, Zhao Z, Wang J, Han X and Wang L (2024), Chinese patent medicine combined with calcium channel blockers in the treatment of essential hypertension:a Bayes network meta-analysis and systematic review. *Front. Pharmacol.* 15:1321405. doi: 10.3389/fphar.2024.1321405

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Chinese patent medicine combined with calcium channel blockers in the treatment of essential hypertension:a Bayes network meta-analysis and systematic review

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Backgroud: The co-administration of Chinese patent medicine with calcium channel blockers (CCBs) is a prevalent practice in China for treating essential hypertension (EH). However, robust evidence supporting the efficacy and safety of tailored combinations of different Chinese patent medicines with CCBs, according to individual patient conditions, is still limited. This study sought to elucidate the efficacy and safety of these combinations using a systematic review and network meta-analysis.

Materials and methods: Relevant studies were sourced from established databases, incorporating randomized controlled trials published up to 1 February 2023. The ROB2 tool from the Cochrane Collaborative Network was employed to independently assess and cross-verify the quality of the included literature. A network meta-analysis was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) 2020 and PRISMA-Network Meta-Analyses (PRISMA-NMA) guidelines. A Bayesian network meta-analysis was utilized to gauge the efficacy and safety of distinct integrations of Chinese patent medicine and CCBs. Primary outcomes were interpreted using a paired fixed-effect meta-analysis. Publication bias was appraised through Egger's test and represented with funnel plots. All statistical analyses were executed within the R statistical framework.

Results: Following rigorous selection, data extraction, and bias evaluation, 36 articles were incorporated. Tianma Gouteng Granule, when combined with CCBs, displayed superior efficacy in reducing systolic blood pressure (SBP). In terms of diastolic blood pressure (DBP) reduction, Songling Xuemaikang Capsule combined with CCBs emerged as the most effective. Regarding enhancement of antihypertensive effective rates, Qinggan Jiangya Capsule paired with CCBs demonstrated optimal results. For diminishing Traditional Chinese Medicine syndrome scores, the Qiangli Dingxuan Tablet and CCBs combination proved most beneficial. When aiming to reduce total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C) levels, Tianma Gouteng Granule

and CCBs showcased superior results. In contrast, the combination of Songling Xuemaikang Capsule and CCBs was more effective in reducing LDL-C, tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6).

Conclusion: This study underscores variability in outcomes from combining Chinese patent medicine and CCBs for hypertension, emphasizing the importance of personalized medicinal combinations, especially Tianma Gouteng Granule and Songling Xuemaikang Capsule. The results offer robust evidence to inform clinical guidelines for essential hypertention and significantly aid clinician in seleting appropriate Chinese patent medicines for treatment.

KEYWORDS

ethnopharmacological research, calcium channel Blockers(CCBs), hypertension, network meta-analysis, review studies, therapeutic efficacy

1 Introduction

Essential hypertension (EH) stands as the leading preventable risk factor for cardiovascular disease (CVD), contributing significantly to conditions such as coronary heart disease, heart failure, stroke, and cognitive impairment (Forouzanfar et al., 2017). The 2017 Global Burden of Disease Study revealed that high systolic blood pressure (SBP) is a primary risk factor, responsible for 10.4 million deaths and impacting 218 million people in terms of disability-adjusted life years (Stanaway, 2017). Among those aged 18 and above, the incidence rate of EH is 27.9%, with a pre-hypertension rate of 39.1% (Forouzanfar et al., 2015). Age plays a crucial role in EH prevalence: it is around 34.38% in middle-aged individuals and surges to nearly 90% in those over 80 years (Beaney et al., 2020), posing a substantial financial and psychological burden on societies and families.

While the primary treatment for EH centers around conventional medications known for their efficacy, ACE inhibitors (ACEI), angiotensin II receptor blockers (ARB), also referred to as sartans, Calcium Channel Blockers (CCBs), and thiazide diuretics are recognized as first-line antihypertensive drugs (James et al., 2014). However, specific challenges like particular contraindications and inadequate control in certain hypertension variants persist. For instance, ACEI and ARB are not advised for pregnant women due to heightened risks linked to renal teratogenicity (Mancia et al., 2013). CCBs target calcium channels situated at the plasma membrane, inducing cell depolarization (Godfraind, 2005). They cause vasodilation by blocking calcium entry, hence diminishing the active tone of vascular smooth muscle. This property has rendered CCBs especially beneficial for EH treatment (Morel and Godfraind, 1987). Nevertheless, despite the availability of such potent antihypertensive drugs, only one in every four women and one in five men with EH attain their treatment objectives (Ncd-RisC, 2021).

Traditional Chinese Medicine (TCM) offers a unique therapeutic perspective, especially with its holistic stance and syndrome differentiation-centric treatments. Leveraging antihypertensive Chinese patent medicine tailored to specific syndromes can efficiently regulate blood pressure and relieve related symptoms. For instance, Ilex hainanensis Merr., also known as Shan-Lv medicine, when paired with antihypertensive drugs, exhibits enhanced efficacy in blood pressure reduction than standalone drug treatments (Yang et al., 2018). Similarly, the Songling Xuemaikang Capsule combined with conventional medicine has demonstrated clear efficacy and safety for EH management (Meng et al., 2022). Over the years, pharmacological management of hypertension has advanced. In the management of EH, combining Chinese patent medicines with western medicines provides superior efficacy and safety compared to conventional treatment alone. Clinical studies have endorsed the blending of traditional Chinese medicine with conventional medicine in treating EH, underscoring mutual benefits, sustained pressure-relief effects, minimized adverse reactions, and fewer cardiovascular complications (Chen et al., 2013). CCBs play an important role in the treatment of EH as CCBs is one of the first recommended antihypertensive drugs. The combination of Chinese patent medicines with CCBs has been extensively utilized as an alternative treatment strategy for essential hypertension (EH) and dizziness in China. This strategy could improve the traditional Chinese medicines symptoms of EH patients, including dizziness, impetuosity, insomnia, tinnitus, etc., which is particularly important for improving the quality of life of EH patients (Lin et al., 2023).

Though numerous hypertension guidelines promote combination pharmacotherapy, solid evidence elucidating the clinical effectiveness and safety of Chinese patent medicine when integrated with CCBs for EH treatment remains scarce. Past meta-analyses have typically compared only two treatment methodologies, failing to provide a comprehensive overview of potential synergies between various Chinese patent medicines and CCBs (Yongcheng et al., 2022; Tong et al., 2023). An extant network meta-analysis did not distinctly categorize the conventional medicines, hindering its clinical application (Zhaochen et al., 2022).

Network meta-analysis (NMA) amalgamates both direct and indirect evidence, offering estimates for every treatment pair (Mavridis, 2019). It serves clinicians by ranking interventions based on their efficacy for each assessed outcome (Nikolakopoulou et al., 2020). This research aims to offer an exhaustive systematic review of RCTs focused on the synergy of Chinese patent medicine and CCBs in EH treatment, strictly adhering to the PRISMA 2020 and PRISMA-NMA guidelines (Hutton et al., 2015; Page et al., 2021). By employing the Bayes network meta-analysis, we aspire to gauge the efficacy and safety of

different Chinese patent medicine-CCBs combinations, thereby offering robust evidence to guide clinical decisions.

2 Materials and methods

2.1 Search strategy

All relevant randomized controlled trials (RCTs) including one or more interventions were identified through extensive searches of databases including PubMed, Cochrane Library, Web of Science, ClinicalTrials.gov, CNKI, WANFANG, VIP, and Sinomed. The timeline for the search spanned from the inception of each database up to 1 February 2023. To ensure a thorough examination of studies related to CCBs, the search began with a broad focus on RCTs that explored the integrative use of Chinese patent medicine with conventional medicine. The subsequent screening refined the results to spotlight studies specifically utilizing CCBs as the antihypertensive treatment. The primary search criteria were defined as: (subject = hypertension OR essential hypertension) AND (subject = Chinese and Western medicine OR Chinese patent medicine OR capsule OR tablet OR scatter OR pill OR ointment OR dan OR dropping pill OR granule OR oral liquid). For a more comprehensive retrieval of relevant studies, synonym expansion was also incorporated into the search formula.

2.2 Inclusion and exclusion criteria

We did meta-analysis and bayes network meta-analysis, which means the integration of direct and indirect comparisons, to compare 6 types of Chinese patent medicine for essential hypertension. Our analysis included studies of people with essential hypertension. Because multiple-treatments meta analysis require a reasonably homogeneous sample, we excluded RCTs done in patients with severe cardiovascular, cerebrovascular, and renal diseases; patients with special types of hypertension, such as gestational hypertension, menopausal hypertension, H-type hypertension, etc.

Studies selected were required to be peer-reviewed, published in either Chinese or English, and to employ a RCT design focusing on patients diagnosed with essential hypertension. Interventions assessed combinations of Chinese patent medicine and CCBs, while control groups received only CCBs. The literature we excluded including unable to obtain full text or incomplete information.

2.3 Outcome measures

Outcomes were chosen based on the《Guidelines for clinical application of Chinese patent medicine in treating essential hypertension》and subsequently, RCTs were reviewed. Primary outcomes comprised Systolic and Diastolic Blood Pressure (SBP and DBP), antihypertensive effective rate, and adverse drug reaction events. If data from this scale were not available, SBP& DBP or antihypertensive effective rate is essential. Secondary outcomes were Traditional Chinese Medicine Syndrome Score (TCM syndrome score), Total Cholesterol (TC), Triglyceride (TG), Low-Density Lipoprotein Cholesterol (LDL-C), Quality of Life Score, Tumor Necrosis Factor-α (TNF-α), Interleukin-6 (IL-6), and Vascular Endothelin-1 (ET-1). Notably, outcomes such as high-density lipoprotein, cardiac function, and others were excluded owing to insufficient comparative data. Because bayes network meta-analysis requires reasonable homogeneity we focused on 8-week duration, and if this is not available, we used data from between 4 and 12 weeks (closest to 8 weeks).

2.4 Data extraction

Data from each study were meticulously extracted, encompassing study details (authorship and year of publication), participant demographics (group classifications, sample size, age), interventions (specific drug names and dosing durations), and a broad spectrum of outcomes including measures like SBP, DBP, TCM syndrome score, and various biochemical markers. Outcomes with continuous data were represented by both pre-intervention and post-intervention means and standard deviations, while dichotomous data were tabulated in 2 × 2 formats. To ensure accuracy and objectivity, two independent reviewers undertook the extraction. In cases of disagreement, resolution was sought with the intervention of a third reviewer. Subsequent to extraction, data were reformatted for compatibility with the R package's requirements.

2.5 Risk of bias assessment

The quality of the selected literature was evaluated using the ROB2 tool from the Cochrane Collaborative Network. Two researchers independently carried out the assessment and cross-checked their evaluations. In cases of disagreement, a third researcher made the decision. The ROB2 tool focuses on assessing potential risks of bias in these areas: bias arising from the randomization process, bias due to deviations from intended interventions, bias due to missing outcome data, bias in measurement of the outcome, and bias in selective reporting of outcomes (Sterne et al., 2019). Bias arising from the randomization process is about whether the allocation sequence was random, adequately concealed, or baseline differences between intervention groups suggest a problem with the randomization process. Bias due to deviations from intended interventions included whether participants, carers and people were aware of participants'assigned intervention during the trial. The purpose of this domain is also to address if applicable deviations from the intended intervention are a result of experimental context in addition to the effect of assignment to intervention. Bias due to missing outcome data is about whether data for this outcome were available for all, or nearly all, participants randomized, (if applicable) the result was not biased by missing outcome data; (if applicable) missingness in the outcome was likely to be influenced by its true value. Bias in measurement of the outcome is about whether the method of

measuring the outcome was inappropriate; different intervention groups could used different mesurement or ascertainment for outcome; outcome assessors were aware of the intervention received by study participants; (if applicable) assessment of the outcome was likely to have been influenced by knowledge of intervention received. And at last, bias in selection of the report result addressed whether the trial was analysed according to a pre-specified plan which was finalized before unblinded outcome data were available for analysis; the numerical result being assessed may have been selected, on the basis of the results, from multiple outcome measurements within the outcome domain; the numerical result being assessed is likely to have been selected, on the basis of the results, from multiple analyses of the data. The response options are yes, probably yes, probably no, no, no information. Some signalling questions were logically related each other, i.e., it may be possible to skip next question because an option was selected for a previous signalling question; if a signalling question was skipped due to this logic setting, it was noted as not applicable (NA). If the risk of bias assessment results of all domains were "low risk", then the overall risk of bias was "Low" risk. If the risk of bias evaluation results of some areas were "Some concers" risk and there was no area of "High" risk, then the overall risk of bias was "Some concers" risk. Whenever there was an area where the risk of bias was assessed as "High" risk, the overall risk of bias is "High" risk. Up-to-date information from the developers on RoB 2 and more detail is available via the Risk of Bias tools website: www.riskofbias.info and Cochrane Scientific Committee.

For each source of bias, studies were categorized as having a high, low, or some concerns risk based on, and summarise the answers to signalling questions (Higgins and Page, 2019).

2.6 Statistical analysis

This study conducted a NMA according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) 2020 and PRISMA-Network Meta-Analyses (PRISMA-NMA) guidelines (Hutton et al., 2015; Page et al., 2021). Bayes network meta-analysis was used to evaluate the efficacy and safety of various Chinese patent medicine combined with CCBs, aiming to inform clinical decisions.

The initial phase entailed the design of multiple network geometries to decipher the comparative dynamics between different medications, each integrated with distinct Chinese patent medicines. Subsequently, a consistency analysis juxtaposed direct and indirect therapeutic effect estimates. Given the absence of closed-loop networks in this research, only indirect comparative estimates apply. Anticipating study heterogeneity, we deploy a random-effect model.

We focused on constructing a Bayes model for an NMA concerning primary and secondary outcomes. We guided the model by four Markove chains on R software to perform NMA. When calculating the effect size, dichotomous data was expressed as odds ratio (OR), continuous variables were expressed as mean difference (MD), bayes network meta-analysis set 95% credible interval (CrI). Using I^2 to test heterogeneity, and $p < 0.05$ was considered statistically significant.

Gelman-Rubin-Brooks plots were conducted to examine the convergence in diagnostic model combining interval-based graphical evaluation and quantitative analysis of PSRF. After n iterations, the curve was formed and observed whether the curve is fit with each other and kept stable. A Potential Scale Reduction Factor (PSRF) tended to 1.00 indicating that the degree of convergence was satisfactory (Jiahao et al., 2021), otherwise increase the number of iterations to achieve model convergence. When calculating the rank probability intervention the parameter preferred direction was equal to 1 means a higher value which indicates a better result in the antihypertensive effective rate and the quality of life score. Other outcomes took preferred direction equaling to −1 to indicate a lower value which means better results.

Trace plot was used to test whether the Markov chain reach stable and overlap during the caluation proces. It always showed the fluctuation process of Markove chain during the iterative computation process, which can be expressed in different forms depending on the number of iterations and preset distributions (Dodds and Vicini, 2004; Toft et al., 2007).

We conducted density plots to evaluate the consistent. Density plots were based on a predefined distribution, and after numerical simulation, the distribution of the *a posteriori* values is observed to see if it is consistent with the predefined distribution. The bandwidth value be used as a quantitative assessment. And the smaller the bandwidth, the smaller difference between distribution range of the parameter *a posteriori* values and the preset distribution range. After enough iterations, the Bandwidth tends to 0 and stabilizes (Harpole et al., 2014).

Then the efficacy of the interventions was compared, and then they were ranked. The Surface Under the Cumulative Ranking (SUCRA) score, visualized through a heatmap, offered a perspective on the probable efficacy of an intervention based on the ranking of all interventions. A SUCRA of x% meant that the drug achieves x% of the effectiveness of this imaginary drug, thus larger SUCRAs denoted more eff ective interventions (Leucht et al., 2013).

We conducted sensitivity analyses on the primary outcome to explore potential reasons for heterogeneity or inconsistency. Leave-one-out sensitivity analysis was used by metafor package in R software to perform a "leave-one-out" function, which examined the robustness of the results by repeatedly fitting the specified model, omitting one study at a time. The size of the forest plot squares was plotted in proportion to the sample size of each RCT to see which RCT carried more weight.

Subgroup analyses were used to examine whether there are significant differences in the effectiveness of interventions between different subgroups to explore which interventions are more effective. Contour-enhanced funnel plot was drawn to detect publication bias due to the suppression of non-significant findings (Peters et al., 2008). Plot of influence diagnostics was drawn to compute outlier and influential case diagnostics, which include externally standardized residuals, DFFITS values, Cook's distances, covariance ratios, leave-one-out estimates of the amount of heterogeneity, leave-one-out values of the test statistics for heterogeneity, hat values, and weights (Viechtbauer and Cheung, 2010).

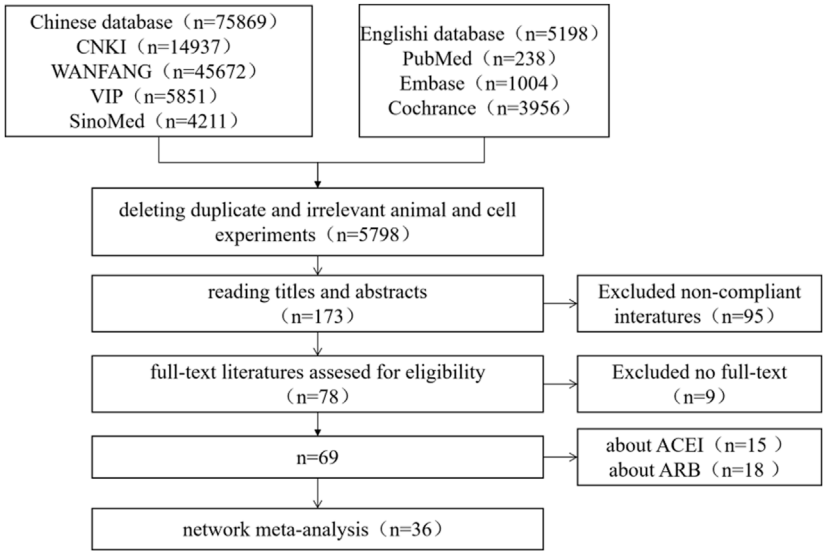

FIGURE 1
Prisma flow diagram for literature screening.

The network meta-analyses were facilitated by the netmeta and gemtc packages (Gert van Valkenhoef, 2021; Guido Schwarzer, 2023). All statistical evaluations are conducted in the R statistical environment (R 4.2.2, www.r-project.org).

3 Results

3.1 Literature search process

An initial search of the literature database yielded 75,869 documents. After removing duplicates in Endnote, Prisma flow diagramand helped to identify RCTs for the remaining 70,756 records. 64,958 records were rejected as animal and cell experiment studies, and 5,798 papers were left. Upon reviewing the titles and abstracts, 173 articles that matched the study criteria were shortlisted. A thorough reading of the full text and further screening based on the inclusion criteria left 78 relevant Chinese papers. Studies with fewer than 2 outcomes were excluded, leaving 69 papers. Out of these, 36 articles focusing on the combination of Chinese patent medicine with CCBs were ultimately selected for this study. The detailed screening process is illustrated in Figure 1.

3.2 Included literature quality evaluation

A total of 3,740 patients participated in studies involving six distinct types of Chinese patent medicine. These medicines include: Songling Xuemaikang Capsule (SLXM), Qiangli Dingxuan Tablet (QLDX), Tianma Gouteng Granule (TMGT), Qiju Dihuang Pill (QJDH), Qinggan Jiangya Capsule (QGJY), and Xinmaitong Capsule (XMT). The essential characteristics of the studies under consideration are detailed in Table 1. Exact information about Chinese patent medicines in our studies can be found in Supplementary Material S1. All the plant names have been checked with http://www. worldfloraonline.org mentioning on 25 May 2023.

3.3 Risk of bias assessment of included studies

Domain 1 assessed the bias stemming from the randomization process. Out of 36 articles, 10 (Gao and Jin, 2013; Zhou, 2013; Li, 2017; Yuan, 2017; Yu, 2019; Liu et al., 2020; Wang, 2020; Liu, 2021; Ye et al., 2021) used the term "random" to describe their stratified block randomization method without more details. Furthermore, one study (Hao et al., 2020) employed a stratified random method. Both these approaches were categorized as having a "Low" risk of bias. 8 (Jia and Liu, 2004; He et al., 2013; Han, 2014; Huang and Fu, 2014; Sun, 2015; Xin and Lin, 2016; Sun, 2019; Qiu et al., 2021) had "High" risk because of the bias arsing from the randomization process. Other 17 literature were "Some concerns" risk.

The assessment result of domain 2 showed that 17 (Du et al., 2009; Zhao, 2012; Gao and Jin, 2013; Liu, 2013; Zhou, 2013; Zhang et al., 2014a; Zhang, 2014; Hang et al., 2018; Huang et al., 2018; Jiao et al., 2018; Xiong, 2018; Liu et al., 2019; Wang, 2019; Yu, 2019; Hao et al., 2020; Ye et al., 2021; Wang et al., 2022) were "Low" risk, the other 17 literature (Jia and Liu, 2004; Wang, 2011; He et al., 2013; Han, 2014; Huang and Fu, 2014; Sun, 2015; Zhu et al., 2015; Li, 2017; Yan, 2017; Yuan, 2017; Chen and Qiu, 2018; Sun, 2019; Du et al., 2019; Liu et al., 2020; Wang, 2020; Liu, 2021; Qiu et al., 2021) were "Some concerns" risk, and the rest were "High" risk.

Assessments for the domain "bias due to missing outcome data" differed according to the integrity and missingness of the data, and the result showed that 33 (Du et al., 2009; Wang, 2011; Zhao, 2012; Gao and Jin, 2013; He et al., 2013; Liu, 2013; Zhou, 2013; Zhang et al., 2014a; Zhang et al., 2014b; Han, 2014; Huang and Fu, 2014; Zhang, 2014; Sun, 2015; Zhu et al., 2015; Xin and Lin, 2016; Li, 2017; Yan, 2017; Chen and Qiu, 2018; Hang et al., 2018; Huang et al., 2018; Jiao et al., 2018; Xiong,

TABLE 1 Characteristics of included studies.

Study	n		Age		Interventions		T	Outcome
					C:Western medicine			
					E:Chinese patent medicine			
					Combine with western mecine			
	C	E	C	E	CPM	WM		
Yu (2019)	47	47	51.19 ± 6.92	50.42 ± 7.23	SLXM	Amlodipine Besylate	4W	①②④⑩
Sun (2015)	58	58	61.5 ± 8.2	61.5 ± 8.2	SLXM	Nifedipine	8W	①②③
Liu et al. (2020)	50	50	49.06 ± 8.77	51.52 ± 10.20	SLXM	Benidipine Hydrochloride	4W	①②④⑤⑥⑦⑨⑬
Li (2017)	49	49	56.39 ± 6.71	56.42 ± 6.70	SLXM	Levoamlodipine Maleate Dispersible Tablet	8W	①②③⑤⑥⑨⑧
Wang (2011)	70	68	52.1 ± 9.3	50.3 ± 8.9	SLXM	Amlodipine Besylate Tablet	4W	①②⑩
Qiu et al. (2021)	50	50	69.01 ± 4.17	68.74 ± 4.25	SLXM	Nicardipine Hydrochloride Tablet	4W	①②③⑪⑫
Xin and Lin (2016)	50	50	—	—	SLXM	Amlodipine Besylate Tablet	12W	①②
Gao and Jin (2013)	60	60	66.31 ± 10.75	65.24 ± 11.32	SLXM	Nifedipine	2W	①②③
Zhang et al. (2014a)	60	60	55.1 ± 15.2	54.2 ± 14.9	SLXM	L-levamlodipine Besylate Tablet	8W	⑤⑥⑦⑨
Zhang et al. (2014b)	81	81	56.2 ± 13.9	54.8 ± 14.6	SLXM	L-levamlodipine Besylate Tablet	8W	①②③⑧
He et al. (2013)	29	30	62.74 ± 3.67	62.42 ± 3.82	SLXM	Nifedipine	4W	①②
Liu 2013)	48	48	43–70	46–67	QLDX	NifedipineSustained-release Tablet	12W	①②③
Xiong (2018)	63	63	59.0 ± 3.1	61.0 ± 3.1	QLDX	NifedipineSustained-release Tablet	12W	①②③⑧
Chen and Qiu (2018)	40	40	50–80	50–80	QLDX	Lacidipine Tablet	6W	③
Huang et al. (2018)	39	39	55.9 ± 5.20	55.1 ± 5.67	QLDX	Lacidipine Tablet	6W	①②③④
Jia and Liu (2004)	34	32	56 ± 10	57 ± 9	TMGT	Levamlodipine Besylate Tablet	4W	③
Ye et al. (2021)	60	60	61.19 ± 5.36	7.21 ± 1.87	TMGT	Levoamlodipine Maleate	8W	①②③⑩⑬
Zhu et al. (2015)	54	53	55.8 ± 5.4	56.3 ± 7.2	TMGT	Nifedipine	12W	③
Zhou (2013)	48	49	65.4 ± 9.5	64.9 ± 10.1	TMGT	Nifedipine	12W	①②③④⑧
Sun (2019)	75	75	69.29 ± 1.31	69.19 ± 2.21	TMGT	Nifedipine	8W	①②③⑧
Hao et al. (2020)	30	30	—	—	TMGT	Felovipine	4W	③⑨
Wang (2020)	55	55	53.81 ± 8.12	52.48 ± 7.29	TMGT	NifedipineSustained-release Tablet	8W	③⑧⑬
Yuan (2017)	52	52	76.28 ± 3.56	76.89 ± 3.71	TMGT	Nifedipine Sustained-release Tablet	12W	①②③⑪⑫⑬
Huang and Fu (2014)	51	48	28.16 ± 7.34	30.94 ± 6.23	TMGT	L-levamlodipine Besylate Tablet	4W	③⑤⑥⑦
Wang (2019)	57	57	74.2 ± 6.9	72.5 ± 7.1	TMGT	Nifedipine	12W	③⑧⑪⑫⑬
Hang et al. (2018)	47	47	57.89 ± 2.35	57.67 ± 2.42	TMGT	L-levamlodipine Besylate Tablet	8W	①②③
Zhang (2014)	34	34	48.56 ± 6.32	46.78 ± 5.71	QJDH	Amlodipine Besylate Tablet	4W	①②③
Du et al. (2019)	30	30	51.2 ± 6.6	51.5 ± 6.9	QJDH	NifedipineSustained-release Tablet	4W	①②③
Jiao et al. (2018)	40	40	64.7 ± 7.2	65.2 ± 6.5	QJDH	Amlodipine Besylate	4W	①②
Du et al. (2009)	60	60	64.63 ± 8.01	63.37 ± 8.16	QJDH	Felodipine Sustained Release Tablet	4W	①②⑬
Zhao (2012)	40	40	65–80	65–80	QGJY	NifedipineSustained-release Tablet	4W	①②⑧
Yan (2017)	100	100	66.2 ± 11.4	65.9 ± 12.4	QGJY	NifedipineSustained-release Tablet	12W	①②③
Wang et al. (2022)	70	70	70.97 ± 7.85	70.56 ± 8.13	XMT	Benidipine Hydrochloride	8W	①②④⑧⑬

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TABLE 1 (*Continued*) Characteristics of included studies.

Study	n		Age		Interventions		T	Outcome
					C:Western medicine			
					E:Chinese patent medicine			
					Combine with western mecine			
	C	E	C	E	CPM	WM		
Liu (2021)	64	64	53.48 ± 7.12	54.36 ± 7.86	XMT	Verapamil	4W	①②③⑧
Han (2014)	45	45	55	55	XMT	Amlodipine Besylate Tablet	8W	③
Liu et al. (2019)	33	33	68.9 ± 4.2	69.4 ± 4.5	XMT	L-levamlodipine Besylate Tablet	12W	①②③⑤⑥

Notes: E: experimental group, C: control group; T:treatment time; W:week; D:day; SLXM:Soling Xuemaikang Capsule; QLDX:Qiangli Dingxuan Tablet; TMGT:Tianma Gouteng Granule; QJDH:Qiju Dihuang Pill; QGJY:Qinggan Jiangya Capsule; XMT:Xinmaitong Capsule.①systolic blood pressure (SBP); ②diastolic blood pressure (DBP); ③antihypertensive effective rate; ④TCM, syndrome score; ⑤total cholesterol (TC); ⑥triglyceride (TG); ⑦low-density lipoprotein cholesterol (LDL-C); ⑧adverse drug reaction events; ⑨quality of life score; ⑩blood pressure variability; ⑪tumor necrosis factor-α (TNF-α); ⑫interleukin-6 (IL-6); ⑬vascular endothelin-1 (ET-1).

FIGURE 2
Risk of bias assessment in the included studies.

TABLE 2 The risk of bias assessment of the RCTs.

	Randomization process (%)	Deviations from intended interventions (%)	Mising outcome data (%)	Measurement of the outcome (%)	Selection of the reported result (%)	Overall Bias (%)
Low risk	30.56	47.22	91.67	63.89	13.89	27.78
Some concerns	47.22	47.22	8.33	16.67	80.56	47.22
High risk	22.22	5.56	-	19.44	5.56	25.00

2018; Du et al., 2019; Liu et al., 2019; Wang, 2019; Yu, 2019; Hao et al., 2020; Liu et al., 2020; Wang, 2020; Liu, 2021; Qiu et al., 2021; Ye et al., 2021; Wang et al., 2022)were "Low" risk, 3 (Jia and Liu, 2004; Yuan, 2017; Sun, 2019) were "Some concerns" risk.

The assignment to the measurement of the outcome showed 23 (Du et al., 2009; Wang, 2011; Zhao, 2012; Gao and Jin, 2013; Zhou, 2013; Zhang et al., 2014a; Han, 2014; Zhu et al., 2015; Li, 2017; Yan, 2017; Hang et al., 2018; Huang et al., 2018; Xiong, 2018; Du et al., 2019; Liu et al., 2019; Wang, 2019; Yu, 2019; Hao et al., 2020; Liu et al., 2020; Wang, 2020; Liu, 2021; Ye et al., 2021) with "Low" risk, 6 (Liu, 2013; Zhang et al., 2014a; Xin and Lin, 2016; Yuan, 2017; Chen and Qiu, 2018; Jiao et al., 2018) with "Some concerns" risk and 7 (Jia and Liu, 2004; He et al., 2013; Huang and Fu, 2014; Zhang, 2014; Sun, 2015; Sun, 2019; Qiu et al., 2021)with "High" risk.

Domain 5 was about the bias in selection of the reported result with 5 (Yan, 2017; Hao et al., 2020; Wang, 2020; Liu, 2021; Ye et al., 2021)

TABLE 3 The data of ROB2 of risk of bias of included studies.

Study	Randomization process	Deviations from intended interventions	Mising outcome data	Measurement of the outcome	Selection of the reported result	Overall bias
Yu (2019)	Low	Low	Low	Low	Some concerns	Low
Sun (2015)	High	Some concerns	Low	High	Some concerns	High
Liu et al. (2020)	Low	Some concerns	Low	Low	Some concerns	Low
Li (2017)	Low	Some concerns	Low	Low	Some concerns	Low
Wang (2011)	Some concerns	Some concerns	Low	Low	Some concerns	Some concerns
Qiu et al. (2021)	High	Some concerns	Low	High	Some concerns	High
Xin and Lin (2016)	High	High	Low	Some concerns	Some concerns	High
Gao and Jin (2013)	Low	Low	Low	Low	Some concerns	Low
Zhang et al. (2014a)	Some concerns	Low	Low	Some concerns	Some concerns	Some concerns
Zhang et al. (2014b)	Some concerns	High	Low	Low	Some concerns	High
He et al. (2013)	High	Some concerns	Low	High	High	High
Liu (2013)	Some concerns	Low	Low	Some concerns	Some concerns	Some concerns
Xiong (2018)	Some concerns	Low	Low	Low	Some concerns	Some concerns
Chen and Qiu (2018)	Some concerns	Some concerns	Low	Some concerns	Some concerns	Some concerns
Huang et al. (2018)	Some concerns	Low	Low	Low	Some concerns	Some concerns
Jia and Liu (2004)	High	Some concerns	Some concerns	High	Some concerns	High
Ye et al. (2021)	Low	Low	Low	Low	Low	Low
Zhu et al. (2015)	Some concerns	Some concerns	Low	Low	Some concerns	Some concerns
Zhou (2013)	Low	Low	Low	Low	Some concerns	Low
Sun (2019)	High	Some concerns	Some concerns	High	Some concerns	High
Hao et al. (2020)	Low	Low	Low	Low	Low	Low
Wang (2020)	Low	Some concerns	Low	Low	Low	Low
Yuan (2017)	Low	Some concerns	Some concerns	Some concerns	Some concerns	Some concerns
Huang et al. (2018)	High	Some concerns	Low	High	Some concerns	High
Wang (2019)	Some concerns	Low	Low	Low	Some concerns	Some concerns
Hang et al. (2018)	Some concerns	Low	Low	Low	Some concerns	Some concerns

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TABLE 3 (*Continued*) The data of ROB2 of risk of bias of included studies.

Study	Randomization process	Deviations from intended interventions	Mising outcome data	Measurement of the outcome	Selection of the reported result	Overall bias
Zhang (2014)	Some concerns	Low	Low	High	High	High
Du et al. (2019)	Some concerns	Some concerns	Low	Low	Some concerns	Some concerns
Jiao et al. (2018)	Some concerns	Low	Low	Some concerns	Some concerns	Some concerns
Du et al. (2009)	Some concerns	Low	Low	Low	Some concerns	Some concerns
Zhao (2012)	Some concerns	Low	Low	Low	Some concerns	Some concerns
Yan (2017)	Some concerns	Some concerns	Low	Low	Low	Some concerns
Wang et al. (2022)	Low	Low	Low	Low	Some concerns	Low
Liu (2021)	Low	Some concerns	Low	Low	Low	Low
Han (2014)	High	Some concerns	Low	Low	Some concerns	Some concerns
Liu et al. (2019)	Some concerns	Low	Low	Low	Some concerns	Some concerns

"Low", 29 (Jia and Liu, 2004; Du et al., 2009; Wang, 2011; Zhao, 2012; Gao and Jin, 2013; Liu, 2013; Zhou, 2013; Zhang et al., 2014a; Zhang et al., 2014b; Han, 2014; Huang and Fu, 2014; Sun, 2015; Zhu et al., 2015; Xin and Lin, 2016; Li, 2017; Yuan, 2017; Chen and Qiu, 2018; Hang et al., 2018; Huang et al., 2018; Jiao et al., 2018; Xiong, 2018; Du et al., 2019; Liu et al., 2019; Wang, 2019)"Some concerns" and 2 (He et al., 2013; Zhang, 2014) "High" risk. At last, overall bias with 10 (Gao and Jin, 2013; Zhou, 2013; Li, 2017; Yu, 2019; Hao et al., 2020; Liu et al., 2020; Wang, 2020; Liu, 2021; Ye et al., 2021; Wang et al., 2022) "Low" risk, 17 (Du et al., 2009; Wang, 2011; Zhao, 2012; Liu, 2013; Zhang et al., 2014a; Han, 2014; Zhu et al., 2015; Yan, 2017; Yuan, 2017; Chen and Qiu, 2018; Hang et al., 2018; Huang et al., 2018; Jiao et al., 2018; Xiong, 2018; Du et al., 2019; Liu et al., 2019; Wang, 2019)"Some concerns" risk and 9 (Jia and Liu, 2004; Zhang et al., 2014b; Huang and Fu, 2014; Zhang, 2014; Sun, 2015; Xin and Lin, 2016; Sun, 2019; Qiu et al., 2021)"High" risk which was an overall judgment for the result from the above 5 areas. More information was available in Supplementary Material.

Based on the evaluations across the aforementioned five domains, the results were determined in line with the ROB2 operational logic. Specifically, 30.56% of the studies exhibited low risk, 47.22% presented medium risk, and 22.22% had high risk. These findings were depicted in Figure 2. For a more detailed risk of bias assessment for the RCTs, please refer to Table 2 and Table 3.

3.4 Paired meta-analysis of the antihypertensive effective rate

A paired meta-analysis was conducted on the included studies using a fixed-effect model. The analysis indicated that the combination of Chinese patent medicine with CCBs yields greater efficacy in enhancing the antihypertensive effective rate compared to CCBs alone. This difference was statistically significant, as illustrated in Figure 3. To assess publication bias for the primary outcome's effective rate, a funnel plot was generated. The plot revealed that most data points cluster around the center and top but display asymmetry, suggesting potential publication bias, as presented in Figure 4.

3.5 Evidence network map for outcomes

An evidence network diagram was constructed to represent the 12 types of outcomes for 6 Chinese patent medicines combined with CCBs antihypertensive medications. Outcomes with consistent literature information were merged into a single diagram. Edges in the diagram were thicker when more RCTs are included. Points were larger when there's a greater number of patients involved in those studies. All evidence networks and corresponding data are presented in Figure 5.

3.6 Bayesian network model using gemtc package

Utilizing the gemtc package, we built a Bayesian network model with the antihypertensive effective rate reduction as our focal point. After 5,000 preliminary iterations, the observed fluctuations in each Markov chain were minimal, signifying satisfactory convergence of the model. Consequently, there was no necessity to augment the calculation coefficient. Post the 5,000 iterations, the PSRF for every group rapidly converged to 1. In conjunction with the gemtc software, the calculated PSRF for all groups equaled 1.00. This indicated that the computational outcomes between distinct chains are consistent. The

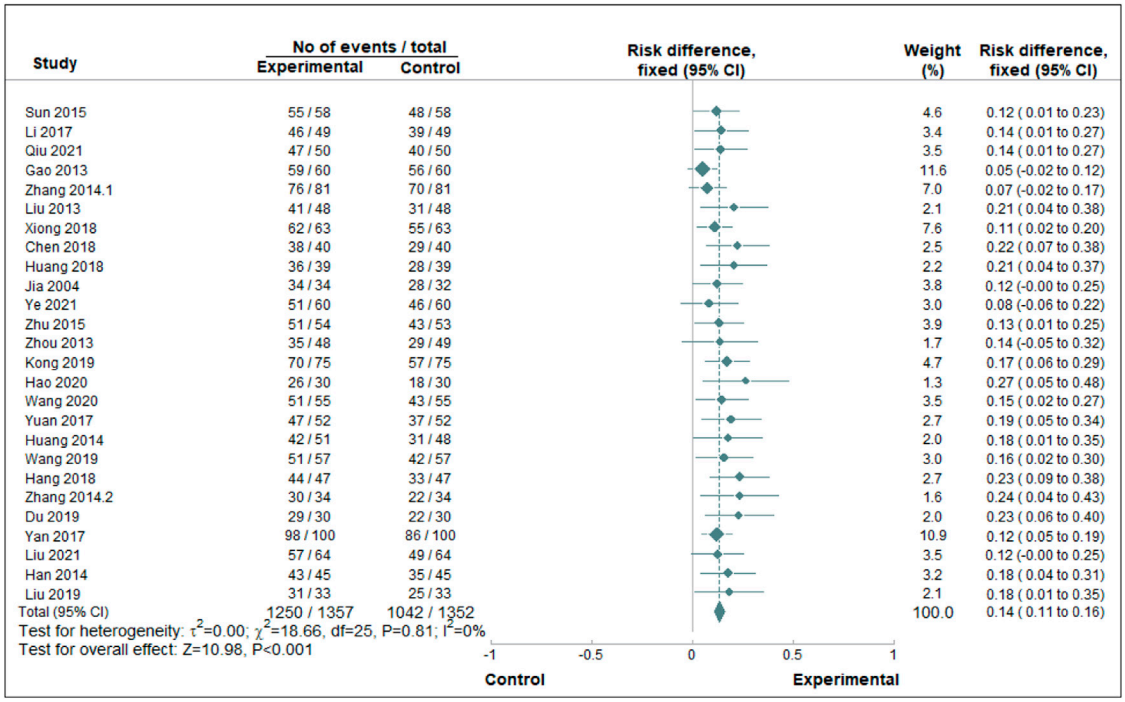


FIGURE 3
Paired meta-analysis of the antihypertensive effective rate. *Notes:* Chinse patent medicine + CCB vs. CCB.

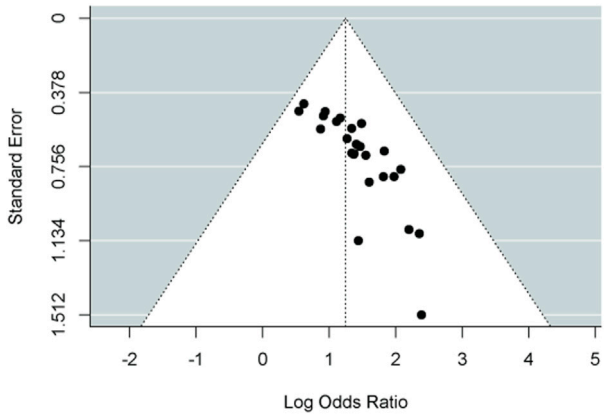


FIGURE 4
Funnel plot of paired meta-analysis of the antihypertensive effective rate.

level of convergence was deemed satisfactory, ensuring that the established Bayesian model can effectively forecast subsequent results. These findings are illustrated in Figures 6, 7; Supplementary Figure S7; Supplementary Figure S8.

3.7 Results of the network meta-analysis

3.7.1 Systolic blood pressure (SBP) analysis

Our study, depicted in Figure 5, evaluated the impact of CCBs with various Chinese patent medicines on systolic blood pressure (SBP). The comprehensive results of the network meta-analysis are as follows:

QGJY [MD = −10.93, 95%*CrI* (−17.28,-4.62)], QJDH [MD = −7.3, 95%*CrI* (−11.91, −2.68)],QLDX [MD = −10.67,95% *CrI*(-16.06,-5.19)],SLXM [MD = −10.28,95%*CrI*(-13.25,−7.3)],TMGT [MD = −12.43,95%*CrI*(-16.86,−8.08)],XMT [MD = −6.99,95% *CrI*(-12.39,-1.56)]. These data are visualized in Figure 8. Each of the aforementioned combinations showcased a superior antihypertensive effect compared to the sole administration of CCBs, with statistical significance at $p < 0.05$.

Further insights are provided by the Surface Under the Cumulative Ranking (SUCRA) scores, which indicate the probable efficacy of each combination in reducing SBP. Tianma Gouteng Granule combined with CCB (TMGT + CCB) emerged as the most likely superior intervention, boasting an 83.99% score. In terms of efficacy, the ranking of the Chinese patent medicine combined with CCB is as follows:

TMGT + CCB (83.99%) > QGJY + CCB (68.51%) > QLDX + CCB (66.73%) > SLXM + CCB (63.29%) > QJDH + CCB (34.06%)>XMT + CCB (33.26%). These comprehensive rankings and data are visualized in Figure 9 and Supplementary Material.

Table 4 shows combined treatments based on outcomes, analyzed in conjunction with SUCRA.

3.7.2 Diastolic blood Pressure (DBP) analysis

The network meta-analysis, depicted in Figure 5, assessed the impact of various Chinese patent medicines combined with CCBs on diastolic blood pressure (DBP). Here's a breakdown of the findings: QJDH [MD = −4.54, 95%*CrI*(−8.99, −0.08)], QLDX

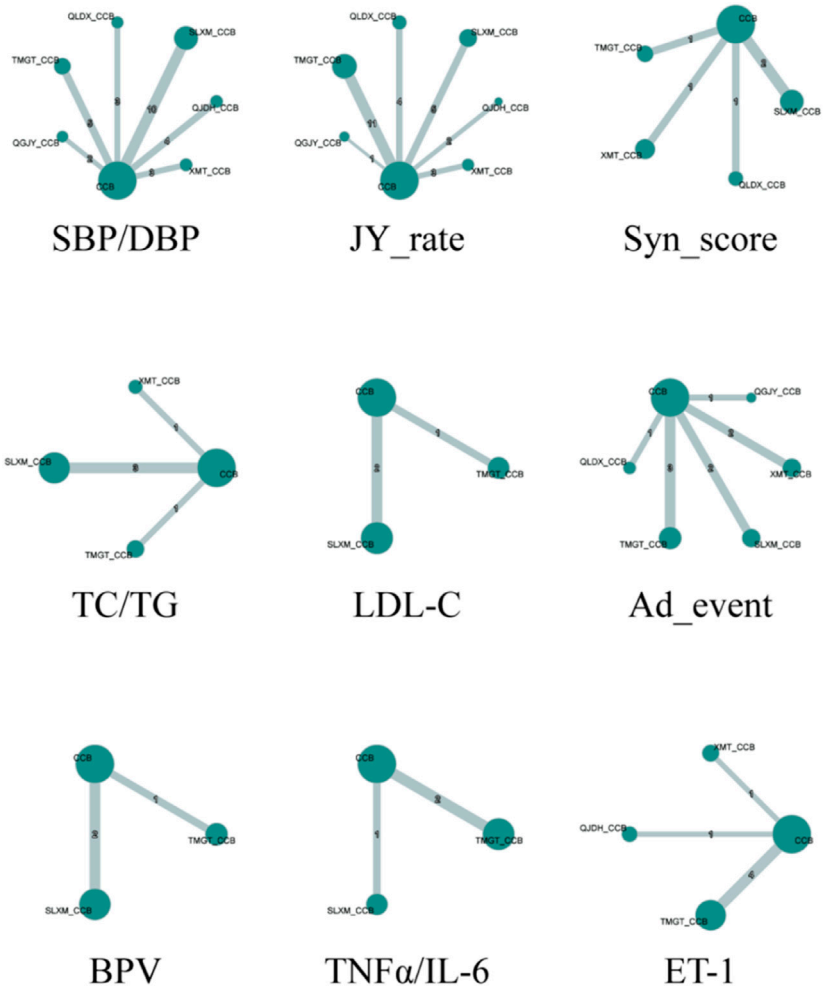


FIGURE 5
Network plot for the antihypertensive effective rate. *Notes*: SBP/DBP:Systolic blood pressure/Diastolic blood pressure; JY_rate: Rate of blood pressure decreases; Syn_score: TCM Syndrome score; TC/TG: Total cholesterol/Triglycerides; LDL-C: Low-density lipoprotein cholesterol; Ad_event: Adverse events; BPV: Blood pressure variability; TNFα/IL-6: tumor necrosis factor α/Interleukin- 6; ET-1: Endothelin-1.

[MD = −5.52, 95%*CrI*(-10.73, −0.3)], SLXM [MD = −7.69, 95% *CrI* (−10.58, −4.79)], TMGT [MD = −5.74, 95% *CrI* (−9.77, −1.72)]. These data are visualized in Figure 8.

However, two combinations, namely, QGJY [MD = −6.21, 95% CrI (−12.58, 0.12)] and XMT [MD = −4.86, 95%CrI (−10.05, 0.36)], did not show a statistically significant improvement over using CCB alone. Despite this, most of these combinations indicated a statistically significant superior antihypertensive effect compared to using only CCB ($p < 0.05$).

A deep dive into the SUCRA scores provided insights into the probable efficacy of each combination in reducing DBP. The results spotlighted SLXM + CCB as the most promising intervention with a score of 82.44%. In terms of efficacy, the Chinese patent medicines combined with CCB are ranked as:

SLXM + CCB(82.44%)>QGJY + CCB(62.17%)>TMGT + CCB(57.41%)>QLDX + CCB(54.85%)>XMT + CCB(47.76%) >QJDH + CCB (43.56%). These comprehensive findings and rankings are visualized in Figure 9.

3.7.3 Antihypertensive effective rate analysis

As presented in Figure 10, the network meta-analysis delved into the efficacy of various Chinese patent medicines when combined with calcium channel blockers (CCBs) to improve antihypertensive effective rates. Here are the key findings:

QGJY [OR = 9.26, 95% CrI (2.02, 70.59)], QJDH [OR = 5.93, 95% CrI (2.11, 19.08)], QLDX [OR = 5.04, 95%CrI (2.66, 10.73)], SLXM [OR = 3.42, 95%CrI (1.88, 6.65)], TMGT [OR = 3.22, 95%CrI (2.28, 4.61)], XMT [OR = 3.71, 95% CrI (1.76, 8.61)]. These combinations showcased a statistically significant advantage in antihypertensive efficacy when compared to using only CCB ($p < 0.05$).

The SUCRA scores further shed light on the probable efficiency of each combination in enhancing antihypertensive rates. Notably,

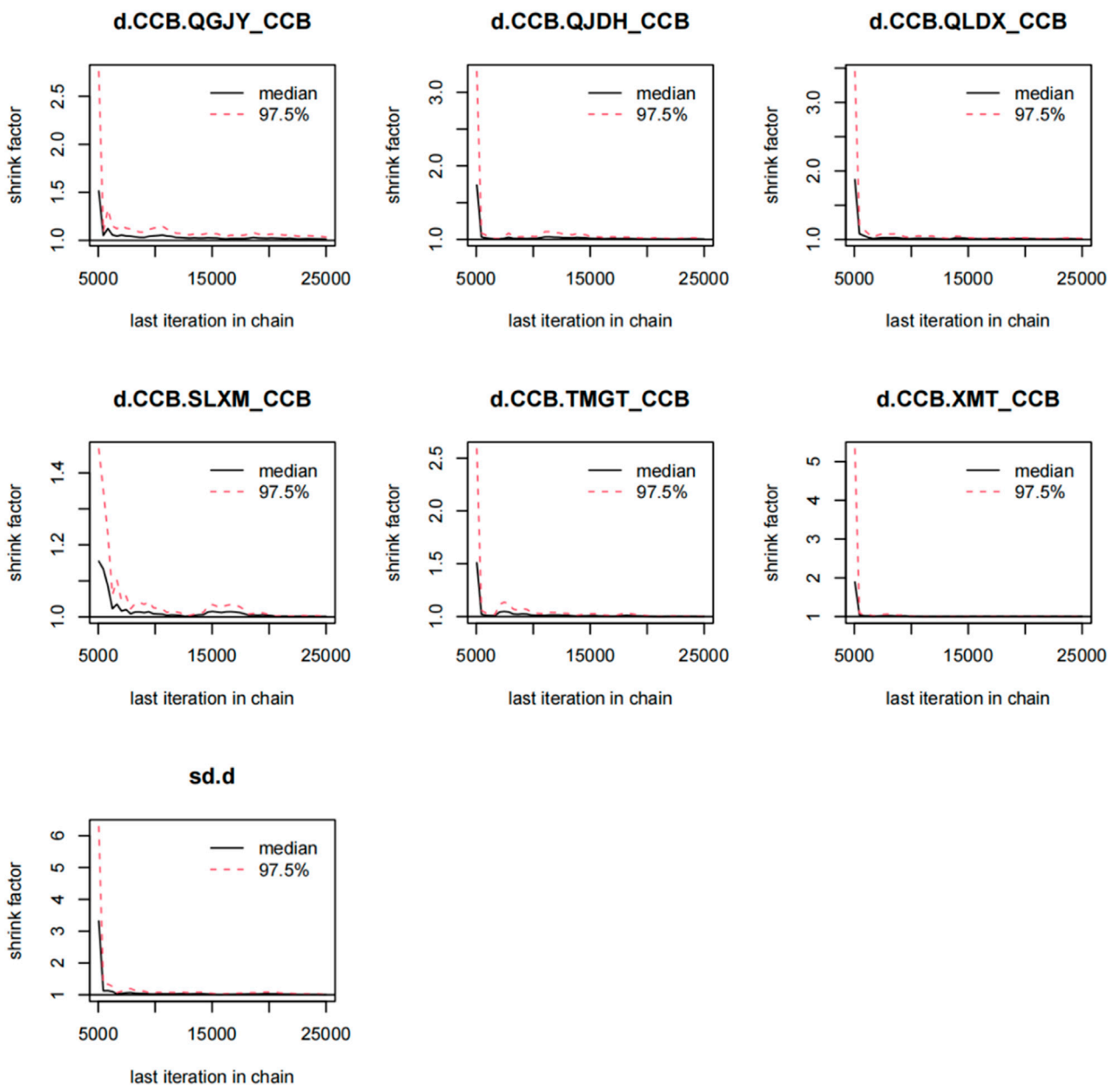


FIGURE 6
Gelman-Rubin-Brooks plot for rate of blood pressure decreases. *Notes*:Chinese patent drugs + CCB vs. CCB.

QGJY + CCB emerged as the top contender with a remarkable score of 84.48%. Here's the hierarchy based on efficacy:

QGJY + CCB (84.48%) >QJDH + CCB (70.89%) > QLDX + CCB (66.34%)>XMT + CCB (48.69%)>SLXM + CCB (42.62%)> TMGT + CCB (36.87%). These insightful findings are vividly portrayed in Figure 9.

See the secondary outcomes in Supplementary Material.

3.8 Safety evaluation

Safety is paramount when evaluating the efficacy of any medical treatment. In our analysis, 10 out of the total studies discussed potential adverse drug reactions/events. There was no significant difference in the rage of adverse events with no heterogeneity (Supplementary Figure S14). The most common adverse events included facial blushing, nauseous, palpitations, dizziness.

However, one of the challenges in consolidating these findings was the varied criteria each study used to determine and classify these adverse reactions/events. Due to these inconsistencies and to ensure clarity, we've opted for a descriptive analysis approach rather than a quantitative one.

For a detailed breakdown of the reported adverse reactions/events from each of these 10 studies, please refer to Table 5. This table provides insight into the type, frequency, and severity of reactions, giving both researchers and medical professionals a clearer picture of the safety profile of these treatments.

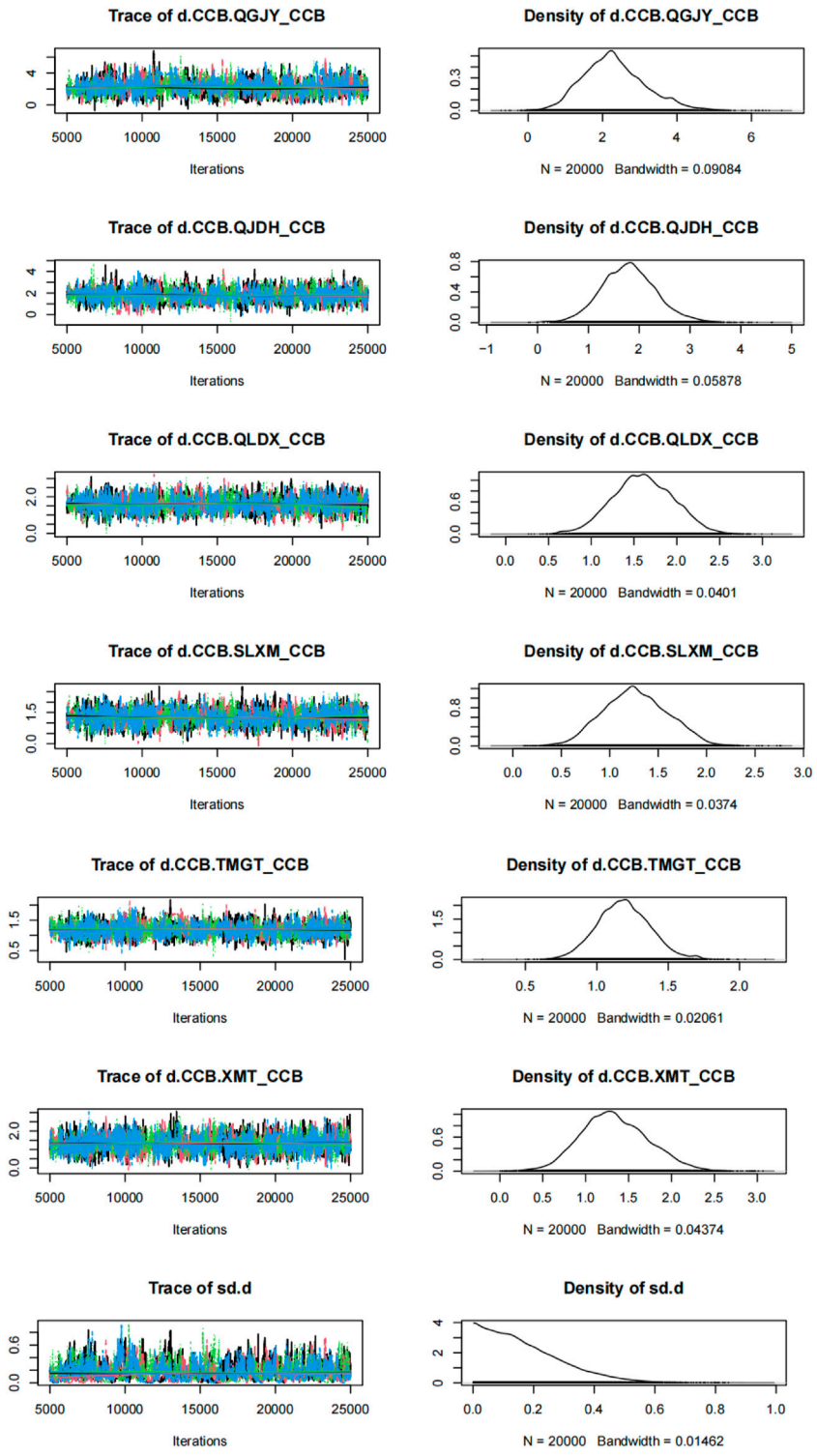
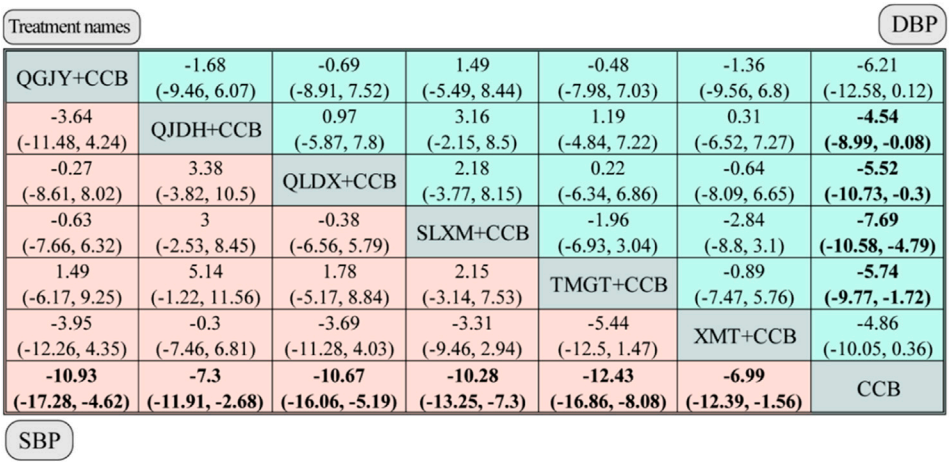


FIGURE 7
Time-series and density plots for rate of blood pressure decreases. *Notes*: Chinese patent drugs + CCB vs. CCB.

3.9 Sensitivity analysis

Using the leave-one-out sensitivity anlysis, we have revealed and examined that no single study altered the pooled effect of RR and MD. Most of the summary results remained the same after systematically excluding specific studies one bye one proving the robustness of our results. Additional results of sensitivity analyses of SBP, DBP, antihypertensive and adverse rate were provided in Supplement.



Treatment names						DBP
QGJY+CCB	-1.68 (-9.46, 6.07)	-0.69 (-8.91, 7.52)	1.49 (-5.49, 8.44)	-0.48 (-7.98, 7.03)	-1.36 (-9.56, 6.8)	-6.21 (-12.58, 0.12)
-3.64 (-11.48, 4.24)	QJDH+CCB	0.97 (-5.87, 7.8)	3.16 (-2.15, 8.5)	1.19 (-4.84, 7.22)	0.31 (-6.52, 7.27)	**-4.54 (-8.99, -0.08)**
-0.27 (-8.61, 8.02)	3.38 (-3.82, 10.5)	QLDX+CCB	2.18 (-3.77, 8.15)	0.22 (-6.34, 6.86)	-0.64 (-8.09, 6.65)	**-5.52 (-10.73, -0.3)**
-0.63 (-7.66, 6.32)	3 (-2.53, 8.45)	-0.38 (-6.56, 5.79)	SLXM+CCB	-1.96 (-6.93, 3.04)	-2.84 (-8.8, 3.1)	**-7.69 (-10.58, -4.79)**
1.49 (-6.17, 9.25)	5.14 (-1.22, 11.56)	1.78 (-5.17, 8.84)	2.15 (-3.14, 7.53)	TMGT+CCB	-0.89 (-7.47, 5.76)	**-5.74 (-9.77, -1.72)**
-3.95 (-12.26, 4.35)	-0.3 (-7.46, 6.81)	-3.69 (-11.28, 4.03)	-3.31 (-9.46, 2.94)	-5.44 (-12.5, 1.47)	XMT+CCB	-4.86 (-10.05, 0.36)
-10.93 (-17.28, -4.62)	**-7.3 (-11.91, -2.68)**	**-10.67 (-16.06, -5.19)**	**-10.28 (-13.25, -7.3)**	**-12.43 (-16.86, -8.08)**	**-6.99 (-12.39, -1.56)**	CCB

FIGURE 8
Network meta-analysis for SBP and DBP. *Notes*: different Chinese patent medicine + CCB vs. CCB. The comparison between different interventions is represented by MD and 95%*CrI*, and the ones in bold mean $p < 0.05$.

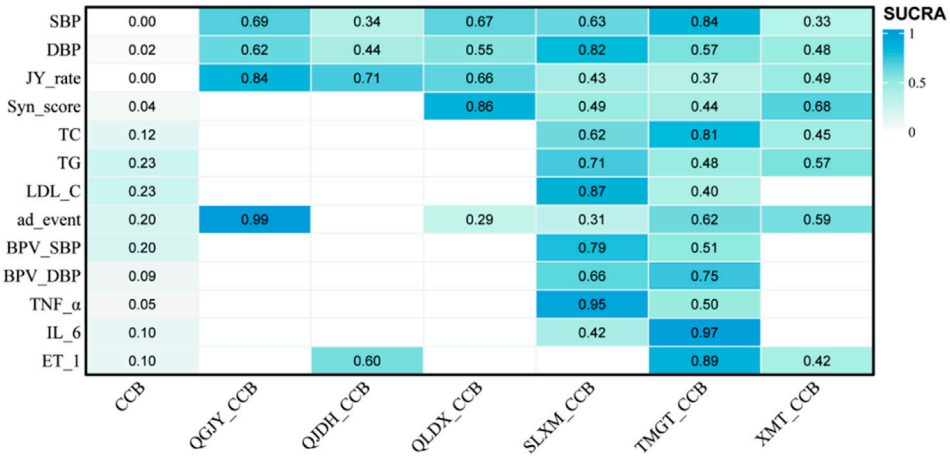


FIGURE 9
SUCRA score heat map of outcomes. *Notes*: SBP: systolic blood pressure; DBP: diastolic blood pressure; JY_rate: antihypertensive effect rate; Syn_score: TCM syndrome score; TC: total cholesterol; TG: triglyceride; LDL-C: low-density lipoprotein cholesterol; ad_event: adverse reactions/events; BPV_SBP: systolic blood pressure variability; BPV_DBP: diastolic blood pressure variability; TNF_α: tumor necrosis factor-α; IL_6: interleukin-6; ET_1: vascular endothelin-1.

3.10 Subgroup analysis

As the amount of data for subgroup analysis was insufficient, subgroup analysis in our study were conducted of SBP, DBP and antihypertensive effective rate. Specific of Chinese patent medicines included SLXM, TMGT, QLDX, QGJY, QJDH and XMT. Statistically significant differences were observed between subgroups, and heterogeneity was reduced in the subgroups (Supplementary Material), suggesting that the type of Chinese patent medicines may be the source of heterogeneity. Other results of the bias analysis were presented in Supplementary Material.

4 Discussion

4.1 Global prevalence of EH and insights into modern and traditional therapeutic approaches

The global implications of elevated blood pressure are concerning. Among the 3.5 billion adults worldwide, blood pressure levels in many are below the optimal range. Alarmingly, about 874 million adults have systolic blood pressure exceeding 140 mmHg, translating to nearly one-quarter of the global adult population grappling with EH (1). The underpinnings of abnormal

TABLE 4 Combined treatments according to different outcomes ranked by SUCRA.

Outcomes	Recommended interventions
SBP	TMGT + CCB
DBP	SLXM + CCB
JY_rate	QGJY + CCB
Syn_score	QLDX + CCB
TC	TMGT + CCB
TG	SLXM + CCB
LDL_C	SLXM + CCB
ad_event	QGJY + CCB
BPV_SBP	SLXM + CCB
BPV_DBP	TMGT + CCB
TNF-α	SLXM + CCB
IL-6	TMGT + CCB
ET-1	TMGT + CCB

Notes: SBP: systolic blood pressure; DBP: diastolic blood pressure; JY_rate: antihypertensive effect rate; Syn_score: TCM, syndrome score; TC: total cholesterol; TG: triglyceride; LDL-C: low-density lipoprotein cholesterol; ad_event: adverse reactions/events; BPV_SBP: systolic blood pressure variability; BPV_DBP: diastolic blood pressure variability; TNF-α: tumor necrosis factor-α; IL-6: interleukin−6; ET-1: vascular endothelin-1.

blood pressure can primarily be attributed to irregular blood pressure regulation. Ensuring physiological blood pressure requires the synchronized functioning of various elements within an intricate neurohumoral system. This includes factors such as the renin-angiotensin-aldosterone system, natriuretic peptides, endothelium, sympathetic nervous system, as well as inflammation and immune responses (Oparil et al., 2018).

Modern medicine has recognized the importance of vascular tone in regulating blood pressure and distributing blood flow within the body. Among the therapeutic arsenal, CCBs are particularly effective for hypertensive vessels, owing to their specificity to increased inactivated channels and the presence of a greater number of these channels (Morel and Godfraind, 1987). Beyond mere blood pressure control, CCBs also play a pivotal role in preventing the structural changes induced by EH in both the heart and arteries.

Complementing this modern understanding, TCM provides a unique perspective. While EH is not directly named in TCM, its clinical manifestations align with symptoms described as "dizziness" and "headache". The TCM diagnosis points to various pathogenic factors, such as hyperactivity of liver yang, syndrome of static blood blocking collaterals, liver wind stirring, and kidney essence deficiency. Guided by its foundational principles of holistic treatment and syndrome differentiation, TCM emphasizes tailored treatments. Consequently, antihypertensive Chinese patent medicines, when prescribed in line with syndrome differentiation, have demonstrated efficacy in reducing blood pressure and alleviating clinical symptoms.

Recognizing and addressing EH is paramount, not just for individual health, but from a broader public health perspective. As supported by a 2009 meta-analysis, optimal management of EH, whether through modern antihypertensive drugs or TCM approaches, is instrumental in curtailing cardiovascular risks and fostering longevity in the global populace (Law et al., 2009).

4.2 Comparative efficacy of combined treatments for EH

Building on our understanding of the global prevalence of EH and the synergy of modern and traditional therapeutic approaches, it is essential to delve into the efficacy of combined treatments.

In the paired meta-analysis, our data presents compelling evidence supporting the enhanced therapeutic efficacy of

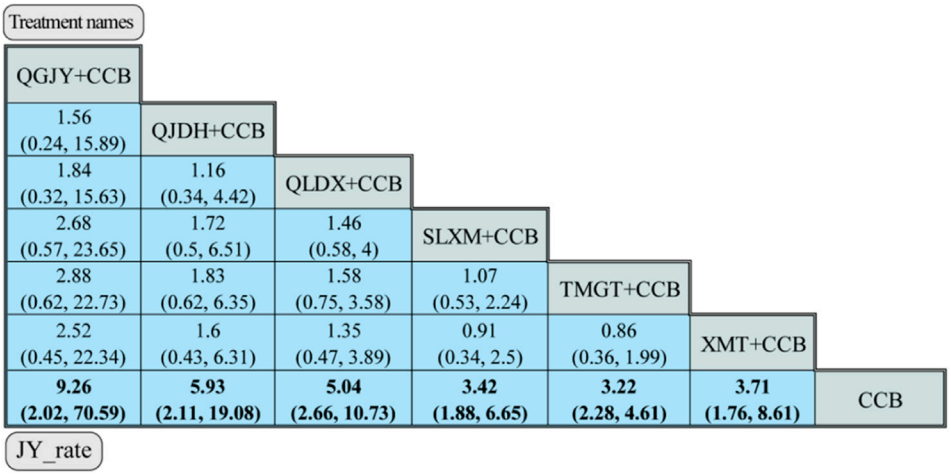


FIGURE 10
Network meta-analysis for JY_rate. *Notes*: different Chinese patent medicine + CCB vs. CCB. The comparison between different interventions is represented by MD and 95%*CrI*, and the ones in bold mean $p < 0.05$.

TABLE 5 Overview of drug safety information.

Study	Intervention	n	Adverse effects/events		P	Outcome
		E/C	E	C		
Li (2017)	SLXM + CCB vs. CCB	49/49	mild diarrhea、dizziness、palpitations in 3patients	nauseous、headache in 2 patients	0.646	Not mentioned
Zhang et al. (2014b)	SLXM + CCB vs. CCB	81/81	Palpitations、nauseous、dizziness、fatigue in 2 patients	diarrhoea、abdominal pain、insomsonia、facial blushing、sweat in 4 patients	>0.05	Not mentioned
Xiong (2018)	QLDX + CCB vs. CCB	63/63	facial blushing in 3 patients, palpitations in 2 patients, lower limb oedema in 2 patients, gingival hypertrophy in 1 patient	facial blushing in 4 patients, palpitations in 3 patients, lower limb oedema in 1 patient, gingival hypertrophy in 1 patient	>0.05	Not mentioned
Zhou (2013)	TMGT + CCB vs. CCB	63/64	hypotension in 2 patients, dry cough in 3 patients, facial blushing in 4 patients, ankle oedema in 3 patients	hypotension in 2 patients, dry cough in 4 patients, facial blushing in 3 patients, ankle oedema in 3 patients	>0.05	Not mentioned
Sun (2019)	TMGT + CCB vs. CCB	75/75	headache in 1 patient, nauseous in 2 patients, angina pectoris in 1 patient	Headache in 5 patients, nauseous in 6 patients, angina pectoris in 4 patients	0.012	Not mentioned
Wang (2020)	TMGT + CCB vs. CCB	55/55	adverse effects in 2 patients	adverse effects in 9 patients	<0.05	Not mentioned
Wang (2019)	TMGT + CCB vs. CCB	57/57	no obvious side effects	no obvious side effects	—	Not mentioned
Zhao (2012)	QGJY + CCB vs. CCB	40/40	no obvious side effects	facial blushing in 1 patient, headache in 3 patients	—	Not mentioned
Wang et al. (2022)	XMT + CCB vs. CCB	70/70	palpitations in 2 patients, headache in 1 patient, facial blushing in 1 patient, gastric distention in 1 patient	diarrhoea in 2 patients, gastric distention in 2 patients, palpitations in 1 patient, headache in 1 patient	0.753	Not mentioned
Liu (2021)	XMT + CCB vs. CCB	64/64	facial blushing in 1 patient, increased heart rate in 1 patient	facial blushing in 1 patient, increased heart rate in 6 patients, abdominal distension in 5 patients	<0.05	Not mentioned

combining Chinese patent medicine with CCBs in addressing EH. Specifically, when compared to the CCB-only group, the combined treatment approach exhibited a notably improved curative effect (RD = 0.14, 95%CI = 0.11, 0.16). Remarkably, this outcome demonstrated minimal heterogeneity (I^2 = 0%).

Broadening our lens to the network meta-analysis, further differentiations emerge regarding the efficacy of various combinations:

SBP Reduction: Tianma Gouteng Granule combined with CCB stands out in its efficacy.

DBP Reduction: The combination of Songling Xuemaikang Capsule with CCB takes the lead.

Antihypertensive Efficacy: Qinggan Jiangya Capsule, when combined with CCB, showcases superior results.

TCM Syndrome Score: Patients treated with Qiangli Dingxuan Tablet combined with CCB observed the most significant reduction.

Lipid Profile Improvements: While Tianma Gouteng Granule and CCB excelled in reducing TC, the pairing of Songling Xuemaikang Capsule with CCB emerged as more potent in lowering LDL-C.

Inflammatory Marker Reduction: In terms of mitigating TNF-α and IL-6 levels, the combination of Songling Xuemaikang Capsule with CCB proved most effective.

It is crucial to note that the number of RCTs concerning secondary clinical outcomes was limited, which may impact the robustness of comparisons in those areas.

These insights underscore the importance of nuanced, individualized therapeutic strategies. The differential efficacy outcomes of the varied combinations offer clinicians a spectrum of options, allowing them to tailor EH management according to patient-specific needs, bridging the time-tested wisdom of TCM with the precision of modern pharmacology.

4.3 Mechanisms underpinning the efficacy of TCM capsules in EH management

Having highlighted the comparative efficacy of combined treatments for EH, it becomes imperative to probe deeper into the mechanisms and metabolites of key TCM capsules that stood out in the meta-analysis. This can provide insights into how these traditional remedies potentiate the effects of modern drugs like CCBs.

Xinmaitong Capsule: This capsule is known for promoting blood circulation, clearing blood stasis, and nourishing the heart. This intricate formulation boasts a blend of medicinal botanical drugs, including but not limited to, *Angelica sinensis (Oliv.) Diels, Salvia miltiorrhiza Bunge, Ilex pubescens Hook. and Arn.*, and *Pueraria lobata (Willd.) Ohwi.* Research by Wei Chengke (Wei et al., 2017) showed that this oil can protect vascular cells and help reduce blood pressure. Another significant metabolite, Tanshinone IIA from *S. miltiorrhiza Bunge*, aids in blood circulation (Li, 2017). Li Wendi's study (Li and Sun, 2021)further highlighted its ability to regulate blood pressure, possibly by interacting with certain cellular pathways.

Tianma Gouteng Granule: Recognized for its calming and heat-clearing properties, its primary botanical drugss are *Gastrodia elata Bl.* and *Uncaria rhynchophylla (Miq.) Miq*., from Gastrodia, has potent antihypertensive effects (Chen et al., 2016). It has been shown to counteract certain vasoconstricting agents and promote vasodilation, leading to reduced blood pressure (Shan et al., 2017). Additionally, Cheng Xiankun's research (Cheng et al., 2019) highlighted rhynchophylline's capacity to optimize internal circulation and manage blood pressure in hypertensive model rats.

Songling Xuemaikang Capsule: This capsule calms the liver and subsiding yang, tranquillizing with heavy prescription, promotes blood circulation, and removes blood stasis. Comprising mainly of Pinea *Wolf* and Pueraria lobata (*Willd.*) *Ohwi*, etc., clinical studies have shown its efficacy in treating mild hypertension, with the potential to replace control drugs (Lai et al., 2022). Its mechanism involves regulating the RAAS pathway (Liu et al., 2015), modulating gene expression (Zhao et al., 2013), and safeguarding vascular endothelial cells by inhibiting specific cellular pathways. It further mitigates oxidative stress injuries in rat aorta through modulating CAV1 and IGF1R gene expressions (Shi et al., 2018).

Qinggan Jiangya Capsule: Known for its liver-calming and heat-clearing effects, its main botanical drugs are *Polygonum muliflorum Thunb*. and *S. miltiorrhiza Bunge*. Wang Xiayun's study (Wang et al., 2017) emphasized its role in vascular dilation and reducing specific vascular growth factors. Liang Yanfei (Liang et al., 2021) further showcased its ability to decrease blood pressure while ensuring liver, kidney, and heart functions remain intact.

Qiangli Dingxuan Tablet: This tablet is designed to calm the liver wind. Main botanical drugs include *G. elata Blume* and Eucommia ulmoides Oliv., which have numerous antihypertensive metabolites like lignans and phenylpropanoids. Research has indicated that these metabolites can work through various pathways to manage blood pressure, such as inhibiting specific enzymes and combating vasoconstriction (Taubert et al., 2002; Gu et al., 2011).

4.4 Clinical significance

Past meta-analyses have compared Chinese patent medicines combined with western medicines in the treatment of EH, which showd significant reduction in both SBP and DBP compared to western medicines. There were significant beneficial effects in SLXM combined with antihypertensive drugs compared to the antihypertensive drugs using alone. However, no compelling evidence was found to demonstrate its superior efficacy compared to other Chinese patent medicines. RCTs about QLDX for the treatment of EH showed that SBP and DBP were significantly lower than in the placebo group (Yang et al., 2015; Ji et al., 2022; Zhang et al., 2022; Lin et al., 2023). While our results showed that TMGT and SLXM combined with CCB showed more effective in our NMA, and QLDX played an important role in reducing TCM Syndrome Score.

In furnishing healthcare professionals with a solid, evidence-based framework for treatment decision-making, the research challenges the prevailing one-size-fits-all medical paradigm, advocating for an alignment more intimately connected with the foundational doctrines of TCM. Based on our evidence, clinicians choose the most appropriate treatment plan based on the patient's specific situation, enabling individualized medicine. The ideal integration of Chinese patent medicine and CCBs pivots on specific symptomatology, as discerned through the seasoned expertise of traditional Chinese medical practitioners. Grounded in the comprehensive principles of TCM and reflecting a tangible application in ethnopharmacological exploration, the findings accentuate the indispensability of personalized medical interventions.

Based on the results of our study, not only can provide high quality evidence for the formulation of clinical practice guidelines for EH, but also can guide clinical practice, and is of great significance for clinicians to choose proprietary Chinese patent medicines.

4.5 Limitations, biases, and implications for future trials

While this study offers the most updated review and network meta-analysis concerning Chinese patent medicine combined with CCB in the treatment of EH, several limitations merit attention. Firstly, the included RCTs did not retrieve the registration scheme, with the allocation concealment and blinding methods often omitted, potentially leading to biases. Furthermore, there's a limited number of RCTs available for certain secondary outcomes, emphasizing the need for more comprehensive research and potentially undermining the credibility of some findings due to potential publication bias. And the number of studies and the patients in studies was limited, and many studies were not high quality and showed a degree of heterogeneity. There was an asymmetry in funnel plots, which is not necessarily a result of publication bias, but rather higher efficacy in small trials than in large trials for a variety of reasons.

It is also worth noting that all the included RCTs are exclusively Chinese literature, which means the study lacks data in other languages. This linguistic focus might introduce inherent biases or overlook crucial global perspectives. Additionally, potential heterogeneity in the clinical environments of the trials could introduce inconsistencies, although the consistency of the paired meta-analysis results of combined western medicine mitigates some of these concerns.

In addition, most of the patients in literature were middle-aged and elderly, and there were few studies on the efficacy of young EH patients which could lead to bias. Besides, whether the different duration of intervention causes the difference in efficacy should be studied which was absent in our study.

According to the SUCRA ranking in our study, different proprietary Chinese patent medicines had advantages in improving different outcomes, which may involve the simultaneous use of multiple drugs in clinical practice. Therefore, when the therapeutic effect is well established, economic evaluation such as cost-benefit analysis should be combined to provide valuable insights into the feasibility of these combined therapies. And for western doctors, treatment based on syndrome differentiation may be difficult, if the decision is only according to the outcomes, the syndrome will be ignored, and there may be wrong medication.

Looking forward, it is imperative to expand the linguistic and cultural scope of research. Future endeavors should incorporate studies from diverse linguistic and cultural backgrounds, ensuring a comprehensive global perspective. A deeper exploration into the

molecular mechanisms through experimental research could elucidate the cellular interactions when blending these traditional medicines with western antihypertensives. Broadening clinical trials to target a wider variety of hypertensive patient profiles can pave the way to determine the best combinations for specific patient needs. Moreover, once therapeutic efficacy is well established, economic assessments such as cost-benefit analyses could offer valuable insights into the financial viability of these combined treatments, especially in settings where resources are limited.

However, the silver lining in this study is the innovative comparison of the curative effects of different Chinese patent medicines when combined with a specific type of antihypertensive western medicine. This provides clinicians with a more personalized approach to treatment, bolstered by our evidence-based analysis. As a beacon for future research, this study underscores the significant potential of integrating traditional practices with modern medicine, paving the way for more harmonized and effective healthcare solutions.

5 Conclusion

In conclusion, the results of our network meta-analysis provide evidence of the need for EH. This thorough review decisively underscores the nuanced therapeutic potentials and variances when integrating Chinese patent medicine with CCBs in the treatment of EH. The effectiveness of such combinations distinctly depends on the particular Chinese patent medicine and the unique clinical presentations of patients. Compared to traditional western therapy, the addition of Chinese patent medicine can simultaneously reduce cardiovascular risk factors, lower blood pressure, improve eradication rates and reduce side effects. Notably, Tianma Gouteng Granule and Songling Xuemaikang Capsule combined with CCB have the most prominent overall efficacy. The results of this study should be referenced by policymakers and the formulation of clinical practice guidelines, and should be applied in clinical practice in treatment of essential hypertension.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

LC: Writing–review and editing, Writing–original draft, Formal Analysis, Data curation, Conceptualization, XL: Writing–review and editing, Writing–original draft, Formal Analysis. YL: Writing–original draft, Data curation. TJ: Writing–original draft. DL: Writing–original draft. CR: Writing–original draft. TY: Writing–original draft. YW: Writing–original draft. ZZ: Writing–original draft. JW: Writing–original draft. XH: Writing–review and editing, Supervision, Methodology, Conceptualization. LW: Writing–review and editing, Supervision, Methodology, Conceptualization.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This research was funded by the Science and Technology Innovation Project of China Academy of Chinese Medical Sciences, under the project "Chinese Medicine Clinical Basic Discipline Innovation Team Project" (CI 2021B003) and "Research on Common Methodology for the Development of Key Technologies and Treatment Guidelines for the Whole Cycle of TCM Standardization" (CI 2021A00701-1). "Theoretical study on the dynamic spatio-temporal Chinese medicine diagnosis and treatment law of hypertension under the model of "cause-mechanism-symptom-treatment"" (CI2021A00105) and "The Seventh Batch of National Famous Elderly Chinese Medicine Han Xuejie Expert Academic Experience Inheritance Project" (Z0814).

Acknowledgments

We express our gratitude to Xing for his invaluable assistance with statistical analysis and R-based graphical plotting. His expertise significantly enhanced our research outcomes.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2024.1321405/full#supplementary-material

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Glossary

ACEI	angiotensin converting enzyme inhibitors
Ad_event	adverse drug reaction events
ARB	angiotensin II receptor blockers
BPV	blood pressure variability
C	control group
CAV1	caveolin 1
CCB	calcium channel blockers
CI	confidence interval
CNKI	China National Knowledge Infrastructure
CrI	credible interval
CVD	cardiovascular disease
D	day
DBP	diastolic blood pressure
E	experimental group
EH	essential hypertension
ET-1	vascular endothelin-1
IGF1R	insulin like growth factor 1 receptor
IL-6	interleukin-6
JY_rate	antihypertensive effective rate
LDL-C	low-density lipoprotein cholesterol
MD	mean difference
NMA	network meta-analysis
OR	odds ratio
PPARγ	peroxisome proliferator-activated receptor γ
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analysis
PSRF	potential scale reduction factor
QGJY	Qinggan Jiangya Capsule
QJDH	Qingju Dihuang Pill
QLDX	Qiangli Dingxuan Tablet
RAAS	rein angiotensin aldosterone system
RCTs	randomized controlled trials
RD	risk difference
ROB2	risk of bias 2
SBP	systolic blood pressure
SD	standard deviation
SLXM	Songling Xuemaikang Capsule
SUCRA	surface under the cumulative ranking
Syn_score	TCM syndrome score
T	treatment time
TC	total cholesterol
TCM	Traditional Chinese Medicine
TG	triglyceride
TMGT	Tianma Gouteng Granule
TNFα	tumor necrosis factor-α
VIP	China Science and Technology Journal Database
W	week
XMT	Xinmaitong Capsule

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TYPE Review
PUBLISHED 18 March 2024
DOI 10.3389/fphar.2024.1365911

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EDITED BY
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RECEIVED 05 January 2024
ACCEPTED 26 February 2024
PUBLISHED 18 March 2024

CITATION
Li C, Li Y, Huang X, Li S, Sangji K and Gu R (2024), Traditional Tibetan medicine: therapeutic potential in lung diseases.
Front. Pharmacol. 15:1365911.
doi: 10.3389/fphar.2024.1365911

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Traditional Tibetan medicine: therapeutic potential in lung diseases

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Lung diseases have become a major threat to human health worldwide. Despite advances in treatment and intervention in recent years, effective drugs are still lacking for many lung diseases. As a traditional natural medicine, Tibetan medicine has had a long history of medicinal use in ethnic minority areas, and from ancient times to the present, it has a good effect on the treatment of lung diseases and has attracted more and more attention. In this review, a total of 586 Tibetan medicines were compiled through literature research of 25 classical works on Tibetan medicine, drug standards, and some Chinese and English databases. Among them, 33 Tibetan medicines have been studied to show their effectiveness in treating lung diseases. To investigate the uses of these Tibetan medicines in greater depth, we have reviewed the ethnomedicinal, phytochemical and pharmacological properties of the four commonly used Tibetan medicines for lung diseases (rhodiola, gentian, sea buckthorn, liexiang dujuan) and the five most frequently used Tibetan medicines (safflower, licorice, sandalwood, costus, myrobalan). It is expected to provide some reference for the development of new drugs of lung diseases in the future.

KEYWORDS

traditional Tibetan medicine, natural medicine, lung diseases, pharmacological properties, bioactive components

1 Introduction

Due to factors such as environmental pollution, smoking, economic development, an accelerated pace of life, and poor lifestyle, the incidence of lung diseases has increased year by year in recent years, and lung diseases have become an important disease threatening human health worldwide (Xiao and Wei, 2018) and is a major cause of death and disability worldwide (Collaborators, 2020). Despite recent advances in patient care and intervention, many lung diseases still lack effective treatments and remain incurable (Ryter et al., 2012). Chronic obstructive pulmonary disease, asthma, tuberculosis, interstitial lung diseases, lung cancer, bronchiectasis, lung abscess, and pneumothorax are the most common lung diseases at present (Xiao and Zheng, 2018). Among them, three respiratory diseases such as chronic obstructive pulmonary disease (COPD), lung cancer, and asthma have been among the top 10 causes of death worldwide (Levine and Marciniuk, 2022). In recent years, several types of lung diseases have received widespread attention and have become a public health problem. The latest data from the World Health Organization on mortality and causes of death showed that the prevalence of COPD will continue to rise over the next 40 years as smoking rates rise in developing countries and populations in high-income countries age, with

projections of more than 5.4 million deaths/year from COPD and related diseases by 2060 (Geng and Wang, 2021). In 2020, there were 2.21 million new cases and 1.8 million deaths due to lung cancer worldwide with age-standardized incidence rate (ASIR) of 22.4/100,000 (male: 31.5; female: 14.6) and age-standardized mortality rates (ASMR) of 18.0/100,000, (male: 25.9; female: 11.2/100,000). As the projections, there will be 3.8 million incident cases and 3.2 million deaths globally due to lung cancer in 2050 (Sharma, 2022). Interstitial lung disease (ILD) is a group of heterogeneous lung diseases that includes more than 200 parenchymal lesions characterized by extensive fibrosis or inflammatory abnormalities of the lung parenchyma (Ryerson and Collard, 2013). Statistically, up to one-third of patients with ILD develop severe pulmonary fibrosis and are usually diagnosed 2–9 months after the onset of symptoms (Wijsenbeek et al., 2019). Besides, pneumonia is a major health problem worldwide due to its high incidence and susceptibility to complications and high mortality rate among infectious diseases. In particular, the global pandemic of the novel coronavirus pneumonia (COVID-19) that broke out at the end of 2019 has caused unassailable damage to human society in terms of life and health. As of 4 February 2024, more than 775 million confirmed cases of COVID-19 and more than 7.0 million deaths have been reported globally (https://covid19.who.int/). Available studies show that neo-coronavirus infection may cause non-negligible long-term health damage (Chen et al., 2023).

Many of the available therapies for lung diseases have limited effectiveness or are associated with side effects (Kaczynska et al., 2023). Clofazimine, Amikacin, Tigecycline, and Linezolid are currently used to treat *Mycobacterium* abscessus complex-lung disease. However, these drugs have varying degrees of side effects, including gastrointestinal disturbances, reddish-brown skin discoloration, ototoxicity, hemoptysis, nephrotoxicity, and peripheral neuropathy (Weng et al., 2020). Although, in recent years, new approaches and new drugs have emerged for the treatment of lung diseases, such as biologic therapies, and Lactoferrin (LF). The safety and efficacy are still remaining somewhat controversial (Scallan et al., 2020; Kaczynska et al., 2023). For example, despite the effectiveness of TNF-α inhibitors in improving clinical status and slowing joint disease progression, pulmonary toxicity remains a major concern (Dixon et al., 2010; Komiya et al., 2011; Perez-Alvarez et al., 2011; Jani et al., 2014; Perez-De-Lis et al., 2017). In animal studies, LF appears to be an excellent drug, but in human studies, it is more of an immunomodulator with limited therapeutic potential for lung diseases (Kaczynska et al., 2023).

At present, there are scholars have sorted out Tibetan medicine prescriptions for COVID-19, providing important information for the basic research, clinical application, and drug development of traditional medicines for the treatment of COVID-19 or other infectious diseases (Zhang et al., 2023). Biological activities of Gentianeae in Tibetan Medicine (Chi et al., 2021) and Tibetan medicine Bang Jiang (Li et al., 2023) have been compiled. And presented their protective effects on the respiratory system. However, the above researches only provide a detailed introduction to a certain type of lung disease or Tibetan medicine of the Gentiana genus, and do not provide a comprehensive summary of the treatment of lung diseases with Tibetan medicine. Therefore, this study provides the first available data compilation for the ethnic medical knowledge of Tibetan medicine for the treatment of lung diseases. In this paper, based on the literature survey of Tibetan medicine monographs and drug standards, the Tibetan medicine, and Tibetan prescription used in the traditional Tibetan medical system, here, we summarise the methods indicated for lung diseases. Five hundred and eighty-six vegetal (medicinal) species were found to treat lung diseases in the traditional Tibetan medicine system. Among them, Rhodiola, Gentian, Sea buckthorn, and Liexiang dujuan are the ones that the most frequently used in Tibetan hospitals. Among the Tibetan medicine prescriptions recorded in the literature, safflower, licorice, sandalwood, sandalwood, and mirobaran are used most frequently. Therefore, this article provides a detailed introduction to the above drugs, including traditional usage, metabolites, pharmacological effects, and clinical research.

Traditional Tibetan medicine (TTM) is rich in content and complete in theory, and its achievements in physiology, pathology, therapeutics, and pharmacology have not only made great contributions to Chinese medicine but have also long attracted the attention of world medicine. On the other hand, many of the therapies nowadays for lung diseases have limited effectiveness or are associated with side effects. As a result, it is hoped that this review can provide a reference for the development of new drugs with no side effects and significant therapeutic effects for the treatment of lung diseases, thereby improving the quality of life of patients and prolonging their survival time.

2 Methods

We manually searched 25 Tibetan herbal monographs and drug standards, including Jing Zhu Materia Medica, Chinese Dictionary of Ethnic Medicines, Tibetan Medicine Drug Standards, Prescription Medicines for Ethnic Medicine Formulations, Diqing Tibetan Medicine, and National Tibetan Medicine Standard Complete Book, to collect information on Tibetan herbal medicines and prescriptions for the treatment of lung diseases. The data collected from this literature included names, original species, families, medicinal parts, and therapeutic diseases. The botanical names of original plants are mainly from the references, and verified through the "Flora of China (http://frps.eflora.cn/)" database based on their Chinese names. The database of "The Plant List (http://www.theplantlist.org/)" is used to standardize their Latin names. To understand the most commonly used Tibetan medicines for lung diseases, data mining of Tibetan prescriptions was carried out using Traditional Chinese Medicine Inheritance Support System (TCMISS) (Version 2.5) software. All the collected prescriptions were entered into the TCMISS software, and the frequency of use of each drug was ranked from the largest to the smallest by clicking on the "Frequency of Use Statistics" module. In addition, we obtained the active ingredients and biological/pharmacological effects of the selected species through Chinese databases (e.g., Wanfang, Weipu, China Knowledge Network) and international databases (e.g., ISI Web of Science, MEDLINE, Science Direct, Google Scholar) using their Chinese names, English or Latin names as search terms.

10.3389/fphar.2024.1365911

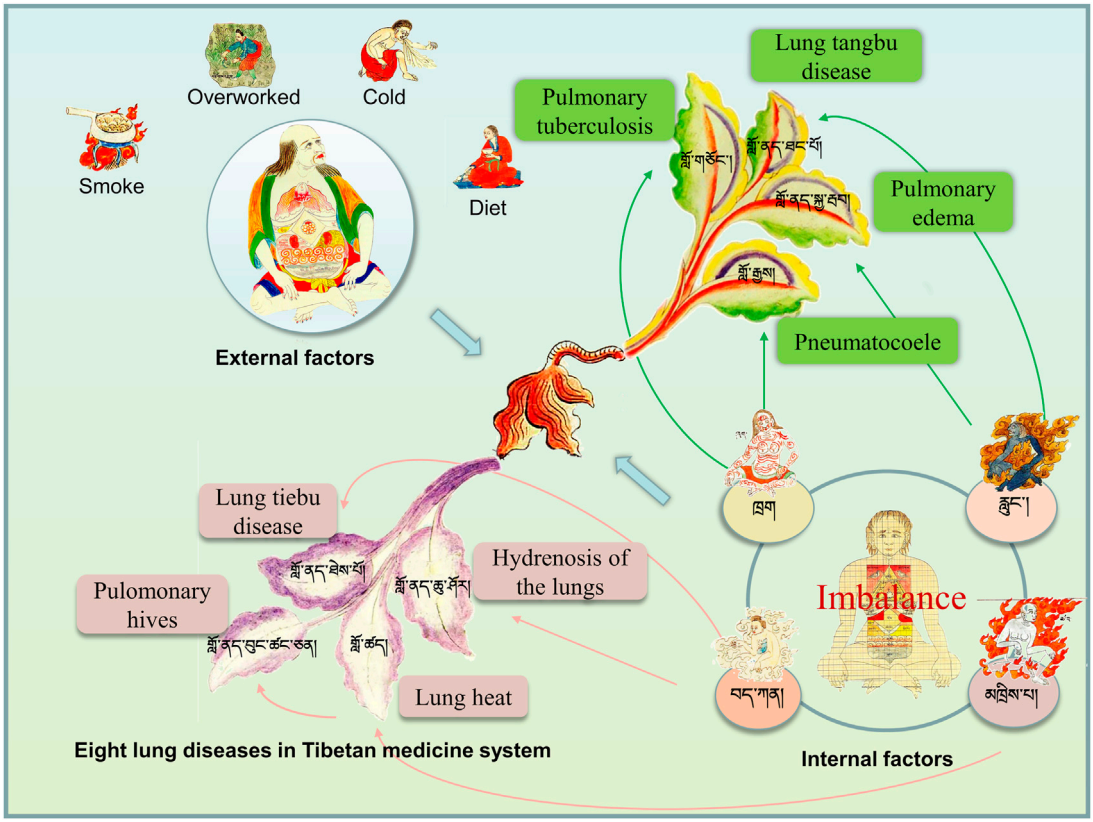

FIGURE 1
Understanding of pulmonary diseases in traditional Tibetan medicine system. Lung diseases are caused internally by the imbalance of the body's "Loong (རླུང་།)", "Tripa (མཁྲིས་པ།)", "Baekan (བད་ཀན།)", and "Blood (ཁྲག)" and foutr external causes.

3 Results

3.1 Understanding the pathogeny of lung disease in TTM theory

TTM is one of the oldest known medical systems in the world. It has a long history of more than 2,000 years. TTM originated from a local folk tradition called "Bon", which dates back to 300 B.C. It is a fusion of theories from early Chinese medicine, Indian medicine (Ayurveda), and Arabic medicine, and has gradually developed into a unique system of medicine (Li et al., 2018a). The Tibetan medical system is very broad in content. Its basic theories include the doctrine of the three factors, the doctrine of the five sources, the five elements, and physiology, pathology, diagnosis, treatment principles, and treatment methods. Among them, the theory of three factors is the core of Tibetan medicine theory, which explains the physiological activities, pathogenesis, and treatment mechanism of the human body by the properties of three substances, namely, "Loong (རླུང་།)", "Tripa (མཁྲིས་པ།)", "Baekan (བད་ཀན།)". It believes that these three substances are the material basis of the human body and the energy of life activities. In the physiological state, the three substances maintain coordination and balance to keep the normal physiological activities of the human body; in the pathological state, the three are out of balance under the influence of various substances, leading to various diseases.

According to Tibetan medicine, the lung is a ministerial organ, the second most important of the five organs. It is written in the Secret Department of "Si Bu Yi Dian (The Four Medical Tantras)" that lung diseases are caused internally by the imbalance of the body's "Loong (རླུང་།)", "Tripa (མཁྲིས་པ།)", "Baekan (བད་ཀན།)", and "Blood (ཁྲག)". External causes include invasion of bodily fluids into the lungs, eating rotten and acidic food, old ghee by the barrel, overly salty food, smoking, long-term colds, and overexertion. In Tibetan medicine, lung diseases are divided into lung "tangbu" disease (གཟུང་རདངས་པ།) and pulmonary edema (གཟུང་རདངས་ཆུ་རྒྱས།) caused by "Loong (རླུང་།)", lung heat (གཟུང་ཚོད།)caused by "Tripa (མཁྲིས་པ།)", lung hydropathy and lung "tiebu" disease (གཟུང་རདངས་ཐེ་མ་པ།) caused by"Baekan (བད་ཀན།)", pulmonary tuberculosis (གཟུང་གཅེར།) and pneumatocoele (གཟུང་རླུང་ལས།) caused by "Blood (ཁྲག)", and pulmonary honeycomb (གཟུང་རདངས་བུ་ཚངས་ཅན།) caused by lung heat for a long time (Figure 1). The lungs are where "Baekan (བད་ཀན།)" exists, and the condition is milder in the summer and more severe in the winter, more comfortable during the day and more severe at night.

Most of the lung diseases recorded in "Jing Zhu Materia Medica" are lung fever diseases. In Tibetan medicine, lung fever is caused by external wind or excessive inhalation of smoke and dust, or excessive consumption of spicy and thick flavors, resulting in an imbalance of the three major substances (Loong, Tripa, Baekan) of the body, which turn into three evils and offend the lung, and then turn into

TABLE 1 The most frequently medcines for treating pulmonary diseases in traditonal Tibetan medical system.

No.	Latin name	Tibetan name	Family	Types of plants	Vegetal products	Treated pulmonary diseases
1	*Rhodiola crenulata* (Hook. f. et Thoms.) H. Ohba	སྡེ་རམ་ར་ཐང་།	Crassulaceae	Herb	Rhizome, root, whole herb	Lung disease, lung-heat cough Jia and Zhang. (2016)
2	*Gentiana veitchiorum* Hemsl	སྨན་ལྗང་ནག་པོ།	Gentianaceae	Herb	Flower, root, rhizome	Lung heat, cough with lung heat Herbalism. (2002)
3	*Hippophae rhamnoides* L	སྐུར་པ།	Elaeagnaceae	Shrub	Dried ripe fruit or decoction and paste of fruit	Tracheitis Gema. (2016)
	Hippophae. thibetana Schlechtend					
	Hippophae rhamnoides subsp. *Sinensis* Rousi					
	Hippophae neurocarpa S. W. Liu et T. N. He					
4	*Rhododendron anthopogonoides* Maxim	དུ་ལི་ཤ།	Ericaceae	Shrub	Dried flower, leaf	Tracheitis, emphysema China. (1995)
	R. cephalanthum Franch					
	R. primulaeflarum Bur. Er Franch					
5	*Carthamus tinctorius* L	སྨན་ལྗང་ནག་པོ།	Comopsite	Herb	Flower	Pneumonia, pulmonary tuberculosis Herbalism. (2002)
6	*Aucklandia lappa* Decne	སྨན་ལྗང་ནག་པོ།	Comopsite	Herb	Dried root	Pneumonia Herbalism. (2002)
7	*Arctium lappa* L	བཟའ་ཁ་བཟང་།	Comopsite	Herb	Dried ripe fruit	Wind-heat type common cold Zhong and Song. (2020)
8	*Artemisia scoparia* Waldst. Et Kit	ཚོར་བ་ངར།	Comopsite	Herb	Whole plant	Lung heat Control. (1996)
9	*Aster tataricus* L. f	ལུ་གུ་སེ་གོ	Comopsite	herb	Flower Sequence	Influenza Jia and Zhang. (2016)
10	*Glycyrrhiza uralensis* Fisch	སྨན་ལྗང་ནག་པོ།	Leguminosae	Herb	Dried roots and rhizomes	Lung disease Dimaer. (2012)
11	*Santalum album* L	སྨན་ལྗང་ནག་པོ།	Santalaceae	Tree	Heartwood	Lung heat, pneumonia, lung abscess Yang. (1987)
12	*Terminalia chebula* Retz	སྨན་ལྗང་ནག་པོ།	Combretaceae	Tree	Dried and ripe fruit	Astringing lun for relieving cough Zhong and Song. (2020)
	Terminalia chebula Retz var. *tomentella* Kurz					
13	*Ephedra saxatilis* Royle ex Florin	སྨན་ལྗང་ནག་པོ།	Ephedraceae	Herb	Dried grassstem	Bronchial asthma Zhong and Song. (2020)
14	*Schisandra sphenanthera* Rehd. et Wils	སྨན་ལྗང་ནག་པོ།	Magnoliaceae	Vine	Dried ripe fruit	Maintenance of lungs Zhong and Song. (2020)
15	*Houttuynia* cordata Thunb	སྨན་ལྗང་ནག་པོ།	Saururaceae	Herb	Fresh whole grass or dried ground parts	Abscess of lung, pneumonia, pulmonary tuberculosis Luo. (1997)
16	*Lithospermum erythrorhizon* Sieb. et Zucc	འཇའ་རུ་མེ་གོ	Boraginaceae	Herb	Dried root	Abscess of lung, cough with lung heat Ma. (2012)
17	*Oxytropis falcata* Bunge	སྨན་ཁ་སྐག་ཤ།	Leguminosae	Herb	Dried flower	Lung heat Control. (1996)
18	*Delphinium trichophorum* Franch	གཁ་བྱ་རུ་ཀྱི་ཁ་ལྗང་།	Ranunculaceae	Herb	Aboveground part	Pandemic fever, cough with lung heat Jia and Zhang. (2016)
19	*Hypecoum erectum* L	པདཀ་པ་ཏ།	Papaveraceae	Herb	Whole palnt	Cough from pneumonia Editorial Committee of Tibetan Medicine Journal Chinese Academy of Sciences. (1991)

(Continued on following page)

TABLE 1 (*Continued*) The most frequently medcines for treating pulmonary diseases in traditonal Tibetan medical system.

No.	Latin name	Tibetan name	Family	Types of plants	Vegetal products	Treated pulmonary diseases
20	*Meconopsis punicea* Maxim	ཨཉིལ་དམར་པོ།	Papaveraceae	Herb	Whole palnt	Pneumonia, lung heat Luo. (1997)
21	*Foeniculum vulgare* Mill	ཐྲ་རྒན་པོ།	Umbelliferae	Herb	Fwhole plant	Lung heat, pneumonia, pulmonary tuberculosis Yang. (1987)
22	*Asparagus cochinchinensis* (Lour.) Merr	ལུ་གསོག	Liliaceae	Herb	Tuber	Atrophic lung disease, abscess of lung Jia and Zhang. (2016)
23	*Fritillaria cirrhosa* D. Don; *Fritillaria unibracteata* Hsiao et K. C. Hsia	ཨ་བའུ།	Liliaceae	Herb	Dried bulb	Tracheitis, cough with lung heat Jia. (2005)
	Fritillaria przewalskii Maxim					
	Fritillaria delavayi Franch					
24	*Lycium barbarum* L	འཕང་སྐྱི།	Solanaceae	Tree	Velamen	Pulmonary tuberculosis Jia and Zhang. (2016)
25	*Vitex trifolia* L	_	Verbenaceae	Shrub	Fruit	Pulmonary crests, Pneumonia Jia and Zhang. (2016)
26	*Fagopyrum dibotrys* (D. Don) Hara	བུར་བོ།	Polygonaceae	Herb	Dried rhizome	Lung cancer Jia. (2005)
27	*Geranium wilfordii* Maxim	གལ་ས་ཁང་།	Geraniaceae	Herb	Aboveground parts	Lung disease Zhong and Song. (2020)
28	*Gymnadenia conopsea* (L.) R. Br	དབང་ལག	Orchidaceae	Herb	Dried tuber	Lung disease, pulmonary cough and wheeze (Wang. (2004)
29	*Tinospora cordifolia* Miers	སྐ་ཏ་རུ་ས།	Menispermaceae	Vine	Rattan	Lung disease Wang. (2004)
30	*Vitis amurensis* Rupr	སྐ་ཏ་རུ་ས།	Vitaceae	Vine	Fruit	Pneumonia, cough of pulmonary furuncles, lung heat, lung disease Jia and Zhang. (2016)
31	*Usnea longissima* Ach	སྐ་ཏ་རུ་ས།	Usneaceae		Dried thallus	Lung heat Zhong and Song. (2020)
	Usnea diffracta Vain					
32	*Tremella fuciformis* berk	སྐ་ཏ་རུ་ས།	Tremellaceae		Subentity	Cough due to deficiency of the lung; dry pulmonaray cough; atrophic lung disease Luo. (1997)
33	*Cordyceps sinensis* (Berk.) Sacc	སྐ་ཏ་རུ་ས།			The fungus of the ergot family Cordyceps sinensis parasitizes on the dried complex of the larval larvae and carcasses of the Batmoth family insects	Lung disease, bronchitis; pulmonary tuberculosis, pulmonary tuberculosis Jia and Zhang. (2016)

heat in the lung, or excessive internal heat. Furthermore, because of the wind and cold, the hair orifices are closed and the fire and heat cannot escape through the hair orifices, resulting in the heat becoming more and more intense and burning the lung qi, causing the disease (Yang, 2013). Tibetan medicine has a unique understanding and insight into modern lung diseases. According to TTM theory, as the body is disturbed by external factors such as season, diet and living, the three factors in the body, Loong, Tripa, Baekan becomes out of balance, and the function becomes biased, which causes the COVID-19 (Liu et al., 2020). Chronic obstructive pulmonary disease is called pulmonary "tiebu" disease (གཏེ་བུ་ནད་ཟེར་པོ།) in Tibetan medicine system, which is a kind of lung disease caused by the invasion of "Baekan (བད་ཀན།)" into the lung. The symptoms include vomiting a large amount of green turbid sputum, shortness of breath, heaviness and fullness of the upper body, and a little peace after the sputum is released (Ren and Li, 2015).

3.2 Collation and analysis of natural Tibetan medicines

This paper recorded the use of 586 natural Botanical Tibetan medicines in the traditional system of Tibetan medicine for the treatment of various lung diseases [see Supplementary Material]. The scientific names, families, medicinal parts, diseases treated, and reported biological activities of some natural medicines are given in Table 1 and Table 2. These medicines are distributed in 77 families. The most common families were: Rosaceae (8.6%), Composite (7.7%), Ericaceae (5.3%), Leguminosae (5.3%), Gentianaceae (4.8%), Papaveraceae (4.4%), Liliaceae (3.9%), Cruciferae (3.6%), Labiatae (2.7%), Ranunculaceae (2.6%) (Figure 2). Moreover, herbs (73.7%) were the main source of these natural Tibetan medicines, followed by trees and shrubs (11.4%, 10.6%), animals (4.8%), vines 2.7%, lichens and fungi (1.0%, 0.5%) (Figure 3). Among the various

TABLE 2 Biological activities of medcines in the traditonal Tibetan medical system.

NO.	Name	Biological activities	Bioactive components
1	*Rhodiola crenulata* (Hook. f. et Thoms.) H. Ohba	Protecting against lung injury in COPD mice Carozzi et al. (2017); Zhang et al. (2019), Anti-lung cancer Zhang et al. (2013a); Huang et al. (2023), acute lung injury Liu et al. (2017a); Huang et al. (2017), protective effect of hyperoxia-induced lung injury Zhu et al. (2021a), protective effect against intermittent hypoxia (IH)-induced lung injury Wu et al. (2019), protective effect on lung tissue damage in rats with acute plateau advancement Wang et al. (2022c), improves the inflammatory response of lung tissue in rats with severe pneumonia Wang et al. (2021a), inhibits emphysema development in mice Tang. (2022)	Salidroside
2	*Gentiana veitchiorum* Hemsl	Inhibiting the migration of lung cancer A-549 cells (Chen et al. (2018), therapeutic effects on pulmonary fibrosis (Zheng. (2010), anti-pulmonary fibrosis Shi et al. (2016)	Gentiopicroside
3	*Hippophae rhamnoides* L., *Hippophae. thibetana* Schlechtend, *Hippophae rhamnoides* subsp. *Sinensis* Rousi, *Hippophae neurocarpa* S. W. Liu et T. N. He	Inhibition of proliferation of A549 cell line Jia et al. (2020), and treatment of chronic bronchitis Ren. (2019)	Total flavonoids of sea buckthorn
4	*Rhododendron anthopogonoides* Maxim., *R. cephalanthum* Franch., *R. primulaeflarum* Bur. Er Franch	Inhibitory effect on the growth of Stretococcus pneumoniae College. (1973a), cough suppressing, expectorant and asthma calming effects (College. (1973b); Hu. (2015)	Hyperoside, 4-phenyl-2-butanone
5	*Carthamus tinctorius* L	Anti-pulmonary fibrosis Luan et al. (2019); Luan et al. (2021), anti-pulmonary fibrosis and lung cancer Qiao et al. (2020), improvement of lung function in COPD rats Bao et al. (2021), anti-acute lung injury rats (Wang. (2020)	SYA, safflower yellow, HSYA, AHSYB
6	*Aucklandia lappa* Decne	Anti-lung cancer Hao et al. (2010); Qiao et al. (2020)	Parthenolide, dehydrocostus lactone, costunolide
7	*Arctium lappa* L	Inhibiting neuronal apoptosis in rats with pneumococcal meningitis (Leng and Li. (2022), anti-lung cancer (Miao et al. (2021), inhibiting inflammation in lung tissue and growth of H460 lung cancer cells Yang et al. (2005); Wang. (2014); Kang et al. (2017), preventing and treating acute lung injury Zhang et al. (2010), anti-lung cancer activity Takasaki et al. (2000)	ATG, chlorogenic acid
8	*Artemisia scoparia* Waldst. Et Kit	Protective effects on human lung epithelial cell A549 induced by RSV Ma et al. (2021), treating acute lung injury (Wang et al. (2018)	ASTF
9	*Aster tataricus* L. f	Alleviating the pathological damage caused by *Mycoplasma* pneumoniae infection in mouse lung tissue Wang et al. (2022d), improving the inflammation of lung tissue in young SD rats with asthma Ai and Li. (2022), inhibiting the proliferation and invasion of lung cancer cells Yao et al. (2022)	Asterone
10	*Glycyrrhiza uralensis* Fisch	Anti-cough and expectorant, improvement of lung function Wang et al. (2021a), anti-pneumonia Xie et al. (2009); Xie and Guan. (2009), anti-acute lung injury in mice Furusawa et al. (2009); Chu et al. (2012); Ren et al. (2016), inhibiting apoptosis Tang et al. (2007); Wang et al. (2012), protective effect against acute lung injury Li. (2018b); Chen. (2019), inhibiting collagen fiber synthesis and fibroblast proliferation (He et al. (2006); He et al. (2008); He et al. (2009), anti-pulmonary fibrosis Ye et al. (2007), anti-acute radiation lung injury Yang. (2014), anti-pulmonary fibrosis Li. (2018a), reducing chronic lung injury Bu et al. (2010), inducing apoptosis in non-small cell lung cancer cells Oh et al. (2019)	Licochalcone B, liquiritigenin, isoliquiritin, liquiritin, glycyrrhizin, monoammonium glycyrrhizinate, glycyrrhetic acid, diammonium glycyrrhizinate, Licochalcone A

(Continued on following page)

TABLE 2 (*Continued*) Biological activities of medcines in the traditonal Tibetan medical system.

NO.	Name	Biological activities	Bioactive components
11	*Santalum album* L	Toxic effects on A549 human lung adenocarcinoma cells MATSUO and MIMAKI. (2020)	(7R,8R)-5-O-demethylbilagrewin, bilagrewin
12	*Terminalia chebula* Retz., *Terminalia chebula* Retz var. *tomentella* Kurz	Anti-actinomyces pneumoniae Kang et al. (2014), inhibition of lung cancer A549 cell proliferation Lin et al. (2011); Bao et al. (2012); Tian et al. (2015); Ravi et al. (2016)	
13	*Ephedra saxatilis* Royle ex Florin	Treating lung tissue damage caused by influenza A virus Geng. (2019); Deng et al. (2020), alleviating lung injury and inflammatory reaction caused by viral infection Xiaoting et al. (2020), anti-lung cancer Shao et al. (2021), reducing lung coefficient in rats with pulmonary fibrosis Gao. (2010)	Pseudoephedrine, ephedrine, ESP-B4
14	*Schisandra sphenanthera* Rehd. et Wils	Inhibiting the process of pulmonary fibrosis Wei et al. (2017), inhibiting the growth of pneumonia strain (CV6) Hakala et al. (2015), reducing inflammatory damage in lung tissue Sun et al. (2021), treating acute lung inflammation Bae et al. (2012)	Schisandrin B, schisandra chinensis lignans
15	*Houttuynia* cordata Thunb	Anti-pulmonary fibrosis (PF) Zhu et al. (2021b), alleviating lung inflammatory injury Xu et al. (2015)	Polysaccharides
16	*Lithospermum erythrorhizon* Sieb. et Zucc	Radiosensitization effect on Lewis lung cancer Luo et al. (2004)	Shikonin
17	*Oxytropis falcata* Bunge	Inhibitory effect on idiopathic pulmonary fibrosis (Zhang et al. (2022a)	TFOFB
18	*Delphinium trichophorum* Franch	Alleviating pulmonary fibrosis Arunachalam et al. (2022)	Diterpenoid alkaloids
19	*Hypecoum erectum* L	Alleviating structural changes in lung tissue Zhang et al. (2016b), anti-SARS-CoV-2 effects Zhong et al. (2021)	Hypecorinine, hyperectine, leptocarpinine
20	*Meconopsis punicea* Maxim	95% ethanol extract from it can significantly improve lung injury Huang. (2016)	-
21	*Foeniculum vulgare* Mill	Protecting against acute Lung Injury Lee et al. (2015)	-
22	*Asparagus cochinchinensis* (Lour.) Merr	Inhibitory effect on Lewis lung cancer Li et al. (2000)	ACP
23	*Fritillaria cirrhosa* D. Don;*Fritillaria unibracteata* Hsiao et K. C. Hsia, *Fritillaria przewalskii* Maxim., *Fritillaria delavayi* Franch	Inhibitory effect on A549 cell Liu et al. (2023)	-
24	*Lycium barbarum* L	Inhibiting pulmonary inflammatory response Li et al. (2018), regulating the expression of apoptosis related proteins and proto-oncogenes in human embryonic lung fibroblasts induced by beryllium sulfate Wang et al. (2017), inhibiting the development of pulmonary fibrosis Liu et al. (2016), inhibiting the proliferation of human lung adenocarcinoma cells (A549) Wang et al. (2016)	LBP, lyciyoui-sides
25	*Vitex trifolia* L	Inhibiting the proliferation and promoting apoptosis of human NSCLC line H322 Li et al. (2020b), inhibiting the growth and inducing apoptosis of human lung cancer A549 cells Hu. (2015), inhibiting human small cell lung cancer NCI-H446 cell line lung cancer stem cells Cao. (2014), inhibiting lung cancer Lu1 cells D ıaz et al. (2003)	Vitexicarpin, FVTF, polymethoxy flavones
26	*Fagopyrum dibotrys* (D. Don) Hara	Anti-pneumonia effect Liu. (2023), anti-mycoplasma pneumoniae Liu. (2022)	Luteolin
27	*Geranium wilfordii* Maxim	Protective effect on acute lung injury Wang et al. (2022a), inhibiting the proliferation of human lung cancer cell line A549 Li et al. (2016c), inhibitory effect on pneumonia pathogen Wang et al. (2013),	Geraniin

(Continued on following page)

TABLE 2 (*Continued*) Biological activities of medcines in the traditonal Tibetan medical system.

NO.	Name	Biological activities	Bioactive components
		cytotoxic activity on lung cancer cells (A549) Li et al. (2013)	
28	*Gymnadenia conopsea* (L.) R. Br	Inhibiting silicosis progress during early exposure periods Shang et al. (2017)	-
29	*Tinospora cordifolia* Miers	Cytotoxic to A549 (Mittal et al. (2020)	-
30	*Vitis amurensis* Rupr	Inhibitory effect on the proliferation of lung cancer cells in C57BL/6 mice (Lee et al. (2006), inhibitory effect on the proliferation of A549 cells Diao. (2019), decreaseing OVA-induced lung tissue damage and mucus production in sensitized mice Li et al. (2006)	Heyneanol A, 3α-angloyloxypterokaurene L3
31	*Usnea longissima* Ach., *Usnea diffracta* Vain	Inhibitory effect on acute lung injury, protective effect on pulmonary fibrosis in mice (Su. (2015), inhibitory effect on A549 cells Guan. (2020)	Usnic acid
32	*Tremella fuciformis* berk	Inhibiting cellular apoptosis and autophagy in A549 cells Shi et al. (2018)	Tremella polysaccharides
33	*Cordyceps sinensis* (Berk.) Sacc	Inhibiting airway inflammation and improving lung function in rats with COPD (Guan and Liu. (2008), inhibiting and preventing the occurrence of pulmonary fibrosis Yang et al. (2008). Anti-non-small cell lung cancer Ji et al. (2011)	-

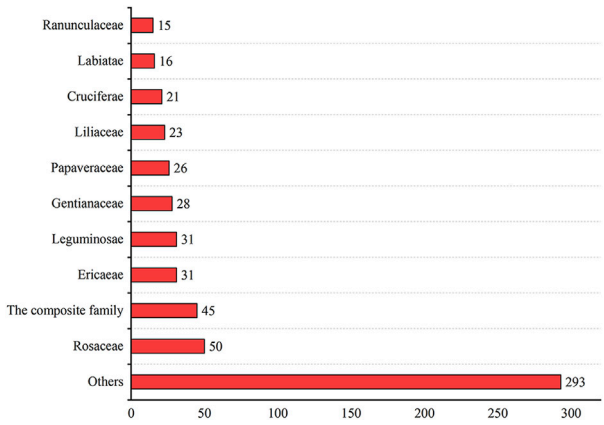

FIGURE 2
The most common families and genera of Tibetan medicine in the treatment of pulmonary diseases.

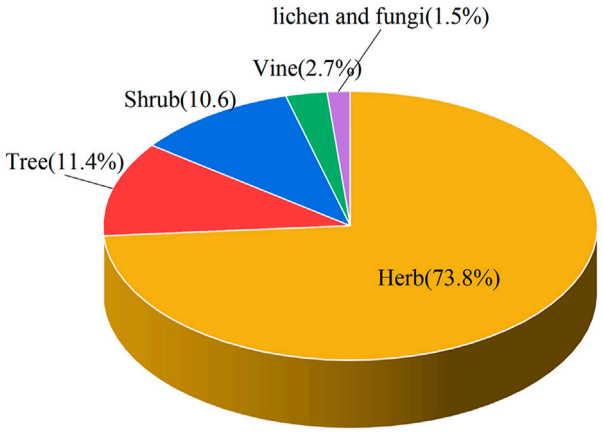

FIGURE 3
The most common source of Tibetan medicine for pulmonary diseases.

vegetal products, the whole plant was used most frequently (30.1%), followed by 17.8% for multipart, 11.1% for stems (including herbaceous stems, rhizomes, tubers, bulbs, *etc.*), flowers and roots (10.6%, 7.5%), fruits and above-ground parts (6.5%, 5.1%), leaves (2.6%), seeds and inflorescences (1.7%, 1.5%) (Figure 4).

In this study, we found that these natural Tibetan medicines were documented to treat a variety of lung diseases, such as lung heat, pneumonia, pulmonary tuberculosis, and lung abscess. Among them, 182 (31.1%) Tibetan medicines could treat more than one type of lung disease, and 126 (21.5%) were not indicated for specific lung diseases. There are 120 (20.5%) Tibetan medicines for lung fever, 46 (7.8%) for pneumonia, and 24 (4.1%) for bronchitis. Plants of the Gentianaceae family were mostly used to treat lung fever, and some plants could also treat pneumonia and lung dryness. Orchids were mostly used to treat lung deficiency. Two plants (*Fagopyrum dibotrys* (D. Don) Hara, *Fagopyrum esculentum* Moench) were indicated to treat lung cancer. Of the 586 Tibetan medicines traditionally used in the treatment of lung diseases, only 7.2% have been experimentally demonstrated to possess various biological and pharmacological activities related to lung diseases (Table 2), such as anti-pneumonia, pulmonary fibrosis, lung cancer, and acute lung injury effects. These findings demonstrated the effectiveness of these species traditionally used to treat lung diseases. However, to date, 544 (92.8%) drugs lacked modern experimental evidence. Therefore, it is necessary for us to conduct more in-depth research on Tibetan medicines to give full play to their efficacy in treating lung diseases.

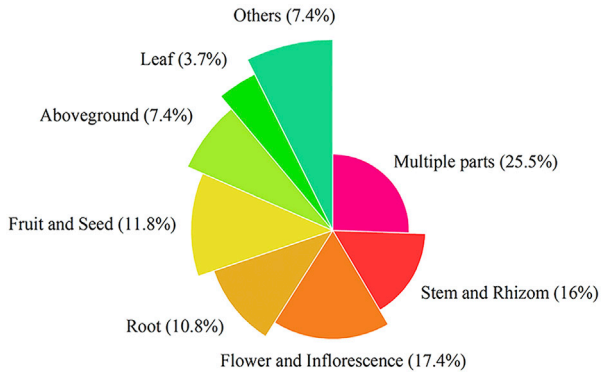

FIGURE 4
The most common vegetal products used of Tibetan medicine for pulmonary diseases.

In the following section, four commonly used Tibetan medicines (Figure 5) for lung diseases and the five Tibetan medicines with the highest frequency of use in compound formulas were introduced in detail, including their names, base origins, uses, metabolites (Figure 8), and pharmacological effects. It hopes that these results can provide a basis for screening new drugs for lung diseases, provide directions and ideas for further research, and promote their global application.

3.3 Commonly used Tibetan medicines for lung diseases

3.3.1 *Rhodiola crenulata* (Hook. f. et Thoms.) H. Ohba

Suo-luo-ma-bao (Tibetan: ས་ཅོ་ན་སྨན་བཏགས།) is one of the most representative Tibetan medicine. It is called Hong Jingtian in Chinese and rhodiola in English. The medicinal parts are the dried roots and rhizomes of *Rhodiola crenulata*. In "Jing Zhu Materia Medica", it was written that Suo-luo purifies lung heat and was divided into three kinds: white, purple, and red, of which the red one was Suo-luo-ma-bao. The "Wendaohe" said: "Solomabao grows in high mountains, rocky mountains, and other places; wherever it is born, the stems are red, harder, most, thick leaves, clusters, turning red in autumn; flowers, fruit pods, seeds are red; roots like human lung color; thick skin, big smell; taste sweet, bitter, astringent, cool; effects nourish the lungs, clear heat, nourish the vital energy." Rhodiola is rich in chemical components, mainly containing tyrosol and its glycosides, flavonoid components, cyanogenic glycosides, coumarins, and so on. Among them, the active ingredient specified in the pharmacopeia is salidroside.

As a commonly used Tibetan medicine for the treatment of lung diseases, there are many pharmacological and clinical studies on rhodiola and its extract salidroside. In recent years, it has been found that rhodiola has various pharmacological activities for the treatment of chronic obstructive pulmonary disease, anti-lung cancer, pulmonary fibrosis, lung injury, *etc. In vitro* studies found that salidroside could protect the *in vitro* cellular model of chronic obstructive pulmonary disease through antioxidant mechanism (Hou et al., 2020). *In vivo* studies have found that salidroside may inhibit NF-κB signaling and reduce pIκBα and p-NF-κBp65 protein expression in the lungs of COPD mice, thus effectively protecting against lung injury in COPD mice (Carozzi et al., 2017). The study by Zhang et al. confirmed that salidroside effectively reduced the protein expression of myostatin, increased the protein expression of myogenin and p-akt protein content, increased cytochrome C oxidase and ATP synthase activities, and enhanced mitochondrial energy metabolism in rats with COPD, thereby improving skeletal muscle dysfunction in rats with COPD, further explaining the rationale for the effect of rhodiogenic on alleviating symptoms in COPD patients (Zhang et al., 2019). Recent clinical studies have confirmed that rhodiola combined with Western medicine for COPD can alleviate symptoms and improve prognosis to some extent. Clinical observation by Li L et al. confirmed that rhodiola injection combined with simvastatin could better improve patients' pulmonary function, arterial blood gas performance, effectively alleviate symptoms, and reduce inflammatory response (Li et al., 2016a). According to a clinical study by Zhao., ligustrazine injection combined with radix injection of large strains improved clinical symptoms and pulmonary function and reduced pulmonary artery systolic pressure better than using atorvastatin calcium tablets (Zhao, 2015). According to Zhang et al., patients treated with rhodiola combined with Western medicine showed a shorter time taken to recover from symptoms such as wheezing, edema, and impaired consciousness, with significant improvement in pulmonary function and APACHE II scores and no significant adverse effects (Zhang, H.Y. et al., 2014). In a study by Qu et al., it was demonstrated that rhodiola combined with conventional Western medical treatment regimens could greatly improve the oxygenation status and symptoms of patients with acute exacerbations of COPD (Qu et al., 2014).

Numerous studies have confirmed that rhodiola extract and salidroside exert anti-lung cancer effects through multiple mechanisms of action (Figure 6). *In vitro* studies have shown that rhodiola extract may inhibit lung cancer cell proliferation, migration, epithelial-mesenchymal transition (EMT), and promote apoptosis by activating the TGF-β/Smads signaling pathway, upregulating E-cadherin and Caspase-3 expression, and inhibiting Vimentin and Bcl-2 expression related (Huang et al., 2023). In addition, Zhu et al. found that salidroside inhibits the proliferation and metastasis of lung cancer cells also by regulating target genes of aberrant microRNAs (miR103–3p/Mzb1) (Zhu et al., 2020). Wang, (2022) found that salidroside played an inhibitory role in the proliferation and migration of human lung cancer A549 cells through AMPK-dependent inhibition of ROS-NLRP3 inflammatory vesicle signaling. Salidroside block AKT and MEK/ERK signaling pathways by upregulating miR-549 expression in A195 cells, thereby inhibiting the proliferation, migration, and invasion of Non-Small-Cell Lung Cancer (NSCLC) cells (Ren et al., 2019). Zhang et al. showed in experimental Lewis lung cancer mice that rhodiola extract might enhance the antitumor immune response by down-regulating the proportion of $CD4^+$ $CD25^+$ Tregs and the mRNA expressions of Foxp3 and TGF-β in the tumor tissues (Zhang et al., 2013a; Zhang et al., 2013b). Li et al. found that salidroside inhibited the growth of human lung cancer A549 cell nude mice transplanted tumors, disrupted tumor cell structure, and promoted apoptosis of tumor cells, presumably by a mechanism that may be related to the inhibition of MAPK/ERK1/2 signaling pathway, upregulation of

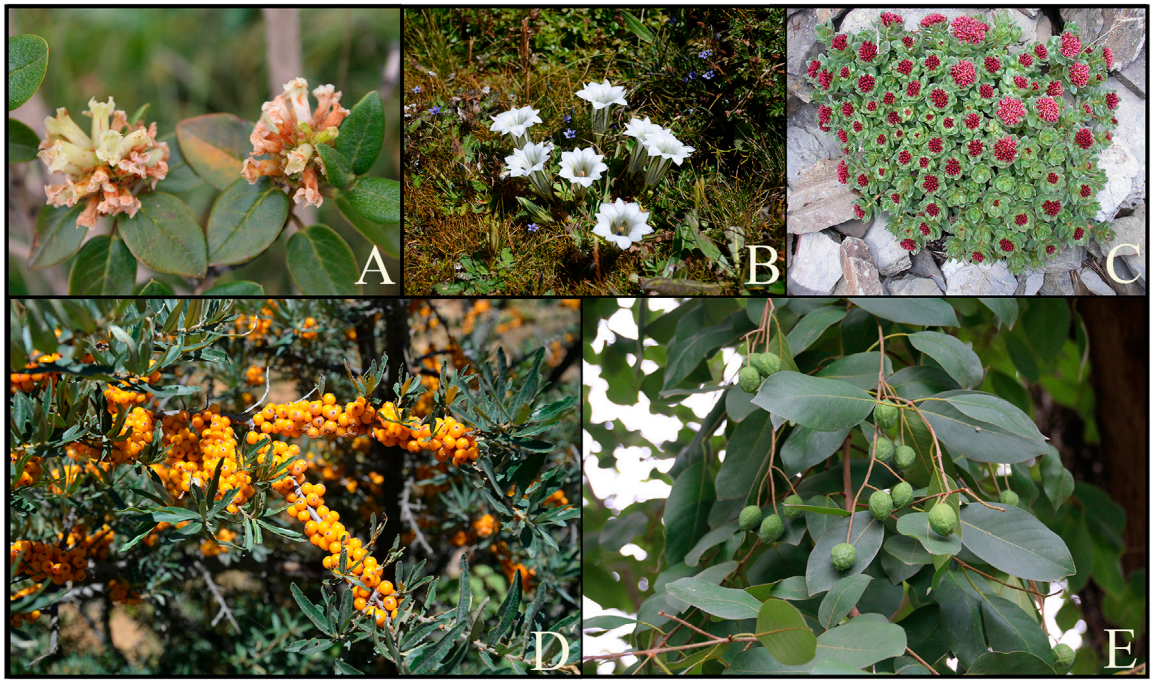

FIGURE 5
The most commonly used medicinal plants in the Tibetan medicine system to treat lung diseases. **(A)** *Rhododendron anthopogonoides* Maxim., **(B)** *Gentiana. szechenyii* Kanitz., **(C)** *Rhodiola crenulata* (Hook. f. et Thoms.) H. Ohba, **(D)** *Hippophae rhamnoides* subsp. *Sinensis* Rousi, **(E)***Terminaalia chebula Retz.*

Bax mRNA and protein expression levels, downregulation of cyclinD1, c-Myc, Bcl-2 mRNA and protein expression levels, and reduction of p-ERK1/2 levels (Li et al., 2020a).

In recent years, there have been many studies on the mechanism of action of rhodiola against pulmonary fibrosis (Figure 7). *In vitro* studies revealed that salidroside induced the transformation of A549 cells to normal cells, reduced cell mortality, and inhibited the expression of the fibronectin EDA fragment (Zhang, Z.Y. et al., 2014a). *In vivo* studies are as follows. Zhang et al. demonstrated that rhodiola prevented bleomycin-induced pulmonary fibrosis in rats through anti-inflammatory, antioxidant, and anti-fibrotic properties (Zhang et al., 2016a). It has been shown (Li, 2015) that salidroside inhibited the bleomycin-induced increase in serum hydroxyproline and TGF-β1, prevented the bleomycin-induced decrease in pulmonary superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT), and reduced pulmonary IV collagen, malondialdehyde (MDA) and TGF-β1 content, thus improving pulmonary fibrosis in rats. It also improved the enzymatic activities of SOD and CAT, and regulated the balance of matrix metalloproteinases (MMPs) and MMP inhibitors (TIMPs), thus improving bleomycin-induced pulmonary fibrosis in rats (Li et al., 2016b). In addition, it has been shown that salidroside reduced serum hydroxyproline, collagen type IV and malondialdehyde levels in lung tissues of mice with bleomycin-induced pulmonary fibrosis, while TGF-β1 levels in serum and lung tissues were significantly reduced, and pulmonary solids and collagen deposition were alleviated (Liu et al., 2017a). Salidroside ameliorate CLP-induced pulmonary fibrosis in mice with acute lung injury from sepsis by downregulating p-JAK2 and p-STAT3 protein expression (Guo et al., 2022); also by regulating collagen-related protein (Huang et al., 2018). Zhang (2015) found that salidroside significantly reduced the expression of MMP-2 and TIMP-1 mRNA and protein ($p < 0.05$) and attenuated the pathological changes of paraquat-induced fibrosis in rats. It has been shown that salidroside can inhibit transforming growth factor β1 (TGF-β1)/Smad-2/-3 and cytosolic nuclear factor κB (NF-κB) pathways, activate Nrf2 antioxidant signaling pathway, and improve pulmonary fibrosis injury in rats (Tang et al., 2016), as well as improve symptoms of acute lung injury and stop the development of pulmonary fibrosis inhibit by inhibiting the expression of TGF-β1-encoded genes and inflammatory cell infiltration (Zhang et al., 2014b).

In vivo studies have shown that rhodiola and its extract salidroside have shown ameliorative effects on acute lung injury, hyperoxia-induced lung injury (Zhu et al., 2021a), intermittent hypoxia (IH)-induced lung injury (Wu et al., 2019), and acute progressive plateau lung injury (Wang et al., 2022a). Salidroside may improve lung function in mice with acute lung injury from CLP-induced sepsis by inhibiting caveolin-1 and TLR/NF-kappa B pathways *in vivo* inhibiting caveolin-1 and TLR/NF-kappa B pathways *in vivo* (Liu et al., 2017b). It has been shown that synthetic salidroside protects against acute lung damage in rats by inhibiting NF-κB protein phosphorylation in lung tissue and by reducing polymorphonuclear (PMN) aggregation in the lung (Huang et al., 2017). Salidroside is protective against hyperoxia-induced lung injury in mice, and their mechanism of action may be related to changes in the expression of the Notch signaling pathway (Zhu et al., 2021b). Rhodiola protects against IH-induced lung

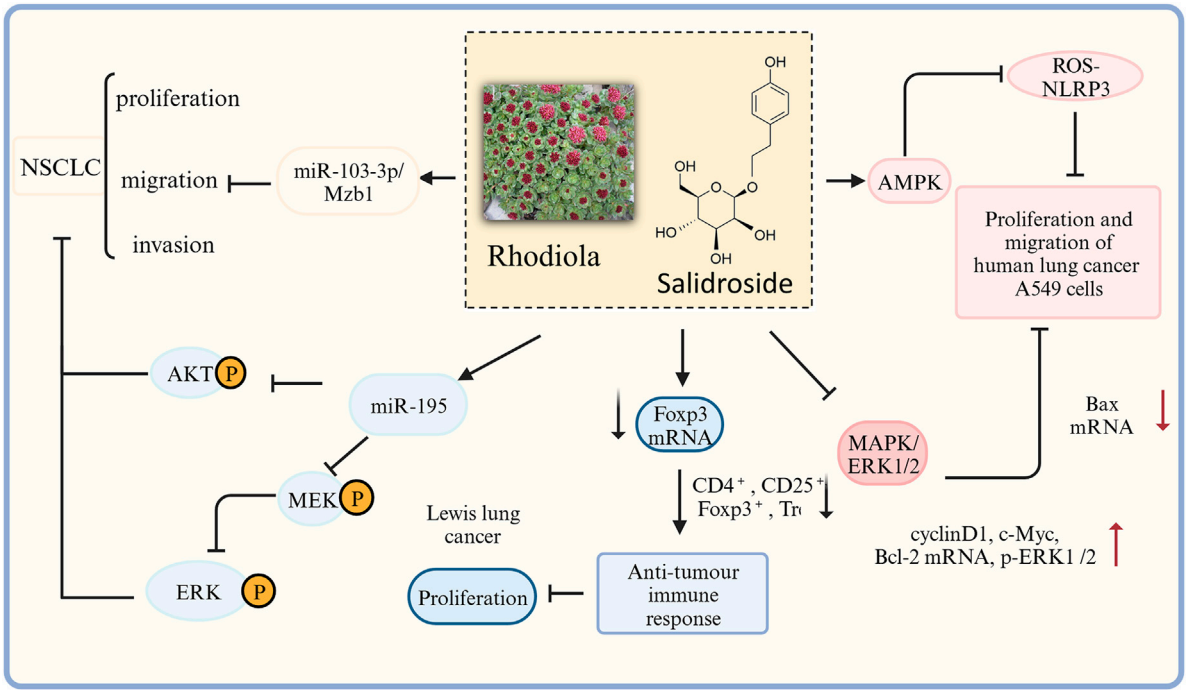

FIGURE 6
The therapeutic effects and potential mechanisms of salidroside on Lung cancer. Salidroside represses proliferation, migration and invasion of human lung cancer cells: through AKT, MEK/ERK, microRNA-103–3p/Mzb1 and MAPK/ERK1/2 signal pathway; salidroside inhibits the proliferation and migration of HUMAN lung cancer A549 cells through AMPK dependent inhibition of ROS-NLRP3 inflammasome signaling.

injury, probably through anti-oxidative stress, mitigation of hypoxic damage, and inhibition of inflammatory response pathways (Wu et al., 2019).

In vivo studies have shown that salidroside improved the inflammatory response of lung tissue in rats with severe pneumonia, reduced the release of inflammatory mediators and inflammatory cell infiltration in a dose-dependent manner, and its effects were associated with reduced levels of NF-κB and p38 signaling pathway activation (Wang et al., 2021a). A study by Zhao et al. explained the anti-inflammatory and immune-enhancing effects of rhodiola. Salidroside showed dose-dependent effects on reducing right heart hypertrophy index (RVHI), serum interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and p-NF-κB/NF-κB and p-p38MAPK/p38MAPK in lung tissues of mice with hypoxic pulmonary hypertension model, and improved cardiopulmonary function in mice (Zhao et al., 2022). Salidroside inhibited the development of emphysema in mice by suppressing cell scorching in lung tissues (Tang, 2022). A clinical study by Lei et al. reported that adjuvant treatment of patients with pulmonary embolism combined with heart failure using rhodiola injection improved the overall effective cure rate, reduced serum amino-terminal brain natriuretic peptide precursor (NT-proBNP) levels, and improved patients' blood gas indices and echocardiographic parameters (Lei et al., 2019). The clinical efficacy can be further improved by using rhodiola injection in addition to the conventional treatment plan for pulmonary embolism combined with heart failure. The above results suggested that rhodiola may be a candidate for the treatment of a variety of lung diseases.

Rhodiola contains multiple components, but currently only salidroside has been studied for its pharmacological effects, and preclinical studies on other components are needed. Preclinical and clinical studies have shown that rhodiola extract and salidroside can treat various lung diseases, including COPD, lung cancer, pulmonary fibrosis, lung injury, pneumonia, emphysema, *etc.* Rhodiola may have a good potential to develop into a candidate drug for the treatment of various lung diseases.

3.3.2 *Gentiana veitchiorum* Hemsl., *G.przewalskii* Maxim., *G. szechenyii* Kanitz., *G. algida* Pall., *G. stipitata* Edgew

Bang-jian (Tibetan: སྨན་ལུང་རྒྱས་པ།)is a common Tibetan medicine used to treat lung diseases. , which comes from a variety of vegetal (medicinal) species in the Gentian family. It is called gentian in English. *Gentiana veitchiorum*, *G. przewalskii*, *G. szechenyii* Kanitz, *G. algida*, *G. stipitata*. are the most commonly used vegetal (medicinal) species of Bang-jian. According to "Jing Zhu Materia Medica", there are three species of Bang-jian according to the color: white flower, blue flower and black flower, which are called " Bang-Jian Gabao", " Bang-Jian Wanbao" and " Bang-Jian Nabao" respectively; white flower gentian grows in the cold zone of high mountains, blooming in late autumn, without stems, with leaves similar to those of *Gentiana macrophylla*, with four to five flowers from the ground, with red luster, and the flowers are united at the base, or born in grassland on the mountain slope, with small leaves and flourishing flowers; blue flower gentian is born in very wet swampy grassland in early autumn, with a similar form as white flower gentian, with small leaves and obvious light blue flowers.

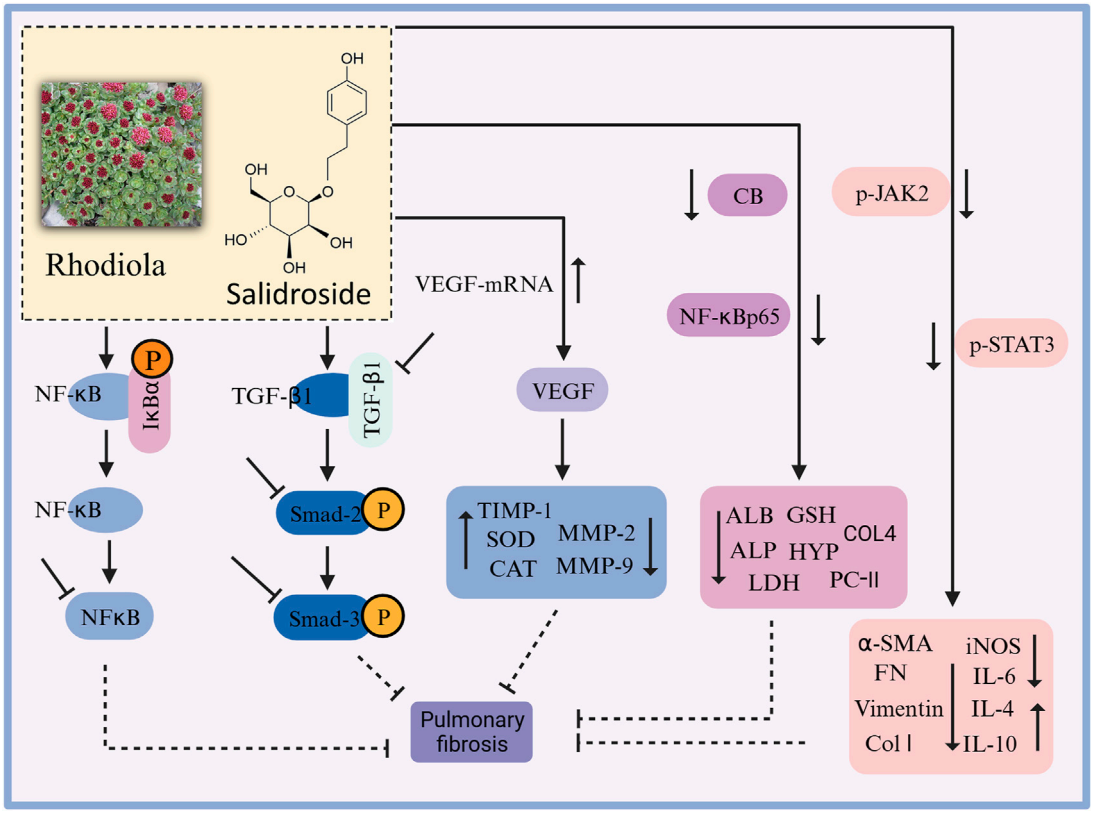

FIGURE 7
The therapeutic effects and potential mechanisms of salidroside on pulmonary fibrosis. Salidroside protects against pulmonary fibrosis: activation of Nrf2-antioxidant signaling, inhibition of NF-κB and TGF-β1/Smad-2/-3 pathways, upregulation of CEGF protein expression, and downregulation of CB, NF-κBp65, p-JAK2, and p-STAT3 protein expressions.

Ranjun duoji said: "Bang-Jian Ga Bao clear lung heat, blue flower Bang-Jian with the same effect and cool nature". Among these five vegetal (medicinal) species, the first two are blue-flowered Bang-jian and the last three are white-flowered Bang-Jian. The dried above-ground parts with flowers are used in the medicine. It is bitter and cool in nature and is used to treat fever, red eyes and sore throat, lung fever and cough, gastritis, meningitis, bronchitis, *etc.*

Fu (2018) divided the mainstream species into two types: among them, type I was represented by *G. szechenyii,* which had little components such as swertiamarin, gentiopicroside, and sweroside while contained gentiournosides D, depressine, gentizechenlioside A and szechenyin A. Type II are mainly from *G. algida*, *G. purdomii* and others containing no iridoid moiety of the benzoyl moiety, but components such as swertiamarin, gentiopicrin, and sweroside were present, and the content of these components in type II is generally higher, and its surrounding chemical composition was more. Flavonoids such as isoorientin, isoscoparin-2″-*β*-*D*-glucopyranoside and isoscoparin were found in both chemical types, and their contents were generally higher in type II.

At present, only *G. veitchiorum* and its extract gentiopicroside have been studied in modern pharmacology. In a vitro experiment, TGF-β was found to stimulate the inhibited epithelial-mesenchymal transition of lung cancer A-549 cells in a dose-dependent manner, and gentiopicroside significantly downregulated the expression of TGF-β and connective tissue growth factor in mouse lung tissue. Therefore, gentiopicroside inhibits the transformation of lung cancer A-549 cells. On the other hand, gentiopicroside has anti-inflammatory and anti-fibrotic effects, in which alveolar epithelial cells and TGF-β may be the main target cells and molecules of gentiopicroside for the treatment of bleomycin-induced pulmonary fibrosis, respectively, and therefore, it may be an ideal candidate for the treatment of pulmonary fibrosis (Chen et al., 2018). By comparing the therapeutic effects of different polar components on mouse chronic bronchitis model and pulmonary fibrosis cell model, effective components of gentian extract for treating chronic bronchitis and pulmonary fibrosis were screened out (Geng, 2010). The results showed that the ethyl acetate and n-butanol extracts of *G. veitchiorum* extracts were effective in the treatment of pulmonary fibrosis. In addition, the blue yuzan granules made from *Gentiana veitchiorumas* the main fraction and Panax ginseng total saponin as a supplement were effective in treating pulmonary fibrosis caused by acute and chronic respiratory system inflammation (Shi et al., 2016). Hou et al. reported that blue yuzan granules may affect the expression of TNF-α through the ERK pathway, thus achieving an inhibitory effect on inflammation, providing a further experimental basis for the mechanism of action of blue yuzan granules in the treatment of chronic bronchitis (Hou et al., 2011).

Gentian originates from various vegetal (medicinal) species and contains numerous metabolites, but only gentiopicroside has been proven to have anti-lung cancer and therapeutic effects on

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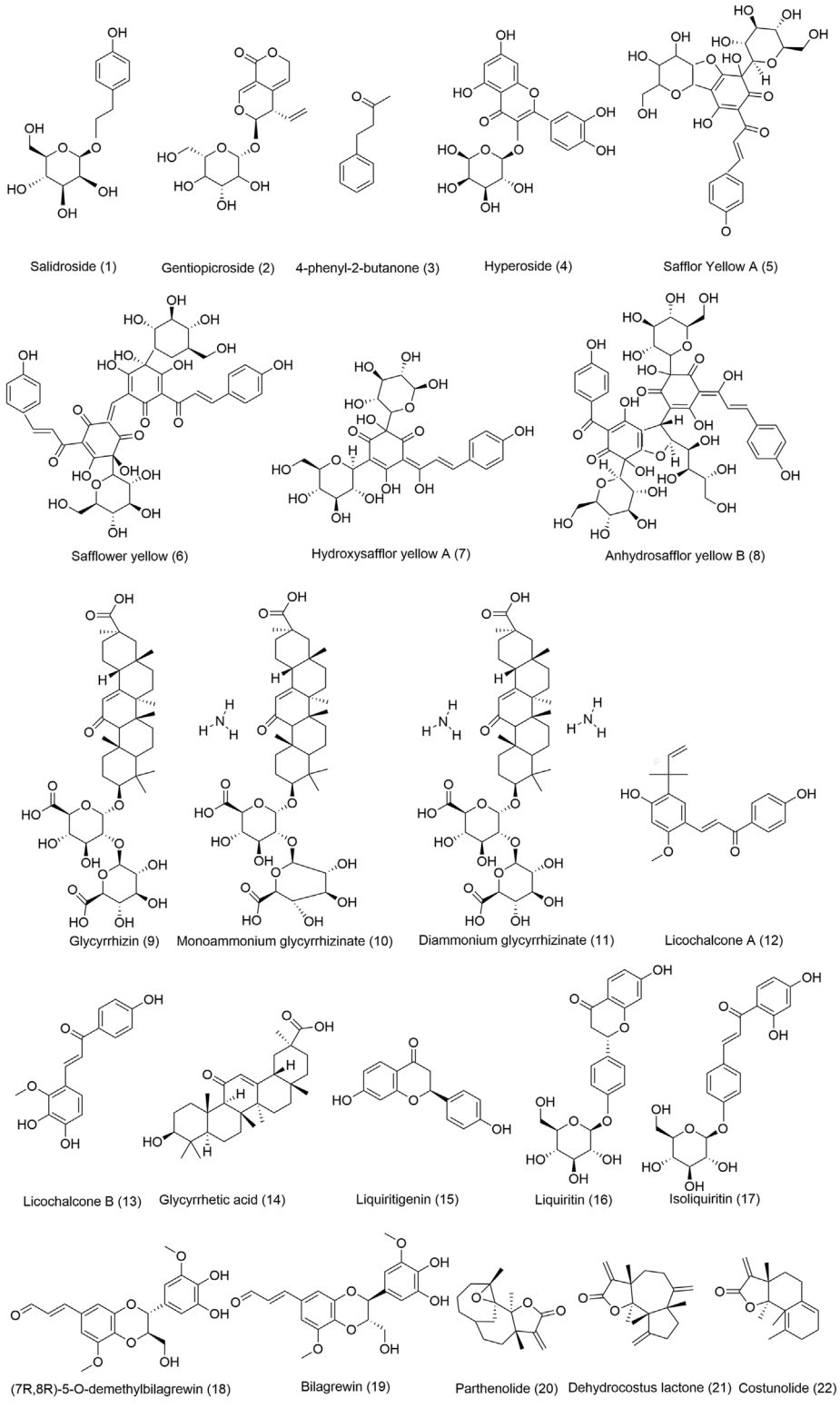

FIGURE 8
Chemical structures of the main TTM bioactive components with anti-lung diseases. (1) Chemical composition comes from rhodiola, (2) Chemical composition comes from *Gentiana veitchiorum,* (3-4) Chemical compositions come from *Rhododendron anthopogonoides,* (5-8) Chemical compositions come from *Carthamus tinctorius* L., (9-17) Chemical compositions come from *Glycyrrhiza uralensis* Fisch, (18-19) Chemical compositions come from *Santalum album* L., (20-22) Chemical compositions come from *Aucklandia lappa* Decne.

pulmonary fibrosis. The crude extracts of *G. veitchiorumas* and blue yuzan granules can treat chronic bronchitis. Further in-depth and comprehensive research is required on the material basis and mechanism of its pharmacological effects.

3.3.3 *Hippophae rhamnoides* L., *Hippophae thibetana* Schlechtend, *Hippophae rhamnoides* subsp. Sinensis Rousi, *Hippophae neurocarpa* S. W. Liu et T. N. He

Da-bu (Tibetan: ཟ་བྲག་པུ།)is called sea buckthorn in English, which is widely used in Tibetan medicine clinics. The most commonly used vegetal (medicinal) species of Tibetan medicine Da-bu include *Hippophae rhamnoides*, *Hippophae Thibetana*, *H. rhamnoides* subsp. Sinensis, and *Hippophae neurocarpa*. Drug Standards of Tibetan Medicine (Commission, 1995) recorded that sea buckthorn paste (Tibetan: ཟ་བྲག་པུ།)is a decoction of the mature fruit of *H. rhamnoides* L. in the family Elaeagnaceae. According to"Jing Zhu Materia Medica", Da-bu can remove lung tumors, transform blood, and treat "Baekan (བད་ཀན།) disease". Da-bu contains many chemical components such as glycosides, flavonoids, phenols, tannins, amino acids, polypeptides and proteins, and organic acids. Among them, total flavonoids and isorhamnetin are the active ingredients stipulated in the pharmacopeia.

Total flavonoids of sea buckthorn are the main pharmacological substance basis for its therapeutic effect. At present, scholars have studied the total flavonoids of sea buckthorn through *in vitro* experiments and network pharmacology, and found that total flavonoids of sea buckthorn have the effect of inhibiting the proliferation of lung cancer cells and treating chronic bronchitis. Jia C et al. investigated the effects of the total flavonoids of *Hippophae thibetana*, *H. rhamnoides*, and *H. neurocarpa* on the proliferation and migration of non-small cell lung cancer A549 cells, and explored their molecular mechanisms of action (Jia et al., 2020). Ren (2019) investigated the pharmacodynamics and mechanism of action of sea buckthorn total flavonoids in the treatment of chronic bronchitis and found that total flavonoids of sea buckthorn also significantly improved the LPS-induced reduction of body mass in mice and reduced the elevation of macrophage, neutrophil and total cell counts in alveolar lavage fluid, which had therapeutic effects on chronic bronchitis. The results of network pharmacology and molecular docking showed that quercetin, isorhamnetin, and kaempferol exerted their therapeutic effects on chronic bronchitis by blocking the Fc epsilon RI signaling pathway, mitogen-activated protein kinase signaling pathway, and vascular endothelial growth factor signaling pathway. Although sea buckthorn is a commonly used Tibetan medicine for treating lung diseases in the Tibetan medical system, current research on its treatment of lung diseases is not sufficient, especially in the absence of pharmacological studies on single pharmacodynamic components. More scholars are needed to devote themselves to the research of the Tibetan medicine sea buckthorn to promote the development of related new drugs.

3.3.4 *Rhododendron anthopogonoides* Maxim., *R. cephalanthum* Franch., *R. primulaeflarum* Bur. Er Franch

Da-li (Tibetan: ཏ་ལི་ས།)comes from a variety of vegetal (medicinal) species. Drug Standards of Tibetan Medicine (China, 1995) recorded that Da-li is the dried flowers and leaves of *Rhododendron anthopogonoides*, *R. cephalanthum*, *R. primulaeflarum*. It is said in the "Thirty Chapters of the Ming Explanation" that Da-li is warm and mild in nature, and is used to treat Baekan disease, painful lung disease, and eruptions. The "Illustrated Book" said: "Da-li born in the shade of high mountains. The trunk is white and the fruit tastes sweet, bitter and astringent. It is used to treat Loong disease, Tripa disease, Baekan disease, and lung disease". In 1971, it was introduced to various parts of China for the treatment of chronic bronchitis (Fan et al., 2016). Da-li contains volatile oils such as 4-phenyl-2-butanone, flavonoids such as hyperoside, and triterpenoids such as ursolic acid (Ji et al., 2002).

Notably, *R. anthopogonoides* and the phytochemicals or extracts obtained from *R. anthopogonoides* have been shown to have some pharmacological activities associated with lung diseases. As a commonly used Tibetan medicine for lung diseases, *R. anthopogonoides* has good efficacy in coughing and phlegm asthma, which are common symptoms of lung diseases. The research group of Lanzhou Medical College for the prevention and treatment of chronic bronchitis found that flavonoids in the ethanolic extract of *R. anthopogonoides* had an inhibitory effect on *Streptococcus* pneumonia (College, 1973a). *In vivo* experiments, *R. anthopogonoides* could reduce mucosal swelling, relax the trachea and bronchi, and accelerate the speed of mucus operation of tracheal cilia, thus having good expectorant and asthma-calming effects (College, 1973a). Li et al. demonstrated that 4-phenyl-2-butanone in the volatile oil of *R. anthopogonoides* has obvious cough suppressant, expectorant and asthmatic effects, which is a central cough suppressant, and its cough suppressant strength is slightly weaker than 1/6 dose of codeine (Li et al., 1980). The phenol red test in mice demonstrated the significant expectorant effect of flavonoids of *R. anthopogonoides* Maxim by intraperitoneal injection, gavage, spray, and intratracheal drip (College, 1973b). Zhang, (1979) found out through *in vivo* experiments that hyperoside, 4-phenyl-2-butanone, a chemical constituent in *R. anthopogonoides*, had cough suppressant effects. In addition, the clinical treatment of chronic tracheitis was clinically treated with anthorhododendrin, and the results showed that the expectorant effect was more prominent (College, 1973a). However, there are no reports of *R. cephalanthum*, *R. primulaeflarum* for the treatment of lung disease. Therefore, it is necessary to conduct phytochemical, pharmacological, and clinical studies on *R. cephalanthum*, *R. primulaeflarum* to verify their efficacy in the treatment of lung diseases and, at the same time, to lay the foundation for the development of new drugs for the treatment of lung diseases. Preclinical studies have shown that some components in the volatile oil of *R. anthopogonoides* have expectorant and asthma-calming effects. Further pharmacological studies can be conducted on other components in volatile oil to fully develop and utilize them.

3.4 The five most frequently used Tibetan medicines in Tibetan medicine prescriptions

3.4.1 *Carthamus tinctorius* L

The Tibetan name of *Carthamus tinctorius* L. is ཟ་ཧོ་ལོ་རགས་པ།, the Chinese name is Honghua, and the English name is

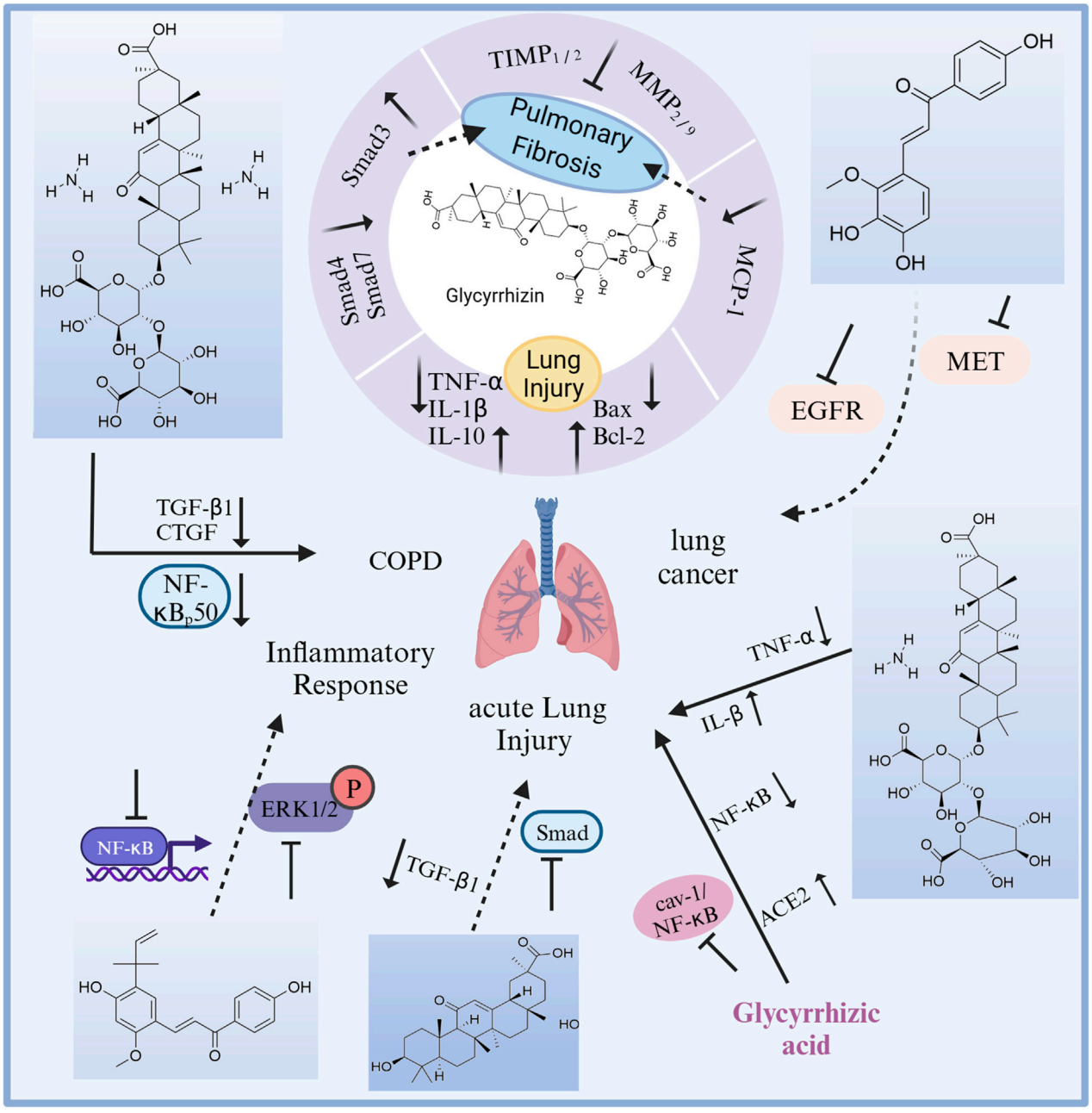

FIGURE 9
The therapeutic effects and potential mechanisms of chemicals from licorice on lung diseases. Glycyrrhizin protects against pulmonary fibrosis: reversing the abnormal expression of Smad family, and downregulation of MCP-1 expressions. Glycyrrhizin protects against lung injury: regulation on inflammation and cell apoptosis.

safflower. Safflower is spicy in taste and warm in nature and is used as a medicine for pneumonia and tuberculosis with dried flowers (Jia and Zhang, 2016). Safflower is native to the 'New Crescent' on the east coast of the Mediterranean and has been cultivated for over 4,500 years (Ren et al., 2017). It is recorded in the "Compendium of Materia Medica" that Zhang Qian introduced safflower to China during his mission to the West (via the Silk Road) during the Han Dynasty. As a result, safflower has been grown and used in China for more than 2000 years. Currently, safflower is widely grown in Asia, Europe, Australia, and America (Ren et al., 2022). Safflower contains a variety of flavonoids, such as safflor yellow A (SYA), safflower yellows B, hydroxysafflor yellow A (HSYA), cathamone, safflomin A, kaempferide, luteolin, etc (Zhong and Song, 2020). Among them, HSYA and kaempferide are the main bioactive prescriptions, which are often used as markers for the quality control of safflower in the pharmaceutical industry and drug standards.

In addition, there have been many reports on the pharmacological activity of safflower for the treatment of lung diseases. *In vitro* studies, safflower injection significantly inhibited proliferation migration and induced apoptosis in A549 cells, and the

related mechanism may be related to the GSK-3β-NF-KB-Snail pathway (Qiao et al., 2020). *In vivo* studies of safflower injection and safflower monomer components are as follows. Several investigators found that safflower injection may reduce bleomycin-induced pulmonary fibrosis in mice by inhibiting the TGF-β1/Smad3 signaling pathway and downregulating α-SMA expression (Luan et al., 2019; Luan et al., 2021). Bao et al. found that safflower yellow significantly improved COPD in model rats by inhibiting the expression of inflammatory factors and regulating the expression of TLR4/NF-KB pathway-related proteins (Bao et al., 2021). Wang found that SYA, HSYA, and anhydrosafflor yellow B (AHSYB) ameliorated acute lung injury by inhibiting the formation of neutrophils (NETs) and the Raf/MEK/ERK pathway (Wang, 2020). The above studies suggested that safflower has potential effects in the treatment of pulmonary fibrosis, lung cancer, COPD, and acute lung injury.

3.4.2 *Glycyrrhiza uralensis* Fisch

The dried roots and rhizomes of *G. uralensis* known as Xiang-an (Tibetan:སྐ་ལ་ལ་རགས་པོ།), Gancao (Chinese name), or licorice (English name), is commonly used herbal medicine. Xiang-an is sweet in taste and mild in nature and treats lung diseases and bronchitis. It is born in sandy soil at 800–2,800 m altitude and is distributed in Golmud in Qinghai, Gansu, Xinjiang, and Inner Mongolia. Flavonoids are one of the main components of licorice extract, and are also important components reflecting the main medicinal value of licorice. The triterpenoid saponins in licorice are its specific markers, among which glycyrrhetinic acid is a high content. Licorice polysaccharide is the third biologically important substance in addition to licorice flavonoids and saponins (Wang et al., 2022b). At present, many prescriptions have been extracted and isolated from licorice, such as isoglycyrrhizinol, licorice coumarin, liquiritin, glycyrrhizin, glycyrrhetic acid, and so on. Among them, liquiritin and glycyrrhizin are often used as markers for quality control of licorice in the pharmaceutical industry and drug standards.

Licorice has a wide range of pharmacological activities, including antibacterial, anticancer, antiviral, anti-inflammatory, memory-enhancing, neuroprotective, and pulmonary protective effects, as well as hypoglycemic and cholesterol-lowering effects. To date, many scholars have conducted antiacute lung injury, chronic lung injury, and pulmonary fibrosis studies on licorice (Figure 9). Total flavonoids are one of the effective sites of licorice against acute lung injury. *In vitro* studies have shown that total flavonoids of licorice inhibited the secretion of eosinophil-activated chemokine-1 by human lung fibroblasts, which was expected to block the recruitment of eosinophils to the site of antigenic inflammation (Jayaprakasam et al., 2009). *In vivo* studies of total flavonoids of licorice and monomeric components are as follows. Total flavonoids of were effective against lipopolysaccharide-induced pneumonia (Xie et al., 2009; Xie and Guan, 2009). It was found that both Glycyrrhiza endophytes and licorice produce cough expectorant, improve lung function and reduce lung injury by upregulating water channel protein expression and downregulating inflammatory factor expression in lung tissue (Wang et al., 2021b). Licochalcone A could treat acute lung injury in mice by blocking the ERK/NF-κB signaling pathway, inhibiting inflammatory factor overexpression and correcting oxidative/antioxidant imbalance (Furusawa et al., 2009; Chu et al., 2012; Ren et al., 2016). In addition, the triterpenoid components glycyrrhizin, glycyrrhetic acid, and diammonium glycyrrhizate also had anti-pulmonary fibrosis and lung injury protection effects. Glycyrrhizin and monoammonium glycyrrhizinate produced protective effects against acute lung injury by regulating the release of pro- and anti-inflammatory factors and by inhibiting apoptosis (Tang et al., 2007; Wang et al., 2012); and by inhibiting the cav-1/NF-κB signaling pathway through upregulation of ACE2 expression (Li, 2018b; Chen, 2019). Glycyrrhizin blocked pulmonary fibrosis by downregulating the expression of monocyte chemokine-1 and inhibiting the secretion of growth factors such as TGFβ1, thereby suppressing collagen fibril synthesis and fibroblast proliferation (He et al., 2006; He et al., 2008; 2009), and by inhibiting the IL-17/TGFβ1/Smad pathway in pulmonary fibrosis (Ye et al., 2007). Glycyrrhetic acid produced anti-acute radiation lung injury by blocking the TGFβ1/Smad signaling pathway (Yang, 2014). Diammonium glycyrrhizate played anti-pulmonary fibrosis effects by reducing the secretion of TGFβ1 and connective tissue growth factor in lung tissue (Li, 2018b), and could reduce chronic lung injury through anti-inflammatory effects (Bu et al., 2010).

In addition, the active ingredients in licorice also can induce apoptosis in lung cancer cells and have anti-viral activity. Li et al. predictive analysis of quality markers (Q-Marker) of licorice showed that flavonoids such as glycyrrhizin, isoglycyrrhizin, glycyrrhizin, triterpenoids such as glycyrrhizin and glycyrrhizic acid, and glycyrrhizin coumarin and glycyrrhizin polysaccharides were resistant to SARS (Li et al., 2021). An *in vivo* study had shown that Licochalcone B could induce apoptosis in non-small cell lung cancer cells (Oh et al., 2019). *G. uralensis* extract and glycyrrhetinic acid have been clinically effective in the treatment of severe acute respiratory syndrome (SARS) and COVID-19 as well as asthma, lung injury during surgical extracorporeal circulation, *mycoplasma* pneumonia, bronchopulmonary dysplasia and COPD, but these studies are only preliminary clinical observations and a large number of clinical observations with rigorous experimental designs are needed in the future (Zhang and Shen, 2020).

In summary, the flavonoids and triterpenoids in licorice have a wide range of pharmacological activities against lung diseases, such as anti-lung injury, anti-pulmonary fibrosis, anti-lung cancer, anti-pneumonia, and anti-COPD. Licorice may have great potential for development and become a candidate drug for treating lung diseases.

3.4.3 *Santalum album* L

The medicinal part of Zan-dan-ga-bao (Tibetan: སྐ་ལ་ལ་རགས་པོ།) is the dried heartwood of *Santalum album*, which is called Tan-Xiang in Chinese, and sandalwood in English. “The Drop of Manna” recorded that it is cool in nature, slow-acting, light and dry, and used for the treatment of lung fever, pneumonia, and lung abscess. *S. album* is not cultivated on the Qinghai-Tibet Plateau and is mostly imported from India, Australia, and Indonesia. It is also grown in Hainan and Taiwan in China. Tibetan medicine classifies sandalwood into three types: white, slightly yellow, and red sandalwood. Santalum album L. is the white one, and the name “white sandalwood” is also used in prescriptions. Tibetan doctors also use *Syringa reticulata (Blume)* var. *mandshurica* (Maxim.) Hara is a substitute for lignum vitae (Zhong and Song, 2020).

Sandalwood mainly contains sesquiterpenoids, monosteroids, and lignin prescriptions. Among them, α-sandalol and β-sandalol are its characteristic chemical components (Zhang et al., 2020). At present, scholars at home and abroad have found that the terpenoids in the volatile oil of sandalwood have a wide range of anti-tumor and anti-cancer effects, and santalol plays an important anti-cancer role in cancer cells. Meanwhile, some *in vitro* studies have reported that the lignans in sandalwood also have some anti-lung cancer cell effects. For example, Matsuo and Mimaki isolated two lignin-like components ((*7R,8R*)-5-*O*-demethylbilagrewin, bilagrewin) from sandalwood and investigated their antitumor activity, showing that both prescriptions were highly cytotoxic against HL-60 human leukemia early juvenile granulocytes and A549 human lung adenocarcinoma cells (MATSUO and MIMAKI, 2020). The above studies suggest that sandalwood may have good potential as a drug candidate for the treatment of lung cancer.

3.4.4 *Aucklandia costus* Falc

Ru-da (Tibetan: ཟུ་ལུ་ལུ་རྒྱ་པོ།), known as Muxiang (Chinese name), costus (English name), is the dried root of *Aucklandia costus* Falc. This medicine is spicy, warm in nature, and has a sharp effect. "Jing Zhu Materia Medica" recorded: "Ru-da treats gas and blood, stomach distention, lung diseases, throat problems, and removes dead flesh. This medicine is divided into black and white. White Ru-da is produced in the land of Kangmu. Black Ru-da is produced in India and the Gondas, and is available in both mountain and cultivated varieties, with an aromatic scent." "Tujian snail's eye" recorded that "Black and white Ru-da like shed antlers". The plant is planted in mountains and hills at altitudes of 2,500–4,000 m. It is native to India and was later introduced to Yunnan, and it is cultivated in most provinces. So far, the chemical components isolated from *Aucklandia lappa* mainly include sesquiterpene lactones and organic acids. Among them, costunlide (CNL), and dehydrocostus lactone (DHC) are usually used as the marker for controlling the quality of costus in the pharmaceutical industry and drug standards.

Many *in vitro* studies have shown that a variety of active ingredients in woody incense have antitumor activity. Numerous studies have shown that vascular endothelial growth factor (VEGF) is overexpressed in many cancer tissues, including liver, lung, colon, ovarian, and breast cancers. Hao LJ et al. found that the volatile oil, DHC, and costunolide inhibited the secretion of VEGF from human lung adenocarcinoma A549 cells (Hao et al., 2010). Parthenolide can significantly induce apoptosis and inhibit the invasion and migration of non-small cell lung cancer H1975 cells, which may be related to PTL's inhibition of the PI3K/Akt signaling pathway (Bai et al., 2019). After the action of feverfew lactone on human lung cancer A-549 cell line, it induces the change of apoptosis, and it completes the mechanism of apoptosis induction through the death receptor pathway and mitochondrial pathway, and the increase of reactive oxygen species level after the action of feverfew lactone may participate in the mechanism of inducing apoptosis (Lv et al., 2015). Also, *in vivo* studies have found that certain chemical components in the costus have some anti-inflammatory activity. For example, DHC might induce heme oxygenase-1 (HO-1) production in RAW264.7 cells via the p38MAPK/Nrf2 signaling pathway, significantly reduce serum levels of IL1β and TNF-α, and decrease nitric oxide (NO) synthase expression induced by CLP mouse lung tissue; CNL, DHC, acetone extracts significantly reduced the production of inflammatory lung eosinophils, type 2 cytokines, and specific Ig E and mucus in allergic mice (Pyun et al., 2018). The above results indicate that DHC, costunolide, and parthenolide all have anti-lung cancer activity, and DHC also has anti-pneumonia activity. Clinical studies can be conducted on the above ingredients to further demonstrate their efficacy in treating lung diseases.

3.4.5 *Terminalia chebula* Retz., *Terminalia chebula* Retz var. *tomentella* Kurz

A-ru-la (Tibetan: ཟུ་ལུ་ལུ་རྒྱ་པོ།) is the dried ripe fruit of *Terminalia chebula*. and *Terminalia chebula* Retz var. *tomentella*., called Hezi in Chinese, myrobalan in English. The medicine is bitter and astringent in taste, warm in nature. "Moon King Medicine Clinic" said that myrobalan is good for all diseases, generates body temperature, helps digestion, and is used for "Loong", "Tripa", "Baekan" and "blood" diseases and the combination of the four diseases. "Jing Zhu Materia Medica" said that myrobalan is the king of all medicines. It is native to India, Myanmar and, other places, and is also distributed in Yunnan, Tibet, Guangdong, Guangxi, and other places in China. As a traditional botanical medicine in China, Chebulan is frequently used in Tibetan medicine and has a variety of pharmacological activities.

The chemical constituents isolated from myrobalan are classified into three main types according to their structure: phenolic acids, tannins, and triterpenes (Yang, 2016). Among them, the phenolic acids include gallic acid and ellagic acid; the tannins include chebulagic acid and chebulinic acid, *etc.*,; the triterpenoids include arjunolic acid, maslinic acid, *etc. In vitro* studies have shown that the pharmacological activities of myrobalan are mainly antitumor activity and antibacterial activity against lung diseases. Tian et al. investigated the effect of aqueous extract of myrobalan on the proliferation of human lung cancer A549 cells and showed that the inhibition rate of human lung cancer A549 cells increased when the concentration of aqueous extract of myrobalan increased, and the mRNA expression of P53 protein increased, so the ability of myrobalan extract to induce apoptosis in A549 cells may be related to the activation of P53 gene (Tian et al., 2015). Bao et al. found that myrobalan extract inhibited the proliferation of lung cancer A549 cells with a significant dose-dependent intensity of action (Bao et al., 2012), and the mechanism may be related to the Tannins prescriptions in myrobalan (Li et al., 2015). Ravi et al. found that the ethanolic extract of myrobalan can be cytotoxic against human breast cancer cell line MCF-7 and lung cancer cell line A-549 with anti-breast and lung cancer activities (Ravi et al., 2016). Lin et al. found that normal doses of chebulinic acid and andrographolide significantly inhibited HSV-1 invasion into human lung cancer cells A549, mainly by inhibiting the interaction of glycosaminoglycans on the surface of A549 cells with the glycoproteins on the surface of HSV-1 virus, and that chebulinic acid and andrographolide were competitive inhibitors of glycosaminoglycans, thus exhibiting some degree of antiviral effect (Lin et al., 2011). In addition, myrobalan has *in vitro* antibacterial activity against *Actinomyces* pleuropneumonia (Kang et al., 2014). At present, only *in vitro* studies have been conducted on the extract of myrobalan, and there is a lack of corresponding *in vivo* and clinical studies to further prove its anti-lung cancer activity.

4 Discussion

Tibetan medical theory suggests that Lung diseases are caused by a combination of internal and external factors. The internal causes are the imbalance of the body's Loong, Tripa, Baekan, and blood. External causes include the invasion of body fluids into the lungs or the consumption of rotten and acidic, overly salty foods, smoking, long-term colds, and overexertion. Tibetan medicine is rich in content and complete in theory. In its long-term practice, Tibetan medicine has accumulated abundant experience in the treatment of lung diseases. It would be very meaningful if these experiences could serve as a reference for the world's medical community.

These natural plants have a wide range of medicinal sources and are distributed in 77 families. Among them, Rosaceae, Composite, Ericaceae, Leguminosae, and Gentianaceae are more common. Vegetal (medicinal) species from different families have the potential to treat different types of lung diseases. In traditional Tibetan medicine, vegetal (medicinal) species of the Gentianaceae family are often used to treat lung fever and pneumonia. Leguminosae are mostly used for lung fever, pneumonia, and tuberculosis. Rosaceae are commonly used to treat lung fever, pulmonary congestion, and pulmonary tuberculosis. Composite are often used for bronchitis, pneumonia, tracheitis, and pulmonary tuberculosis. Ericaceae are usually used to treat lung abscess and tracheitis. It provides new directions and ideas for pharmacological research. For example, further research is necessary to investigate the pharmacological substance basis and mechanism of action of Gentianaceae (medicinal) species against pneumonia.

This review provides a detailed review of four types of Tibetan medicines commonly used in Tibetan hospitals and the five most frequently used Tibetan medicines in Tibetan prescriptions. We found that these Tibetan medicines (excluding costus, sandalwood, and myrobalan) have the potential to treat a variety of lung diseases. Research has shown that costus, sandalwood, and myrobalan have anti lung cancer effect. Pharmacological research on rhodiola and licorice is relatively sufficient, but further clinical studies are needed to demonstrate their efficacy. In addition, salidroside in rhodiola can treat various lung diseases and may become a candidate drug for treating a variety of lung diseases. However, preclinical studies on other metabolites are yet to be conducted. Similarly, gentian contains numerous metabolites, but only gentiopicroside has been proven to have anti-lung cancer and therapeutic effects on pulmonary fibrosis. Most of the studies were on crude extracts, and there were fewer studies on single pharmacodynamic components. For example, total flavonoids from sea buckthorn have been proven to have anti-lung cancer and chronic bronchitis effects. Although gentian is a multi-original species, pharmacological and clinical studies were focused on *G. szechenyii*. There were no pharmacological studies on the other vegetal (medicinal) species of gentian. Correspondingly, the pharmacological researches on liexiang dujuan are rather for ages and all of them are about R. anthopogonoides.

In addition, this paper has excavated and organized Tibetan medicines and prescriptions for lung diseases, and screened more than five hundred natural medicines, including botanical, animal and mineral medicines. We found that some Tibetan medicines (e.g.,: *G. veitchiorum* Hemsl., *Ephedra saxatilis* Royle ex Florin, *Schisandra sphenanthera* Rehd. et Wils.) and their active ingredients (e.g.,: gentiopicroside, ephedrine, pseudoephedrine, schisandrin B, schisandrin lignan) have good anti-lung cancer, treatment of lung injury, inhibition of pulmonary fibrosis, and anti-pneumonia effects. Gentiopicroside inhibits lung cancer A-549 cell transformation; pseudoephedrine treats lung tissue damage by influenza A virus; ephedrine effectively alleviates LPS-induced apoptosis in alveolar epithelial cells A549, inflammatory damage and oxidative stress (Shao et al., 2021), and reduces lung coefficients in rats with pulmonary fibrosis (Gao, 2010); pseudoephedrine inhibited the process of pulmonary fibrosis and reduced the extent of pulmonary fibrosis (Wei et al., 2017). Schisandra chinensis extract inhibits the growth of the C. pneumoniae strain (CV6) (Hakala et al., 2015). Schisandra chinensis lignans significantly reduced inflammatory damage in lung tissue (Sun et al., 2021). These natural medicines will be potential drug candidates for the treatment of lung diseases and deserve further research and development. However, less than 10% of the Tibetan medicines collated have been studied in modern pharmacology for lung diseases, most of which are *in vitro*, and more *in vivo* studies are needed to confirm their effectiveness in treating lung diseases. More than 90% of the drugs have not been studied for lung disease-related bioactivity. For example, Hong-lian (*Lagotis glauca* Gaertn.) is a commonly used Tibetan medicine for the treatment of lung diseases and was found to be used more frequently based on the TCMISS study. However, to date, no biological activity has been reported for this medicine to lung disease. Similarly, Gun-zhu-mu (*Vitis vinifera* L.); sea buckthorn paste; Ba-ya-ba (*Lancea tibetica* Hook. f. et Thomson); Suo-luo-ga-bao (*Pegaeophylon scapiflorum* (Hook. f. et Thoms.) Marq. et Shaw) also lack studies related to lung disease.

Tibetan medicine has great potential for the treatment of lung diseases. However, there are still some shortcomings in the current research. First of all, most of the studies were *in vitro* and *in vivo*, and there was a lack of relevant clinical studies. Clinical studies of these drugs should be carried out in the future to verify their therapeutic effects on lung diseases. Secondly, most of the studies were on crude extracts, and there were fewer studies on single pharmacodynamic components, as well as studies on pharmacodynamic mechanisms still need to be further explored and expanded. Most of the Tibetan medicines collated in this article still need more research to fill the gaps in their chemical composition and pharmacological activities in pulmonary diseases. Therefore, a systematic study including phytochemical analysis, bioactivity screening, biosynthetic pathway elucidation and conformational relationship studies is necessary, and these findings will provide a more rational basis for the utilization and maximization of the intended therapeutic effects of Tibetan medicines.

Currently, many of the available treatments for lung diseases are of limited efficacy or are related to side effects. The Tibetan medicine compiled in this article might have potential as drug candidates for the development of lung diseases. These treasures urgently need to be excavated using modern scientific methods.

5 Conclusion and perspectives

In this review, a total of 586 Tibetan medicines were compiled through literature research of 25 classical works on Tibetan medicine, drug standards, and some Chinese and English databases. Among them, 33 Tibetan medicines have been studied to show their effectiveness in treating lung diseases. Furthermore, using TCMMIS to mine the prescription data, we screened out the five Tibetan medicines with the highest frequency of medication. The ethnopharmacological, phytochemical, and pharmacological

properties of these five Tibetan medicines and the four most commonly used Tibetan medicines in Tibetan hospitals were reviewed. According to modern pharmacological research, these Tibetan medicines have a wide range of pharmacological activities against lung diseases. These include activities such as chronic obstructive pulmonary disease, anti-lung cancer, pulmonary fibrosis, lung injury, pneumonia, *etc.*

Chinese ethnomedicine is a potential resource pool for new drug development, and a large amount of experience in new drug development at home and abroad has proven that screening new drugs from ethnomedicine is far more efficient than random screening in modern biomedicine (Zhang and Du, 2021). These Tibetan medicines have valuable experience in the Tibetan Plateau region of China (including Tibet, Qinghai, Gannan Prefecture in Gansu, Ganzi Prefecture and Aba in Sichuan, and Diqing Prefecture in Yunnan). Modern pharmacology studies and clinical studies should be conducted by screening drugs from them to develop new medicines with fewer side effects and better treatment effects, thereby making a significant contribution to the treatment of these diseases on a global scale.

In addition, at present, some Tibetan medicine resources are scarce, such as G. szechenyii, which belongs to the national first-class endangered Tibetan medicine. These endangered Tibetan medicines need to pay more attention to the protection of their medicinal resources while conducting modern pharmacological research. For example, to meet its pharmacological material basis and clinical application research, some measures on appropriate technologies and industrialization models for the regeneration of Tibetan medicine resources and ecological protection should be carried out.

In summary, we can make fuller and more comprehensive use of Tibetan medicines and realize their great potential.

Author contributions

CL: Formal Analysis, Methodology, Writing-original draft. YL: Funding acquisition, Writing–review and editing. XH: Writing–review and editing. SL: Writing–review and editing. KS: Writing–review and editing. RG: Funding acquisition, Writing–review and editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was supported by the Ministry of Science and Technology of the People's Republic of China (No. 2019YFC1712305), and the National Natural Science Foundation of China (No. 82073964).

Acknowledgments

Upon the completion of the thesis, we would like to express our sincere gratitude to Zhonglian Zhang, who provided a picture of Terminalia chebula Retz.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2024.1365911/full#supplementary-material

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frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 21 March 2024
DOI 10.3389/fphar.2024.1322083

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RECEIVED 15 October 2023
ACCEPTED 27 February 2024
PUBLISHED 21 March 2024

CITATION
Mottaghipisheh J, Kamali M, Doustimotlagh AH, Nowroozzadeh MH, Rasekh F, Hashempur MH and Iraji A (2024), A comprehensive review of ethnomedicinal approaches, phytochemical analysis, and pharmacological potential of *Vitex trifolia* L..
Front. Pharmacol. 15:1322083.
doi: 10.3389/fphar.2024.1322083

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A comprehensive review of ethnomedicinal approaches, phytochemical analysis, and pharmacological potential of *Vitex trifolia* L.

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Plants, renowned for their rich reservoir of metabolites, play a pivotal role in addressing health-related issues. The Verbenaceae family stands out, showcasing immense potential in preventing and treating chronic diseases. *Vitex trifolia* L. (*V. trifolia*), a shrub with a rich history in traditional medicine, particularly in Eastern Asia, has garnered attention for its diverse therapeutic applications. This comprehensive review aims to bridge traditional knowledge and contemporary insights by investigating ethnopharmacology, phytochemistry, and pharmacological effects of *V. trifolia*. The keyword "*V. trifolia*" and its synonyms were searched within the main scientific databases including PubMed, Web of Science, ScienceDirect, Google Scholar, and Baidu Scholar (from 1974 to 2022, last search: 21.10.2023). Phytochemical analyses reveal a spectrum of secondary metabolites in *V. trifolia*, including terpenoids, flavonoids, lignans, phytosterols, anthraquinones, and fatty acids. Notably, terpenoids and flavonoids emerge as the main bioactive metabolites. Pharmacological studies validate its therapeutic potential, demonstrating significant antioxidant, anti-inflammatory, hepatoprotective, anticancer, anti-amnesic, antimicrobial, antiviral, anti-malaria, antispasmodic activities, and reported insecticidal effects. Despite existing literature exploring pharmacological attributes and secondary metabolites of related species, a conspicuous gap exists, specifically focusing on the pharmacological activities and novel methods of

Abbreviations: *V. trifolia* L., *V. trifolia*; GGPP, Geranylgeranyl pyrophosphate; EOs, Essential oils; TPC, Total phenolic content; TFC, Total flavonoid content; SGPT, Glutamate pyruvate transaminase; SGOT, Glutamate oxaloacetate transaminase; ALP, Alkaline phosphatase; NO, Nitric oxide; LPS, Lipopolysaccharide; IL, Interleukin; TNF-α, Tumor necrosis factor; ZnO NPs, Zinc oxide nanoparticles; MCV, *M. contagiosum* virus; HSV, Herpes simplex virus; PGE2, Prostaglandin E2; ERK1/2, Extracellular signal-regulated kinase 1/2; LT_{50}, Lethal time to cause 50% mortality; MTT assay, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide assay.

purification of pure metabolites from *V. trifolia*. This review aimed to fill this gap by delving into traditional medicinal applications, exploring secondary metabolites comprehensively, and providing an in-depth analysis of pharmacological effects of pure metabolites. Combining traditional uses with contemporary pharmacological insights, this article sought to serve as a crucial reference for future research and practical application of *V. trifolia*. This approach contributes substantially to understanding the plant, fostering scientific inquiry, and facilitating its broader application in healthcare.

KEYWORDS

***Vitex trifolia* L., secondary metabolites, ethnomedicine, phytochemistry, bioactivities**

1 Introduction

Plants play a crucial role in health-related problems due to their rich reservoir of bioactive metabolites. These natural metabolites found in various plant species, such as those belonging to the Verbenaceae family, have shown immense potential in preventing and treating chronic diseases, offering a promising opportunity for therapeutic interventions (Hashempur et al., 2018; Alyasin et al., 2020; Conti et al., 2021). Verbenaceae is one of the largest families of the plant kingdom, consisting of trees, shrubs, lianas, and herbs. Verbenaceae comprise of 34 genera and around 1,200 species. *Vitex* is known as one of the largest genera in the family, possessing 270 species mainly distributed in tropical areas, with a few in subtropical regions (Rani and Sharma, 2013; Yao et al., 2016).

Vitex trifolia L. (*V. trifolia*) is a shrub or shrubby tree that may grow up to 6 m in height. It is found in some regions of Asia, China, India, Indonesia, Sri Lanka, Singapore, and Australia. This plant has a rich history in traditional medicine for its effectiveness in treating asthma and respiratory disorders. It has been reported that most plant parts such as the fruit, leaf, root, flower, and stem demonstrated medicinal values; however, its fruit is the most studied and used part (Zaki et al., 2022). Different types of secondary metabolites including terpenoids (mainly labdane-type diterpenes), flavonoids, lignans, phytosterols, anthraquinones, and fatty acids have been reported in *V. trifolia*; whereas terpenoids and flavonoids have been identified as the main bioactive compounds (Yan et al., 2023). Pharmacological studies have further validated its therapeutic potential by demonstrating significant antioxidant, anti-inflammatory, hepatoprotective, anticancer, anti-amnesic, antimicrobial, antiviral, anti-malaria, and antispasmodic activities. Additionally, some studies have reported its insecticidal effects (Tandon et al., 2008). These findings highlight the diverse beneficial properties associated with *V. trifolia* and support its use in traditional medicine for various health conditions.

In recent years, the medicinal potential of *V. trifolia* has garnered significant attention, particularly within traditional medicine. A series of review papers have explored various aspects of this plant species with emphasis on its pharmacological properties (Chan et al., 2016; Kamal et al., 2022; Yan et al., 2023). The most recent review explores the pharmacological attributes and secondary metabolites of *V. trifolia* L. and *V. rotundifolia* L. f., providing insights into their properties. However, despite the existing body of literature, there remains a conspicuous gap in the current discourse, particularly the phytochemical aspects of *V. trifolia*, as an invaluable natural agent, where complementary information regarding the plant parts and extracts utilized, also different separation methodologies could indeed assist futuristic investigations of this species.

The current study is an attempt to fill this gap by investigating the traditional medicine applications of *V. trifolia*, along with an in-depth analysis of its secondary metabolites through an updated literature search strategy, where existing information on chromatographic steps and the plant parts/extracts employed which led to the isolation and identification of its precious secondary metabolites are described in detail.

Moreover, it explores the pharmacological effects of the individual pure metabolites isolated from *V. trifolia* a crucial aspect that has thus far been overlooked in the existing literature. Hence, this article aimed to serve as a vital reference for future research endeavours and the practical utilization of *V. trifolia* Through a comprehensive exploration of both the traditional uses and contemporary pharmacological insights, this review is an attempt to contribute substantially to understanding *V. trifolia* and pave the way for further scientific inquiry and application of this intriguing plant species.

2 Methodology

In this study, a comprehensive literature search was done focusing on *V. trifolia* across various online databases and relevant books. The search employed the term '*Vitex trifolia*' and its synonyms '*Vitex agnus-castus* var. *trifolia* (L.) Kurz, *Vitex indica* Mill., *Vitex integerrima* Mill., *Vitex trifolia* var. *trifoliolata* Schauer, and *Vitex variifolia* Salisb.', (confirmed by http://www.plantsoftheworldonline.org), while targeted prominent databases including PubMed, Web of Science, ScienceDirect, and Google Scholar. Baidu Scholar were also included in the search with a specified time frame from 1974 to 2022 (the last search was conducted on 21.10.2023). The search yielded 889, 283, 1,263, 1,023, and 147 articles in each database, respectively. After this refinement process, a total of 164 articles emerged as pertinent to the scope of this review. This judicious selection ensures that the information is comprehensive and focused, contributing to the literature review robustness.

3 Traditional uses of *V. trifolia*

Various ethnopharmacological studies have demonstrated promising medicinal applications for different plants (Mosavat

et al., 2015; Ayeni et al., 2022; Das et al., 2022). *V. trifolia*, commonly known as the chaste tree or five-leaved chaste tree, has gained recognition as a botanical drug, particularly in Eastern Asia. Throughout the years, ethnomedicinal investigations have documented the diverse therapeutic applications of this plant (Blois, 1958; Ban et al., 2020).

The traditional use of the fruit can be traced back to ancient times, with its earliest recorded mention appearing in Shen Nong's Classic of Materia Medica of China. This historical text was commended for its medicinal properties and addressed as a remedy for various afflictions. Among its benefits, it was believed that it alleviated conditions like cold and heat between the tendons and bones, addressed dampness impediment, enhanced vision by brightening the eyes, strengthened the teeth, unblocked the "nine orifices" (body openings), and even eliminated taeniasis, a condition caused by tapeworm infection. This valuable fruit has a significant place in traditional medicine, connecting its potent healing qualities to improve human health (Wu et al., 2009a). Furthermore, it has been credited with promoting hair growth. Tonga, known for its rich traditional practices, utilized the plant for its remarkable healing properties. Specifically, they harnessed its powers to treat oral infections and inflammations (Limousin and Bessières, 2006).

In Unani medicine, the plant is known as Sambhalu and has been employed to reduce libido (Suchitra and Cheriyan, 2018). In Papua New Guinea, the indigenous population utilizes the stem of *V. trifolia* L. to treat dysentery. The leaves of *V. trifolia*, called Jalanirgundi in traditional Ayurvedic medicine, are commonly prepared as a decoction or used topically as a poultice. They have been employed to alleviate joint pain, inflammation, and rheumatism (Kirtikar and Kirtikar, 1980; Thenmozhi et al., 2015). In New Caledonia, Rotuma, and the Solomon Islands, heated leaves are commonly used to alleviate severe headaches by rubbing them on the forehead or consuming them as an infusion (de Kok, 2007). The fruits of *V. trifolia*, commonly called Manjingzi in the Chinese Pharmacopoeia, have a long-standing history in traditional Chinese medicine. They are known for their wind-heat-dispersing properties, making them valuable in treating various ailments such as headaches, migraines, and ophthalmodynia. The traditional use of Manjingzi highlights its effectiveness in addressing conditions associated with wind heat, providing relief, and promoting overall wellbeing. Besides, the flowers of *V. trifolia* have demonstrated usefulness in treating fever (Talreja and Tiwari, 2020).

4 Phytochemistry

So far, over 180 metabolites have been identified from different parts of *V. trifolia*. Investigation of the chemical profile has led to the isolation of terpenoids (monoterpenes, sesquiterpenes, diterpenes, triterpenes, and phytosterols), ecdysteroids, flavonoids, lignans, phenylpropanoids, anthraquinone, fatty acids, along with xanthones isolated from the endophytic fungi of the fruit (Peng et al., 2021). Among them, the diterpenes special labdane-type are the most significant metabolites in this species. In the following sections, the isolated/identified phytochemicals have been classified based on their main classifications (Figures 1–7); however, more details including plant parts used and chromatographic techniques applied are listed in Supplementary Table S1.

5 Biosynthesis of terpenoids, flavonoids and iridoids

Since the main *Vitex trifolia* compounds identified as terpenoids, flavonoids, and iridoids, thus this section allocates to briefly overview the natural biosynthesis pathways of these compounds. Terpenoids are derived from the mevalonate (MVA) pathway, exhibiting activity within the cytosol, or alternatively from the plasticidal 2-*C*-methyl-D-erythriol 4-phosphate (MEP) pathway. The MEP pathway predominantly serves as the source of hemi-, mono-, di-, and triterpenoids, whereas the MVA pathway is primarily responsible for the synthesis of sesqui- and triterpenoids (Cheng et al., 2007; Maffei et al., 2011) (Figure 8A).

Iridoids represent a vast category of monoterpenoids, distinguished by their skeletal structure consisting of a cyclopentane ring fused with a six-membered ring containing an oxygen atom, commonly known as the iridane skeleton. Typically, these compounds have been identified in plants in conjunction with sugar molecules, rendering their glycosides and allowing for their classification (Villasenor, 2007). The iridoid system is derived from geraniol through a unique folding process, distinct from the folding observed in monoterpenoids. There are over thousand different known natural iridoids, with structural variations primarily arising from hydroxylations, esterifications, and changes in stereochemistry (Figure 8B) (Dewick, 2002).

Flavonoids originate from the phenylpropanoid metabolic pathway and possess a fundamental composition consisting of a C15 benzene ring structure of C6-C3-C6. In recent years, substantial research has been conducted to uncover the intricate mechanisms underlying the biosynthesis of flavonoids in plants (Liu et al., 2021). The entry of *p*-coumaroyl-CoA into the flavonoid biosynthesis pathway signifies the start of the synthesis of specific flavonoids, which begins with the formation of chalcone (Nabavi et al., 2020). Chalcone serves as the initial crucial product in the metabolic pathway of flavonoids, offering a fundamental framework for subsequent synthesis of flavonoids (Zhou et al., 2009). Flavanones have the potential to generate numerous iterations based on this fundamental framework, such as flavones, flavonols, anthocyanidins, and catechins (Figure 9) (Mottaghipisheh et al., 2021).

5.1 Terpenoids

5.1.1 Monoterpenoids and sesquiterpenoids

Most of the *V. trifolia* monoterpenoids are iridoids and their corresponding glycosides (metabolites **1**–**10**). Besides the iridoids, an acyclic monoterpenoid vitexoid (**15**) has been isolated from the fruits and is a characteristic metabolite of *V. trifolia* (Djimabi et al., 2021). Only two aromadendrane-type sesquiterpenoids (**16** and **17**) were found in the fruits (Gu et al., 2007).

5.1.2 Diterpenoids

The *V. trifolia* fruits and leaves can be considered as a rich source of cyclic diterpenes, predominantly containing monocyclic, bicyclic

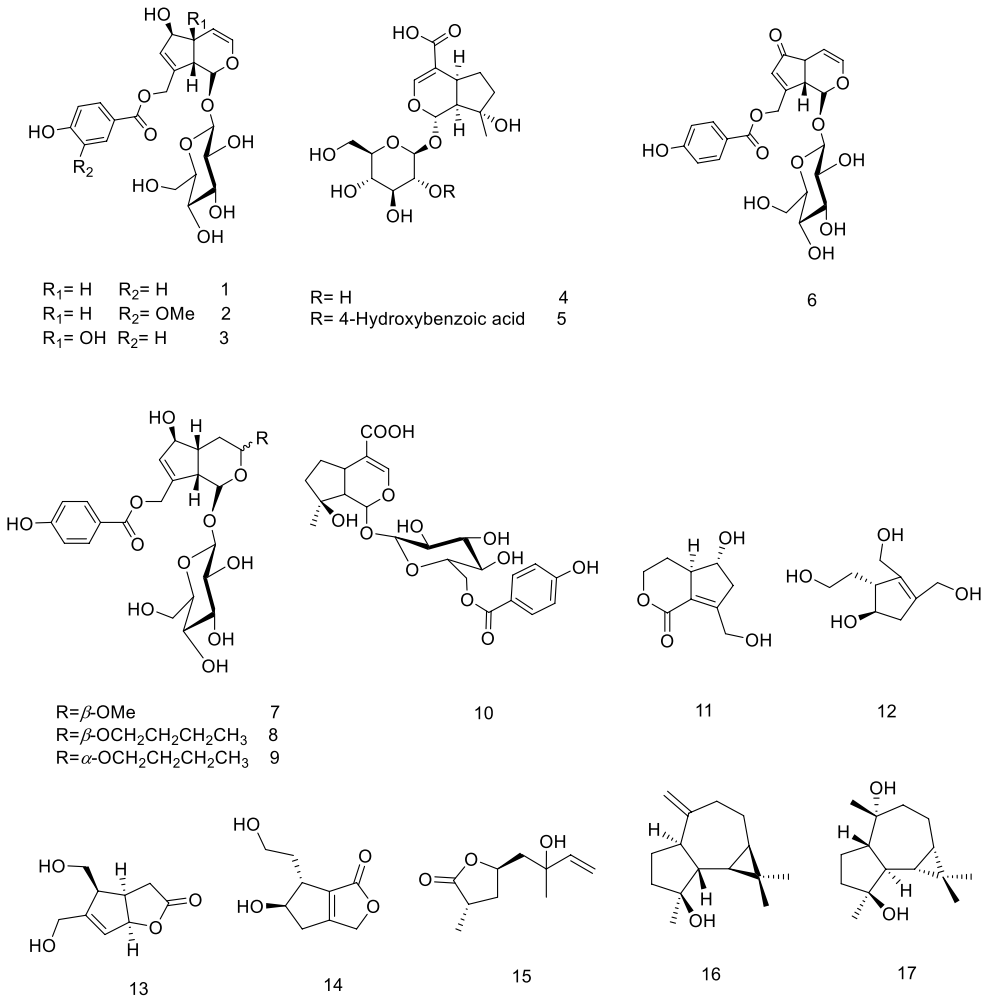

FIGURE 1
Chemical structure of monoterpenoids and sesquiterpenoids isolated from *V. trifolia*.

(labdane, halimane, clerodane), and tricyclic (abietane) skeletons. Noteworthy, all these chemical types were separated from semi-polar fractions or semi-polar soluble extracts (acetone, ethyl acetate) utilizing various chromatographic techniques. Labdane diterpenoids (**18**–**70**) with fifty-three derivatives have been characterized as the highest abundance among other phytochemicals. Except for two glycosylated diterpenoids (**69** and **70**), the remaining metabolites of the genus have been identified in aglycosylated form. Recently, phytochemical investigation on the ethanol extract of the fruits of *V. trifolia* yielded four new labdane diterpenoids vitetrolins A-D (**47**–**48**, **20** and **54**) (Djimabi et al., 2022).

A labdane diterpenoid alkaloid, 9α-hydroxy-13 (14)-labden-16,15-amide **68**, with an α,β-unsaturated-γ-lactam moiety, was isolated from *V. trifolia* leaves (Luo et al., 2017c). Further investigation on the isolation of *V. trifolia* labdane diterpenoid alkaloids led to identification of a cyano-substituted pyrrole cyclic system (**64**–**67**) from the ethanolic extract of the leaf. The precursors of geranylgeranyl pyrophosphate (GGPP), ammonia, and an amino acid may contribute to the biosynthesis of these remarkable metabolites (Luo et al., 2017b).

Halimane diterpenoids (**71**–**81**), known as rearranged labdanes, have further been isolated from this species. According to diterpenoids classification by Roncero and his co-workers, most *V. trifolia* halimane diterpenoids belong to halim-1 (10)-enes (**71**–**73**) and halim-5 (10)-enes (**74**–**80**) groups, described as the largest structures of halimanes (Roncero et al., 2018). The leaves of *V. trifolia* also synthesized three clerodane diterpenoids, named as vitexfolin B (**82**), vitextrifloxide I (**83**), and dysoxydensin G (**84**) (Luo et al., 2017c).

Abietanes diterpenoids (**85**–**89**) have been isolated from the fruit's extracts or non/semi-polar fractions (Ono et al., 2000; Li et al., 2020b; Djimabi et al., 2021). From this plant, aromatic five abietanes, known as the most abundant naturally occurring abietanes, including abietatrien-3β-ol (**86**), ferruginol (**87**), 3β-acetoxyabieta-8,11,13-triene-12-ol (**88**), and vitexifolin C (**89**) have been isolated (Gonzalez, 2015).

Djimabi *et al.* identified helipterol (**90**), possessing a monocyclic scaffold, from the fruit alcoholic extract *V. trifolia*, as the only natural source which is so far reported (Djimabi et al., 2021). Moreover, viterotulin D (**37**), vitetrifolin H (**79**), 15,16-epoxy-9-

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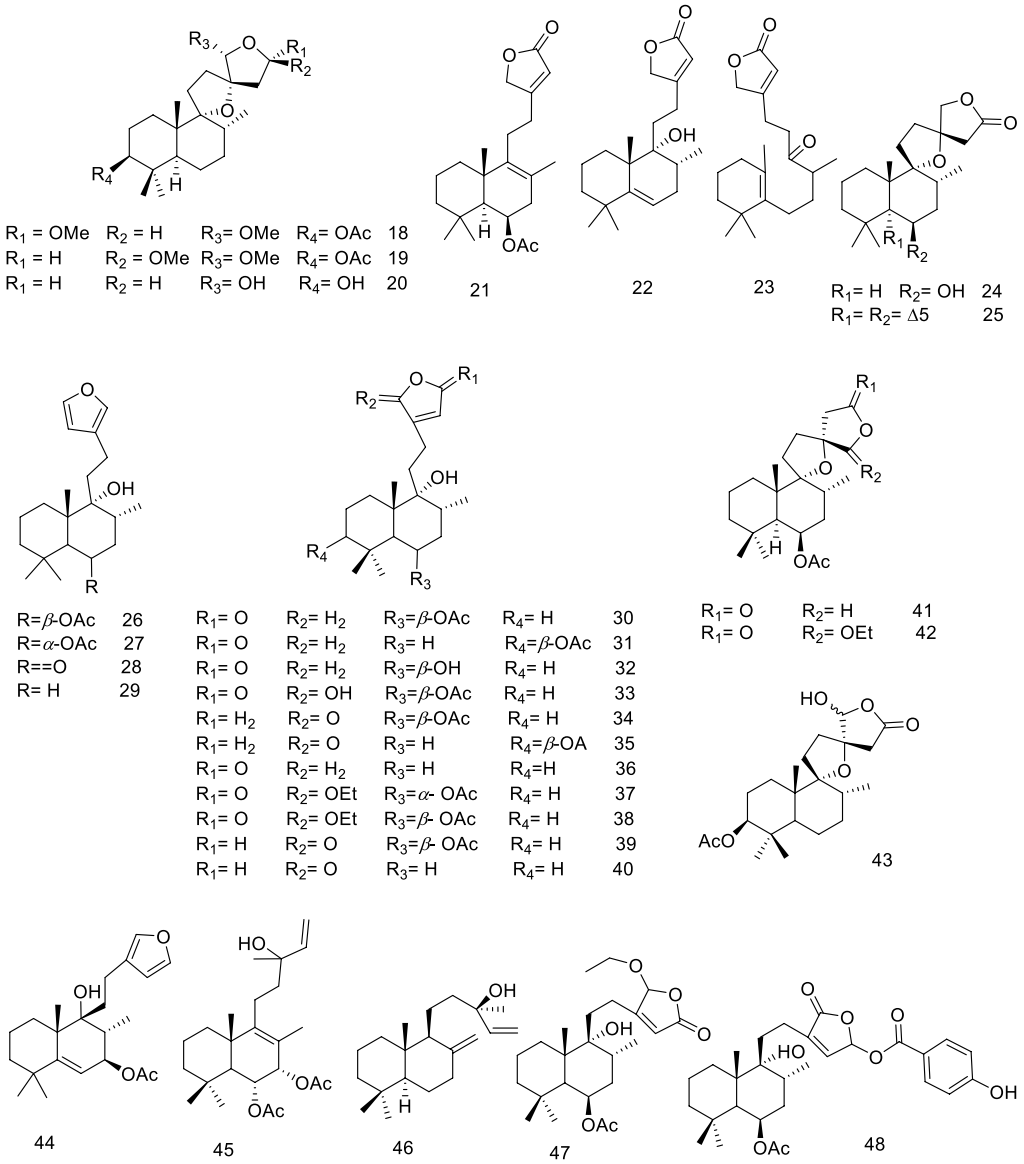

FIGURE 2
(Continued).

hydroxylabda-13 (16),14-diene (**29**), and 3*β*-acetoxyabieta-8,11,13-trien-12-ol (**88**) were screened out to be the specific secondary metabolites (Wu et al., 2009b). The various class of structures is shown in Figure 2.

5.1.3 Triterpenoids and phytosterols

In *Vitex trifolia*, the most abundant subclasses of triterpenoids identified are oleanane, ursane, and lupane, with **92** to **110** representative compounds (Figure 3). Additionally, one metabolite with a taraxerane skeleton (compound **111**) has been isolated, further enriching the chemical profile of this species (Yong-Sheng Chen et al., 2010; Djimabi et al., 2021). Given the abundance of triterpenoids in their free form in *V. trifolia*, Mohamed et al. have successfully identified three triterpenoid saponins (**97–99**) derived from the leaves (Mohamed et al., 2013).

Within the various *Vitex* species, ecdysteroids are of great attention due to their distinctive characteristics advantageous in chemotaxonomy (Sena Filho et al., 2008). Noteworthy ecdysteroids, such as ecdysone (compound **120**), 20-hydroxyecdysone (compound **121**), 20-hydroxyecdysone 2,3-monoacetonide (compound **122**), turkesterone (compound **123**), and polypodine B (compound **124**), have been identified in the leaves of this plant (Thoa et al., 2018). Additionally, this species is known to contain several sterol derivatives—specifically phytosterols (compounds **112–118**) and an ergostanoid (compound **119**)—which have been

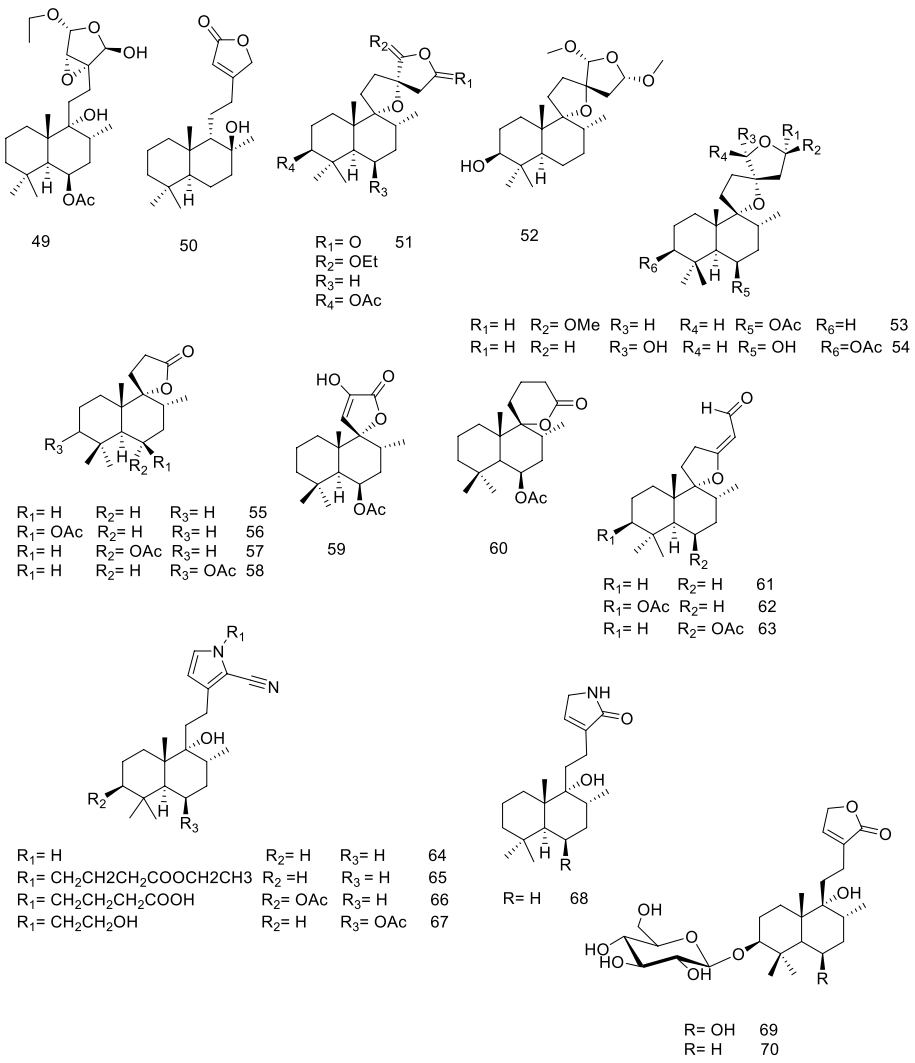

FIGURE 2
(Continued).

reported to contribute to the phytochemical diversity of the plant (Huang et al., 2013; Ban et al., 2018; Li et al., 2020b).

5.2 Flavonoids

Figure 4 presents a detailed representation of the varied flavonoid subclasses identified within *V. trifolia*, including flavones (entities **125–132**), flavonols (entities **133–142**), and flavanones (entities **143–144**). Among these, a particular focus has been noted on methoxylated flavones, specifically compounds **132** and **135–141**. These compounds are characterized by having between two to five methoxyl groups and have been detected primarily within polar extracts or fractions derived from the fruits and leaves of the plant (Lee et al., 2013; Li et al., 2020a).

Further studies have resulted in the isolation of specific methoxylated flavones, including vitexicarpin (compound **135**), artemetin (compound **138**), and chrysoplenol D (compound **140**) from the seeds (Guan et al., 2010). These isolated compounds have been observed to exist either as free-standing molecules or in glucosidic form, often conjugated mainly with glucose units, as seen in structures **126**, **128–131**, and **136–137**. Furthermore, a distinct glucoside, the neohesperidoside moiety, has been discovered in a conjugated form as quercetin 7-*O*-neohesperidoside (compound **134**), within the ethyl acetate extract of the plant (Mohamed et al., 2013). This array of flavonoid derivations not only highlights the chemical complexity of *V. trifolia* but also signifies the potential of the plant as a resource for multifaceted bioactive compounds.

5.3 Lignans, phenylpropanoids and xanthones

Matairesinol 4′-*O*-*β*-D-glucopyranoside **145** was a new lignan isolated from leaves by Ban and his co-workers (Ban et al., 2018). The metabolite **153** dimer structure of dihydro-benzofuran

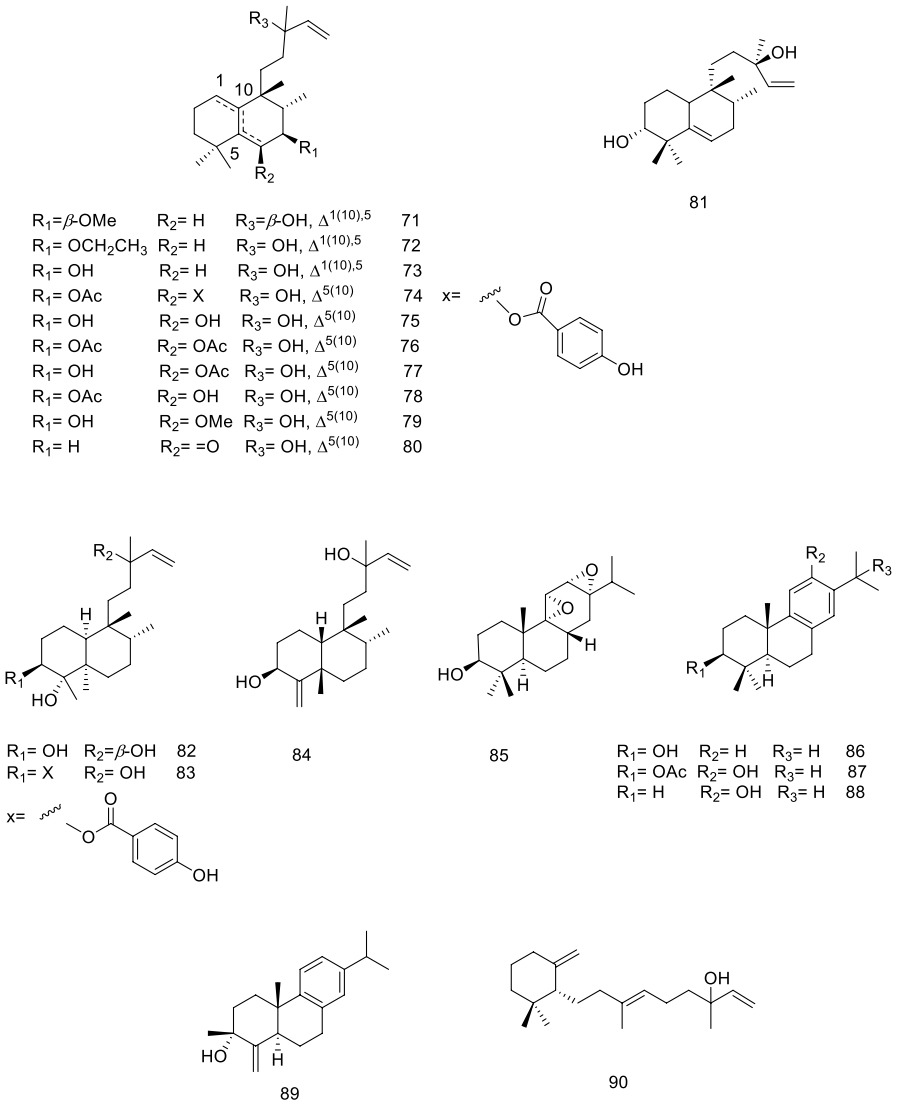

FIGURE 2
(Continued). Chemical structure of diterpenoids isolated from *V. trifolia*.

neolignan was separated from the fruit butanol extract (Gu et al., 2008). Recently, six new xanthone dimers (**161–166**) and seven known analogues (**167–173**) have been reported of ethyl acetate extract of *Diaporthe goulteri* L17, *vitex* fungi, phenylpropanoid, and xanthone metabolites of this species are represented in Figure 5 (Peng et al., 2021).

5.4 Miscellaneous

Only isolated anthraquinone, physcion **174**, was produced through the polyketide pathway (Chemical metabolites from fruits of *V. trifolia*). Benzoic acid and derivatization (**175–182**) instance vanillin **181** were isolated from different plant parts (Djimabi et al., 2021). Furthermore, saturated, and unsaturated long-chain fatty acids (**185–187**) and alcohol (**188**) were obtained from stems and leaves (Figure 6) (Quan-Yu Liu et al., 2014).

The investigation focused on the impact of four distinct drying methods on the antioxidant properties of *V. trifolia*: microwave-drying, oven-drying, sun-drying, and freeze-drying. The findings indicated that the non-thermal method—freeze-drying—and microwave-drying better preserved the antioxidant properties of the leaves compared to oven-drying and sun-drying, which both resulted in decreased antioxidant properties. In terms of total phenolic content (TPC) and total flavonoid content (TFC), *V. trifolia* leaves retained TPC and TFC values of 4,664 ± 109 GAE/100 g and 637 ± 10 mg QE/100 g, respectively. These values significantly surpass those of mature plants, which showed TPC of 3,229 ± 36 GAE/100 g and TFC of 87.0 ± 0.5 mg QE/100 g) (Chong and Lim, 2012; Chandrasekaran et al., 2019).

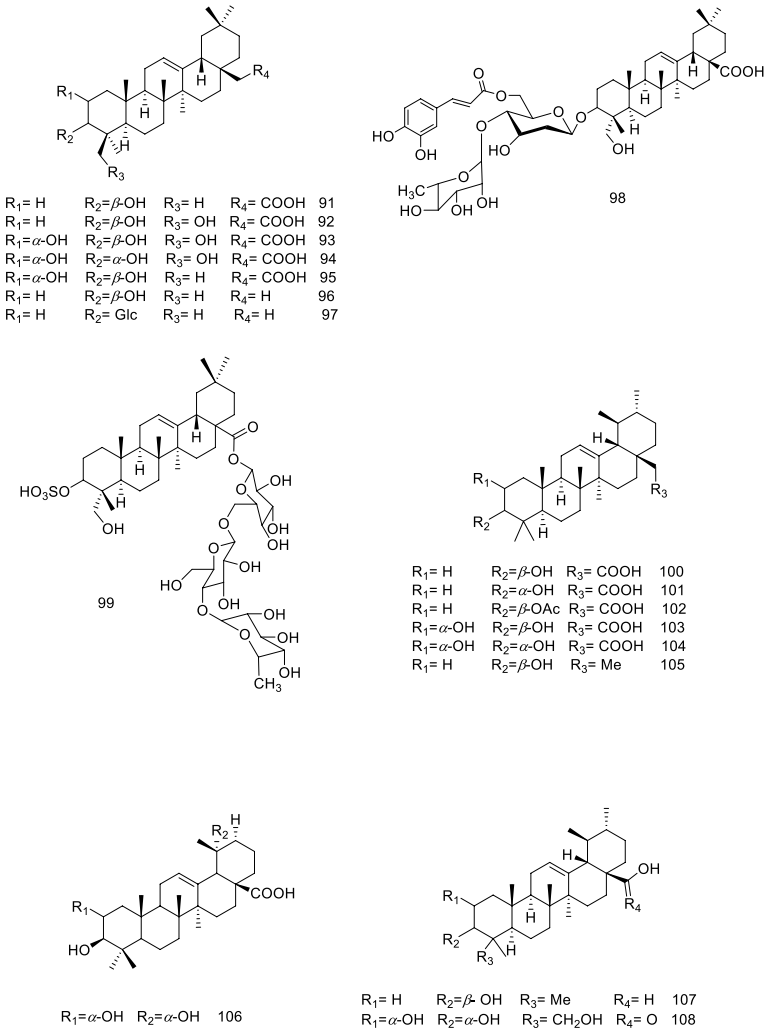

FIGURE 3
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5.5 Essential oils

Extensive analyses have been performed on essential oils (EOs) from various parts of the *V. trifolia* species, including seeds, leaves, aerial components, and flowers. Notably, an Indian research group achieved the highest yield of EOs from leaves at 5% utilizing steam distillation. Figure 7 illustrates the chemical structures of the major metabolites found within these EOs. *β*-caryophyllene (**202**), a volatile sesquiterpene commonplace across numerous *Vitex* species, has been consistently identified in *V. trifolia*, as referenced in nine previous studies, commanding a majority presence in five of these—specifically within the EOs derived from leaves and flowers (Suksamrarn et al., 1991; Musa et al., 2022). This metabolite has a defensive role in many plants, released in both direct and indirect defence mechanisms against insect and pathogens (Barreto et al., 2021). Moreover, 1,8-cineole (**197**) features prominently in *V. trifolia* essential oils. Variations in the yields and the makeup of EOs metabolites, as presented in Supplementary Table S2, are attributable to such factors as the harvest timing, environmental conditions (including altitude, climate, topography, and soil composition), as well as age and genetic type of the plants (Barra, 2009).

Overall, *V. trifolia* is particularly rich in monoterpene hydrocarbons, ranging from 4.04% to 44.57%, and oxygenated monoterpenes between 6.13% and 31.26%. Metabolites such as *cis*-ocimene (**199**) at 44.57%, *α*-pinene (**191**) ranging from 4.04% to 23.87%, sabinene (**193**) from 9.44% to 39.14%, 1,8-cineole (**197**) at 6.13%–31.26%, and terpinyl acetate (**198**) between 9.6% and 13.48% are among the key chemical constituents characterizing these oils.

6 Biological activities of *V. trifolia*

6.1 Bioactivities of the extracts

6.1.1 Antioxidant activity

Furthermore, an examination of the antioxidant activities of ethanol extracts from two *Vitex* species, *V. negundo L.*, and *V. trifolia* after 30 min exposure to DPPH, revealed that *V. negundo*

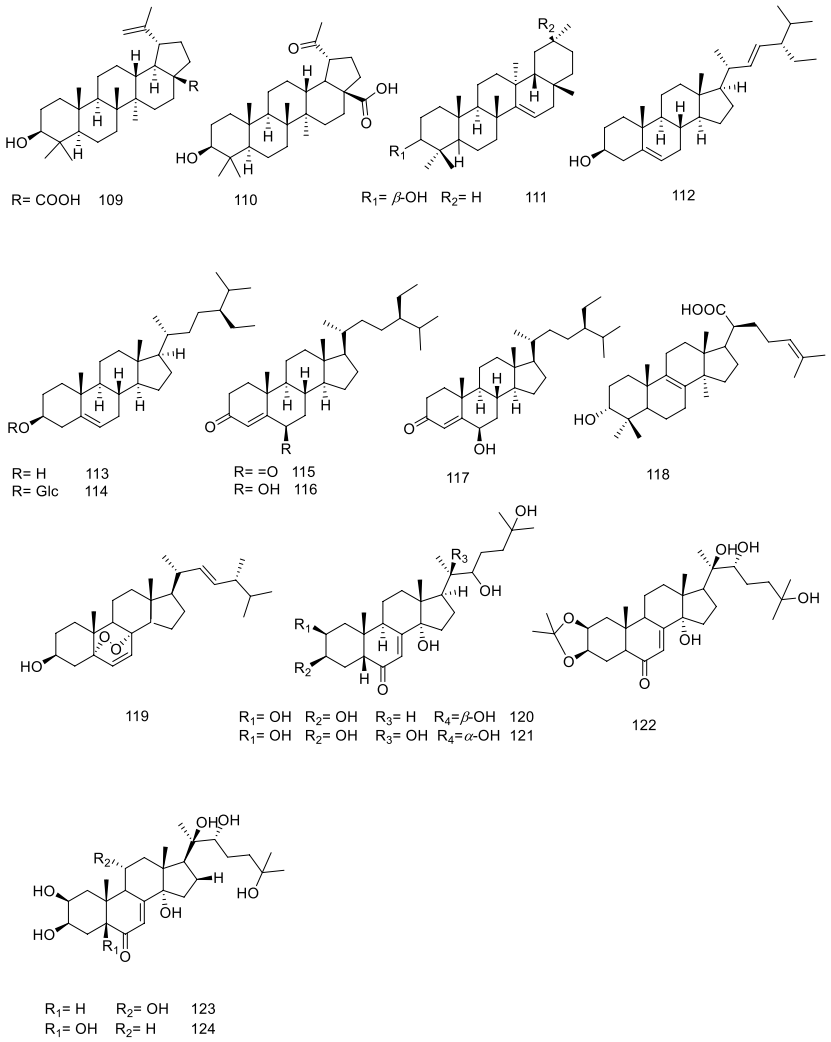

FIGURE 3
(Continued). Chemical structure of triterpenoids and phytosterols isolated from *V. trifolia*.

had greater antioxidant activity ranging from 62.6% to 94.22% with an IC_{50} value between 23.5–208.3 μg/mL. In comparison, *V. trifolia* exhibited a slightly lesser range of antioxidant activity from 60.87% to 89.99% with an IC_{50} value ranging from 40.0 to 226.7 μg/mL. Given the total phenolic and flavonoid content, *V. negundo* showed higher levels with 89.71 mg GAE/g dry weight of the extract and 63.11 mg QE/g dry weight of the extract, respectively, whereas *V. trifolia* had 77.20 mg GAE/g phenolics and 57.41 mg QE/g flavonoids by dry weight of the extract in the *in vitro* study (Saklani et al., 2017).

Additionally, *V. trifolia* demonstrated an IC_{50} value of 64.5 μg/mL in DPPH scavenging activity. This result supports the use of *V. trifolia* as a traditional remedy for ciguatera fish poisoning, endorsing its therapeutic efficacy (Kumar-Roiné et al., 2009).

6.1.2 Hepatoprotective activity

In an *in vivo* study the ethanolic extract of *V. trifolia* flowers at 200 mg/kg b. w. dose exhibited significant hepatoprotective activity against CCl_4-induced hepatic injury in rats after 7 days treatment, demonstrating remarkable hepatoprotective potential. The observed hepatoprotective effects of the tested metabolite were found to be comparable to those of the standard drug, silymarin, administered at a dosage of 100 mg/kg b. w. with 7 days exposure. This similarity is evident from the significant reduction in the serum levels of key liver enzymes, namely, glutamate pyruvate transaminase (SGPT) (342 U/l in the control group vs. 88 U/l in the treatment group), glutamate oxaloacetate transaminase (SGOT) (358 U/l in the control group vs. 136 U/l in the treatment group), and alkaline phosphatase (ALP) (416 U/l in the control group vs. 180 U/l in the treatment group). Additionally, there is a decrease in the levels of total bilirubin (1.2 mg/dL in the control group vs. 0.8 mg/dL in the treatment group) and gamma-glutamyl transpeptidase (GGTP) (251 U/l in the control group vs. 136 U/l in the treatment group), further confirming the hepatoprotective activity of the metabolite (Anandan et al., 2009). This reduction in enzyme levels underscores the therapeutic value of the treatment in maintaining the liver health.

In an *in vivo* study, the acute toxicity assessments of ethanol and aqueous leaf extracts of *V. trifolia* revealed LD_{50} values of 200 mg/kg b. w., p. o. and 300 mg/kg b. w., p. o., respectively. Subsequently,

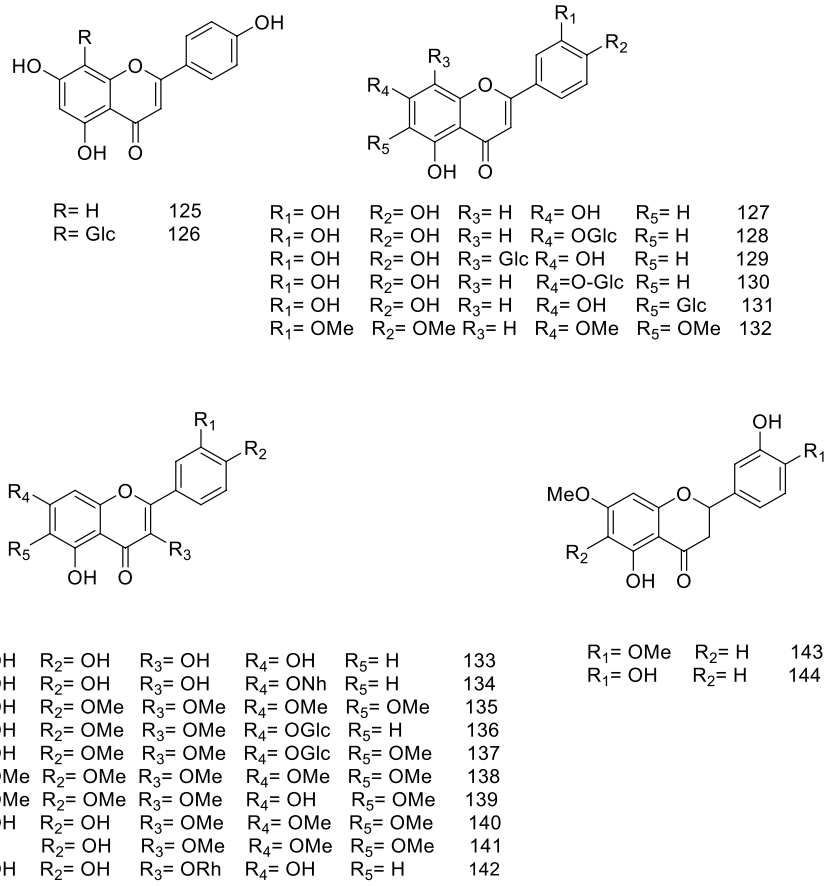

FIGURE 4
Chemical structure of flavonoids isolated from *V. trifolia*.

doses of 20 mg/kg b. w., p. o. and 30 mg/kg, b. w., p. o. of these extracts were selected to evaluate their hepatotoxic activity. The study demonstrated that both extracts, administered for 14 days, significantly reduced the total bilirubin levels. In the context of CCl_4-induced hepatic toxicity (control group), the total bilirubin levels were 2.50 ± 0.04 mg/dL in the CCl_4 group compared to 0.63 ± 0.01 and 0.93 ± 0.01 in the CCl_4 + ethanol extract and CCl_4 + aqueous extract groups, respectively. Furthermore, the serum marker enzymes showed a decrease in CCl_4-induced hepatic toxicity, as indicated by the ALT levels decreasing from 1,322.33 IU/L in the CCl_4 group to 106.43 IU/L and 124.17 IU/L in the CCl_4 + ethanol extract and CCl_4 + aqueous extract groups, respectively. Similarly, ALP levels reduced from 442.10 IU/L in the CCl_4 group to 202.47 IU/L and 236.91 IU/L in the CCl_4 + ethanol extract and CCl_4 + aqueous extract groups, respectively. Concomitantly, there was an increase in the total protein levels in the animal subjects experiencing CCl_4-induced hepatic toxicity, with values of 5.93 ± 0.01 g% in the CCl_4 group compared to 8.24 ± 0.03 and 8.05 ± 0.03 g% in the CCl_4 + ethanol extract and CCl_4 + aqueous extract groups, respectively. These findings suggest the potential hepatoprotective effects of the ethanol and aqueous leaf extracts of *V. trifolia*, as they alleviate CCl_4-induced hepatic toxicity by modulating key biochemical markers (Manjunatha and Vidya, 2008).

6.1.3 Antispasmodic activity

In an *in vivo* study the assessment of viteosin-A (**34**) and vitexicarpin (**135**), the primary active metabolites present in the n-hexane extract of *V. trifolia*, demonstrated that only vitexicarpin exhibited activity in the tracheospasmolytic bioassay. Notably, this activity was observed at a minimum dose of 1.3×10^{-5} M, for 30 min, utilizing sensitized guinea pig trachea stimulated by ovalbumin. The findings suggest that vitexicarpin could potentially hinder the effects of histamine released from sensitized mast cells by stabilizing the membrane function of the mast cells (Alam et al., 2002).

6.1.4 Anti-inflammatory activity

In an *in vivo* study, the anti-inflammatory properties of the hydroalcoholic extract of *V. trifolia* were investigated in rats at doses of 100 mg/kg b. w. and 200 mg/kg b. w. The study demonstrated that the higher dose exhibited an inhibitory effect, as evidenced in the paw volume of 0.37 mL ± 0.01 after 5 h, compared to the control group treated with indomethacin, which had a paw volume of 0.16 mL ± 0.01. The percentage inhibition of inflammation and edema formation at the end of 5 h was 72.23%, while indomethacin demonstrated a higher percentage inhibition of 90.46%. The mean values for total leucocyte count in the group treated with the hydroalcoholic extract were 9,333 ± 448 cells/cm^3, whereas animals treated with indomethacin showed a count of 11,717 ±

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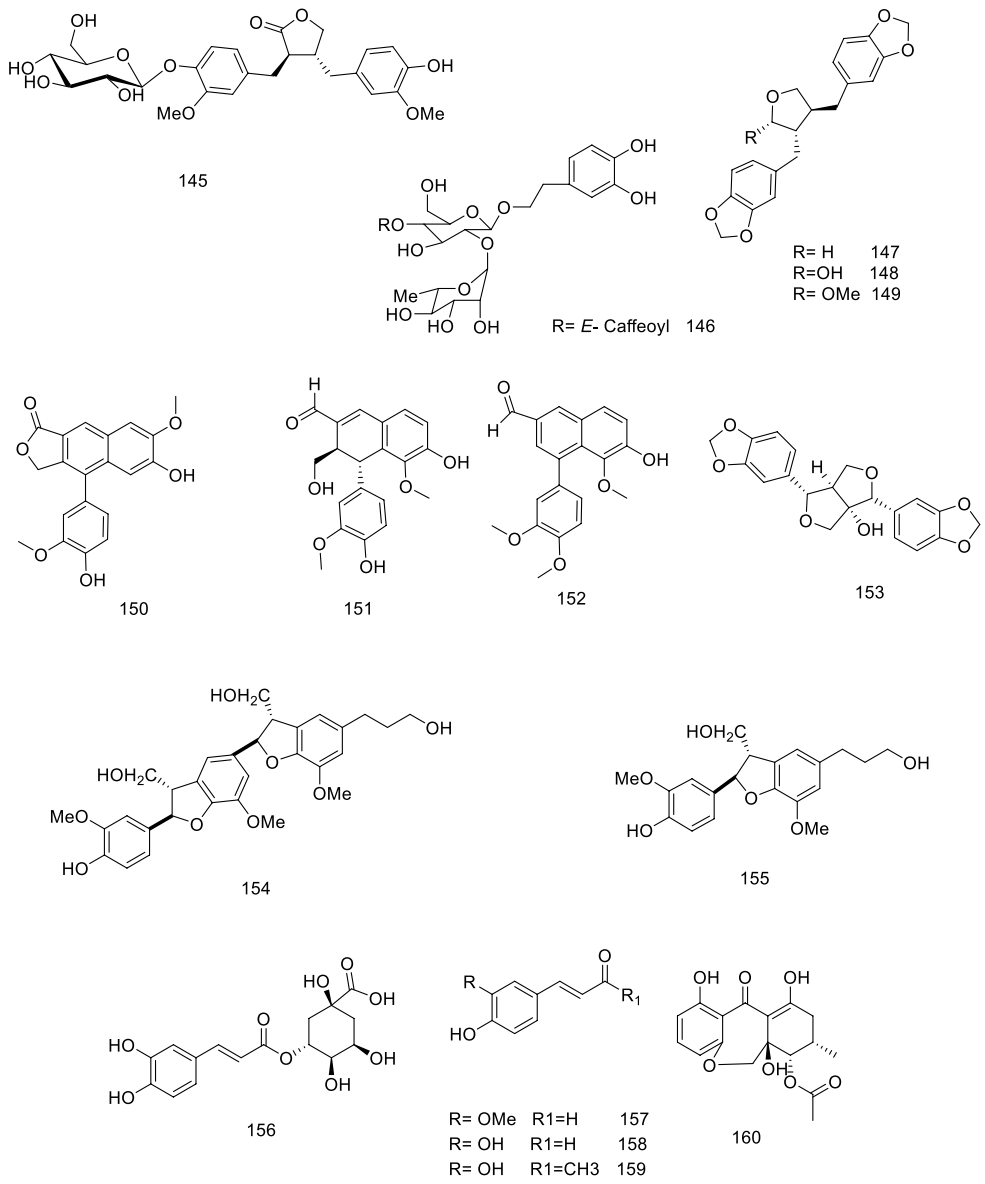

FIGURE 5
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444 cells/cm^3. In the group treated with 200 mg/kg b. w., the percentage of polymorphonucleocytes and lymphocyte count was 23.33% and 73.67%, respectively, while the indomethacin-treated group showed a count of 23.83% and 70.50%, respectively. Furthermore, the groups treated with the hydroalcoholic extract exhibited reduced levels of macrophages, mast cells, and other inflammatory mediators compared to the control group, indicating its potential to mitigate inflammation (Ankalikar and Viswanathswamy, 2017).

Of the 28 aqueous extracts of plants examined in an *in vitro* study, *V. trifolia* demonstrated a notable ability to inhibit NO production without inducing toxicity. Specifically, at a concentration of 0.25 g/L of the tested extract, aqueous extracts of *V. trifolia* exhibited significant NO inhibitory effects in RAW 264.7 cells that were exposed to 10 μg/mL of lipopolysaccharide (LPS) for 24 h (Kumar-Roiné et al., 2009).

The anti-inflammatory properties of leaf extracts from *V. trifolia* were investigated through animal studies. The results revealed that the extract, administered at 200 mg/kg b. w., exhibited dose-dependent anti-inflammatory effects. At this dosage, both aqueous and ethanolic extracts showed a significant activity ($p < 0.0001$) against the acute inflammatory response, demonstrating reductions of 46.91% and 60.49%, respectively. Although these values were less than that of the reference standard (70.27%) on xylene-induced ear edema, the ethanolic extract inhibited edema notably at the 3rd hour (43%) in carrageenan-induced paw edema compared to other treated groups. This effect was consistent in both carrageenan-induced rat paw edema and xylene-induced ear edema

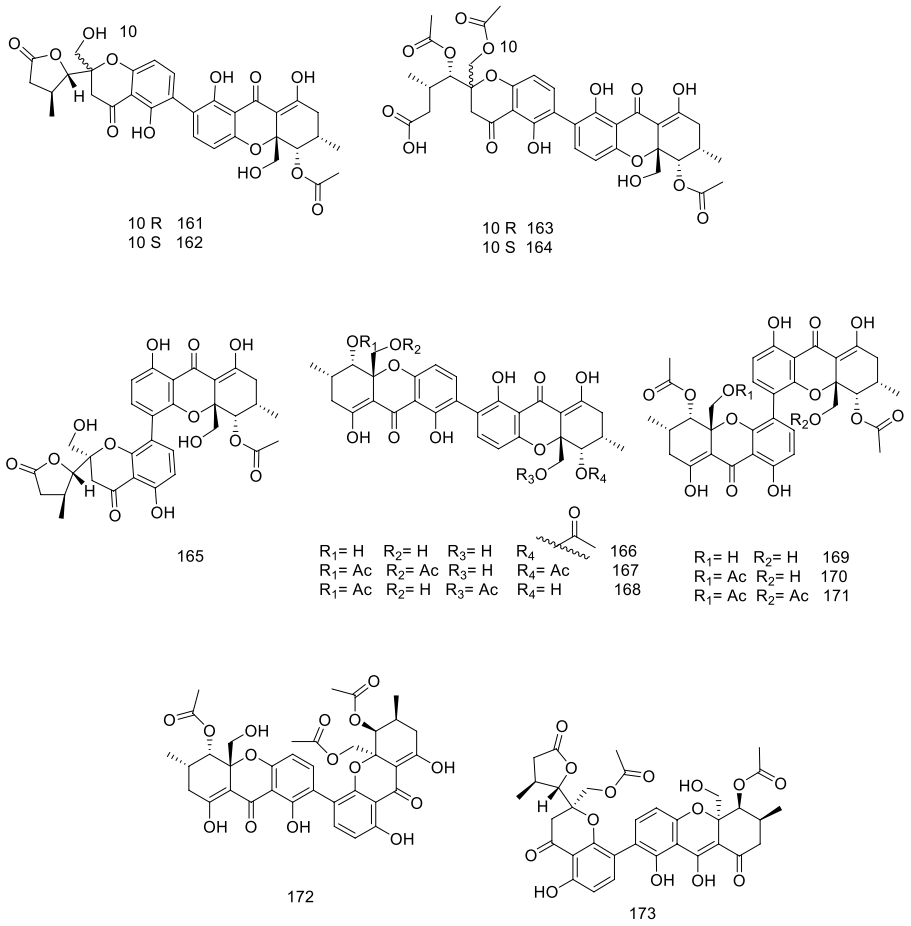

FIGURE 5
(Continued). Chemical structure of lignans, phenylpropanoids and xanthones isolated from *V. trifolia*.

in mice, underscoring the potential of *V. trifolia* as an effective anti-inflammatory agent (Kulkarni, 2011).

An aqueous extract of *V. trifolia* leaf at a concentration of 2,500 μg/mL exhibited an inhibitory activity on the synthesis of interleukin (IL)-1, IL-6, IL-10, and iNOS mRNA with a mild effect on tumor necrosis factor (TNF)-α. These findings indicate that the extract has the potential to modulate the expression of these inflammatory mediators, suggesting its anti-inflammatory properties (Matsui et al., 2009). Furthermore, in an *in vitro* study, it was found that the aqueous leaf extract of *V. trifolia* at a concentration of 5,000 mg/mL and 8 h incubation time displayed a notable inhibitory effect on the expression of multiple inflammatory genes induced by LPS in RAW 264.7 cells. This inhibitory effect was confirmed by a significant decrease in COX-2, CCL-3, and CXCL-10 mRNA production (between 3 and 4-fold compared to the control group). Moreover, the extract exhibited inhibitory activity on LPS-induced p50 mRNA synthesis, further supporting its anti-inflammatory properties (Matsui et al., 2012).

The effect of *V. trifolia* leaf extracts, obtained through different extraction methods, on cytokine production, such as IL-1β and TNF-α in human U937 macrophages, was examined in an *in vitro* study. Among various extraction techniques tested (Soxhlet, ultrasonication, and maceration) and using different solvents, the extracts prepared by maceration in ethanol and ultrasonication in dichloromethane exhibited the highest activity (60%–80% compared to untreated macrophages) in inhibiting IL-1β and TNF-α production in human U937 macrophages after 6 h incubation. These findings suggest that these specific extraction methods and solvents effectively extract bioactive metabolites from *V. trifolia* leaves with potent anti-inflammatory properties in macrophages (Wee et al., 2020).

6.1.5 Anticancer activity and toxicity data

Screening of five samples, namely, *Alpinia galanga* (L.) Willd. (Zingiberaceae), *Piper cubeba* L. f. (Piperaceae), and *Santalum album* L. (Santalaceae), along with *V. trifolia*. at a concentration of 25 μg/mL and incubated for 24 h, revealed a significant inhibitory activity against the T47D breast cancer cell line, with inhibition percentages of 96.4%, 87.6%, 82.6%, and 88.7%, respectively. Epirubicin and doxorubicin were used as positive control, and DMSO for negative control (Dai et al., 2018). The cytotoxic activities of the *V. trifolia* aerial parts were evaluated in an *in vivo* study using three different extracts: methanol, ethyl acetate, and chloroform. The brine shrimp bioassay method was employed for this purpose. The results indicated that the methanolic extract exhibited the highest cytotoxic activity, with an LC_{50} value of

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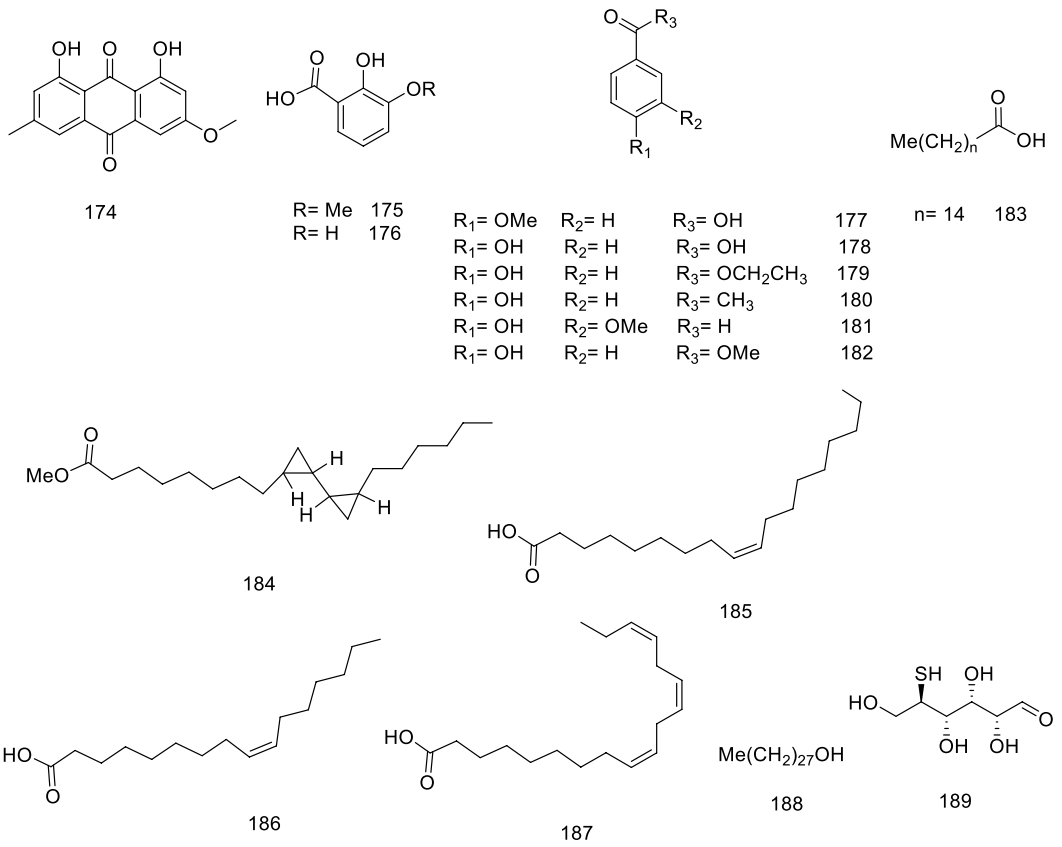

FIGURE 6
Chemical structure of Miscellaneous metabolites isolated from *V. trifolia*.

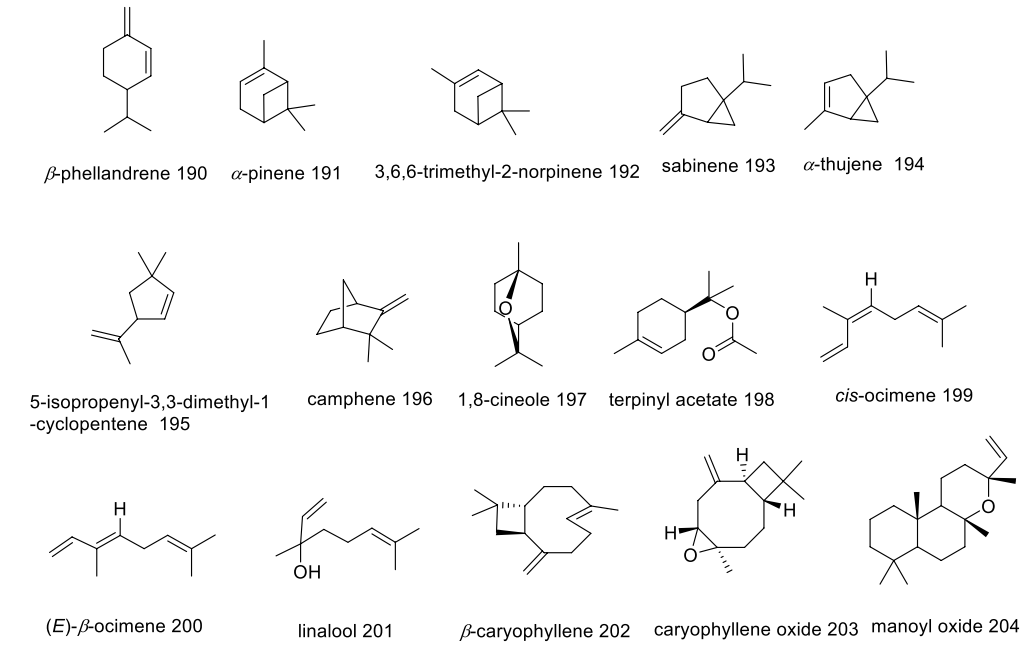

FIGURE 7
Chemical structures of the major secondary metabolites from *V. trifolia* essential oil.

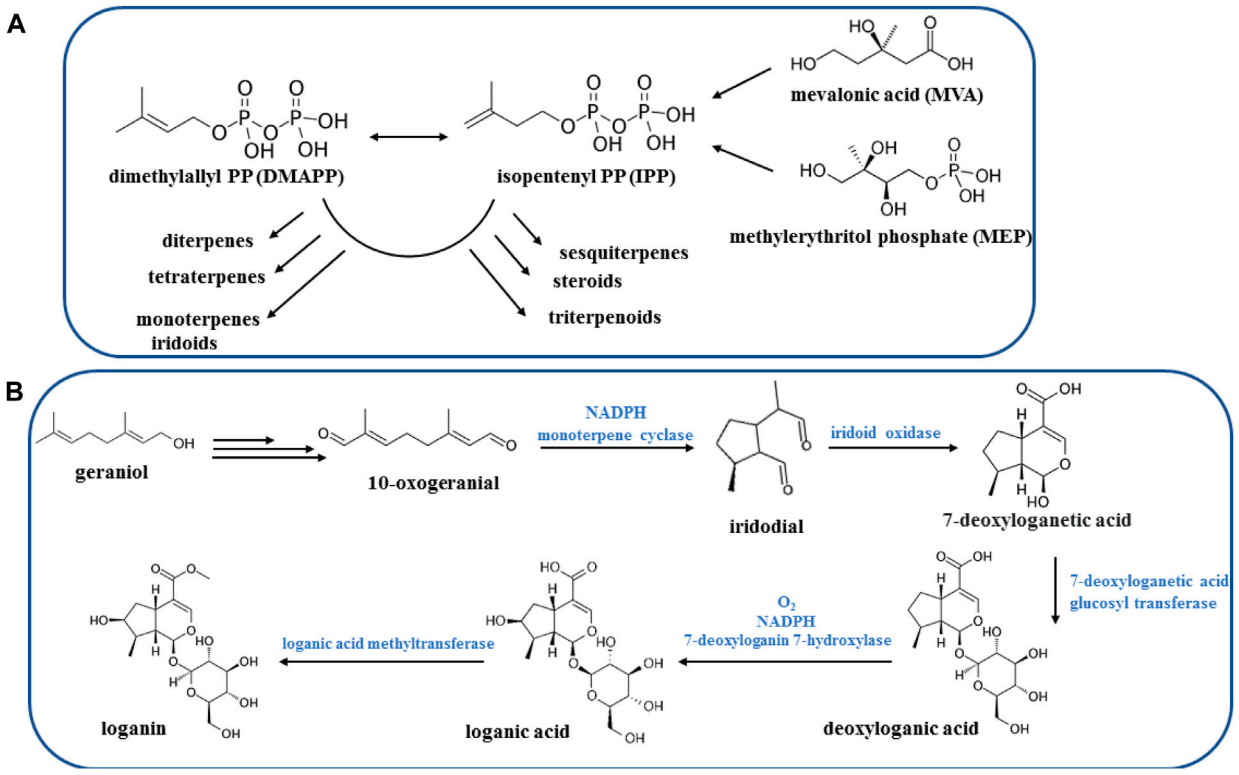

FIGURE 8
Biosynthesis pathways of terpenoids **(A)** and iridoids **(B)**.

140 mg/mL. The ethyl acetate extract showed slightly lower cytotoxicity, with an LC_{50} value of 165 mg/mL. Lastly, the chloroform extract displayed the least cytotoxicity among the three, with an LC_{50} value of 180 mg/mL, compared with potassium dichromate as the positive control (El-Kousy et al., 2012).

The cytotoxicity of different extracts from *V. trifolia* was assessed on brine shrimp and Hep-G2 cell lines. The tested extracts included the crude hot aqueous-methanol extract, chloroform-methanol extract, ethyl acetate methanol extract, and the residue from the methanol extract. Notably, the residue from the methanol extract exhibited the highest cytotoxic activity, showing an IC_{50} value of 6 μg/mL against Hep-G2 cells. The crude hot aqueous-methanol extract, chloroform-methanol extract, and ethyl acetate methanol extract demonstrated IC_{50} values of 10.7 μg/mL, 20.8 μg/mL, and 65.8 μg/mL, respectively, in comparison to the positive control. These findings indicate varying degrees of cytotoxicity against Hep-G2 cells for different *V. trifolia* extracts, with the residue from the methanol extract displaying the most potent activity. Interestingly, the same trend was observed in the brine shrimp assay (El-Sayed et al., 2011).

In an *in vitro* study, the dichloromethane extracts of *V. trifolia* leaf demonstrated significant toxicity against various cancer cell lines, including SQC-1 UISO, OVCAR-5, HCT-15 COLADCAR, and KB with ED_{50} of 2.2 mg/mL, 2.9 mg/mL, 1 mg/mL, and 1.9 mg/mL, after 72 h incubation respectively. These findings highlight the potential of *V. trifolia* leaf extracts as a source of metabolites with cytotoxic activity against different cancer cell lines (Hernandez et al., 1999).

The synergistic anticarcinogenic effects of ethanolic extracts from *V. trifolia* L. and *Triticum aestivum* L. (Poaceae) were investigated in an *in vitro* study. Both extracts demonstrated anti-degranulation properties individually, and their combination synergistically enhanced the anticarcinogenic potential. This was observed through their ability to inhibit 3,8-diamino-5-ethyl-6-phenylphenanthridinium bromide-induced liver microsomal degranulation. Additionally, these extracts exhibited inhibitory effects on cell proliferation in HCT116 and A549 cell lines. These findings highlight the potential of *V. trifolia* and *T. aestivum* extracts as synergistic agents in cancer prevention and treatment (Mathankumar et al., 2015).

Another *in vitro* study investigated the impact of gamma irradiation treatment at a dose of 7.5 kGy (source: ^{60}Co) on the dried coarse powder of legundi leaves, focusing on its potential as an anticancer agent and its chromatogram profile. The IC_{50} values of legundi leaf extract against MCF cancer cells after 72 h of incubation increased from 8.2 μg/mL to 12.1 μg/mL after exposure to the irradiation dose of 7.5 kGy. Similarly, for HeLa cells, the IC_{50} value increased from 7.6 μg/mL to 16.9 μg/mL, and for K-562 cells, it increased from 19.7 μg/mL to 22.4 μg/mL (Winarno et al., 2020).

6.1.6 Anti-amnesic activity

In the passive avoidance and T-maze models, a high dose (20 mg/kg, b. w.) of aqueous *V. trifolia* leaf extract demonstrated a significant ($p < 0.01$) anti-amnesic activity. The extract led to a notably shorter escape latency time (12s) compared to the control

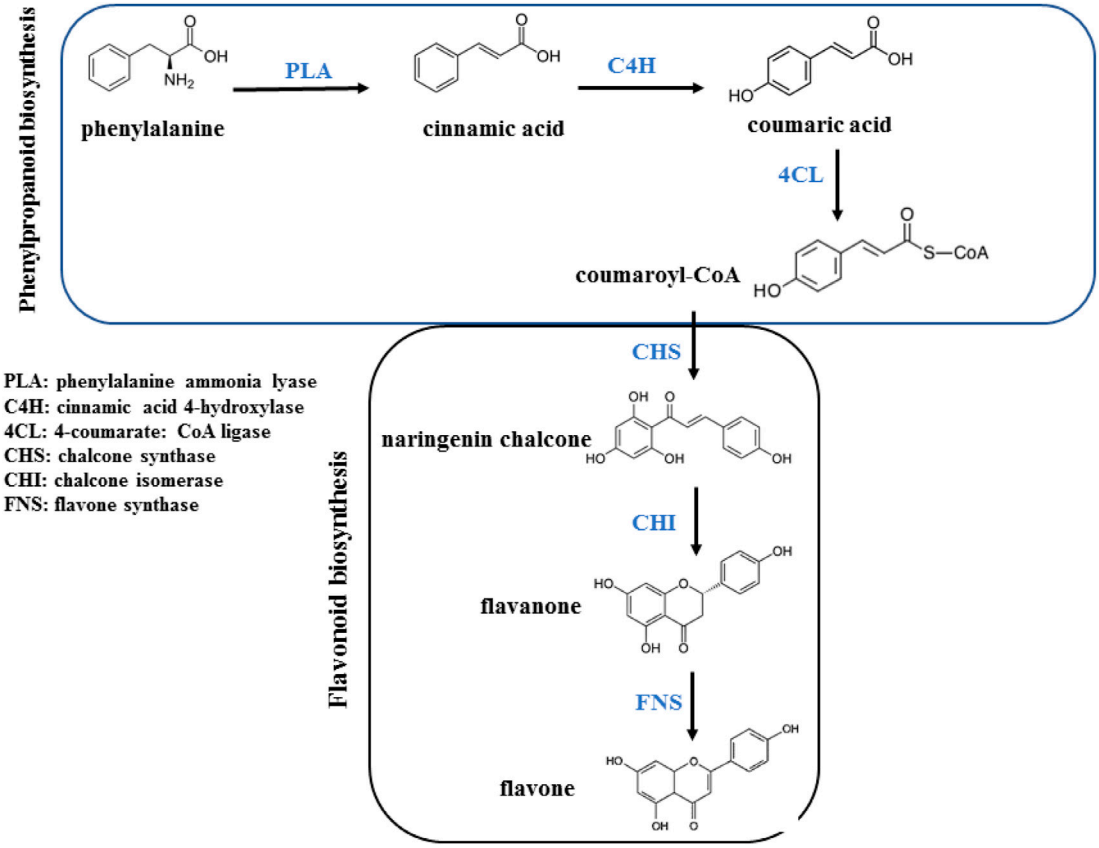

FIGURE 9
Biosynthesis of flavonoid.

group (29s) and showed a maximum percentage of time spent in the probe quadrant by 60.75%. This result was nearly twice as high as that of the control group, indicating improved memory retention compared to both the control and other treatment groups (Mohanbabu et al., 2015).

6.1.7 Larvicidal activity

Vitex trifolia demonstrated effective repellency against mosquitoes at a minimal dosage, as evidenced by Gou et al., in 2020 (Gou et al., 2020). Additionally, a study by Tandon et al., in 2008 compared the essential oils (EOs) of both *Vitex trifolia* and *Vitex agnus-castus* L. and found that although the EO of *Vitex agnus-castus* L. was less effective than that of *Vitex trifolia*, both EOs increased larval duration, larval mortality, pupal duration, and adult deformity, while decreasing adult emergence, fecundity, and egg fertility (Tandon et al., 2008).

Chandrasekaran et al. in an *in vivo* study conducted in 2019, revealed that the EO of *Vitex trifolia* exhibited potent larvicidal activity, resulting in 100% mortality against third instar stages of *Aedes aegypti* and *Culex quinquefasciatus* larvae at 125 ppm. The GC-MS analysis of the *Vitex trifolia* EO identified several bioactive metabolites, including eucalyptol (**197**), which accounted for 16.35% of the total peak area, followed by sabinene (**193**) with 9.44%, and *β*-caryophyllene (**202**) with 8.91%, which might contribute to its larvicidal properties (Chandrasekaran et al., 2019). Comparative evaluations of larvicidal efficacy among different Vitex species found that *Vitex trifolia* exhibited the highest larvicidal activity against *C. quinquefasciatus* larvae, with the most effective results. This was also observed in the investigation of the larvicidal activity of fatty acid methyl ester extracts, where *V. trifolia* demonstrated the highest larvicidal activity among the species tested. Furthermore, a comparative study investigated the effects of the extracts from three *Vitex* species on *Anopheles gambiae* s.s. larvae, revealing that the methanol extract of *V. trifolia* leaves and acetone extracts of *V. schiliebenii* stem bark and leaves, as well as *V. payos* (Lour.) Merr. root bark, exhibited significant potency, causing 100% mortality at a concentration of 100 ppm within 72 h. The larvicidal efficacy of four different *Vitex* species was evaluated against *C. quinquefasciatus* larvae, revealing that *V. trifolia* exhibited the highest larvicidal activity with an LC_{50} value of 41.41 ppm (Kannathasan et al., 2007). Among the species examined for their fatty acid methyl ester extracts, *V. trifolia* demonstrated the highest larvicidal activity with an LC_{50} value of 9.25 ppm, highlighting its potent properties (Kannathasan et al., 2008).

Additionally, a comparative *in vivo* study on the effects of extracts from three *Vitex* species on *A. gambiae s.s. larvae* at concentrations ≤ 50 ppm and 24 h time interval, found significant potency in the methanol extract of *V. trifolia* leaves, acetone extracts of *V. schiliebenii* stem bark and leaves, and acetone extract of *V. payos* (Lour.) Notably, *V. schiliebenii* and *V. payos* extracts demonstrated a faster mortality rate in *A. gambiae* s.s. larvae

compared to *V. trifolia*, indicating their potential as effective agents for controlling *A. gambiae* s.s. larvae, and their promise for further investigation in mosquito control programs to combat malaria transmission (Nyamoita et al., 2013).

6.1.8 Antimicrobial activity

The *V. trifolia* leaf extracts, tested at a concentration of 200 μg/mL for 30 min incubation time, demonstrated varying degrees of inhibition against different microorganisms in an *in vitro* experiment. The inhibition zone sizes (in mm) for each tested organism were as follows: *Bacillus subtilis*: 15.3 mm; *Staphylococcus aureus*: 14.0 mm; *Pseudomonas aeruginosa*: 13.6 mm; *Proteus mirabilis*: 13.5 mm; *Candida tropicalis*: 12.8 mm; and *Escherichia coli* (*E. coli*): 12.5 mm; *Candida albicans*: 12.0 mm. The zinc oxide nanoparticles (ZnO NPs) coated with an extract of *V. trifolia* exhibited improved MIC value compared to uncoated ZnO NP. The increased antimicrobial activity of the *V. trifolia* leaf extract can be attributed to the presence of vitrifolin A (**59**), the major metabolite in the extract. Vitrifolin A is believed to play a crucial role in enhancing the antimicrobial properties of the extract. It achieves this by binding to the surface of nanoparticles, thus leading to a more effective and targeted delivery of antimicrobial agents. This mechanism enables vitrifolin A to exert a stronger impact on the microorganisms, contributing to the overall enhanced activity of the leaf extract against a range of pathogens (Elumalai et al., 2015).

The *in vivo* antibacterial activity of the ethanolic extract of *V. trifolia* leaves was evaluated at concentrations of 1%, 5%, or 25% against *S. aureus* in the *Drosophila* infection model. The results indicated that 5% and 25% concentrations of the extract exhibited comparable activities. Therefore, the findings suggest that a 5% extract concentration would be sufficient to combat *S. aureus* infection (Sukarsih et al., 2021). Moreover, petrol extract (500 μg/disk) and EtOH extract (400 μg/disk) of *V. trifolia* leaves were moderately active against most of the tested Gram-positive and Gram-negative bacteria except *Klebsiella sp*., *Vibrio chole*ra, and *Vibrio mimicus* with a diameter of the zone of inhibition in the range of 8–15 mm (Hossain et al., 2001).

The *in vitro* antibacterial activity of the leaf methanol extracts of *Vitex altissima* L. f, *Vitex diversifolia* Bak., *Vitex negundo* L., *Vitex peduncularis* Wall. ex Schauer and *Vitex trifolia* was examined. *V. peduncularis* showed the highest antimicrobial activity with a zone of inhibition ranging between 11.00 and 22.67 mm; the MIC values were from 62.5 to 1,000.0 μg/mL and the MBC values were from 125.0 to 2000.0 μg/mL (Kannathasan et al., 2011). Methanol, ethanol, and ethyl acetate extract *V. trifolia* were prepared to evaluate the antibacterial activities and showed MIC values of 25, 50, 50, 50, 50, and 25 against *E. coli*, *S. flexneri*, *P. mirabilis*, *P. diminuta*, *E. cloacae,* and *S. aureus* ATCC 6538, respectively (Natheer et al., 2012). Screening of antimicrobial activities on the methanolic extracts of *V. trifolia*, against common freshwater pathogens showed an inhibition zone of 15 mm for *A. hydrophila* and 11 mm for *S. agalactiae*, with no inhibition against *E. cloacae*. Preliminary phytochemical screening of the plant extract showed the presence of tannins, flavonoids, and glycosides (Manaf and Daud, 2016). Antibiofilm screening of *V. trifolia* against *H. pylori* was moderately active (15 mm) with around 60% inhibition at 100 μM (Prasad et al., 2019).

In another *in vitro* study, the antifungal and cytotoxic activities of hexane, methanol, and distilled water extracts of *V. trifolia* were screened against six standard organisms. Results showed that all three extracts were active against *Ceratocystis paradoxa* with MIC in the range of 1.25–5.0 mg/mL, and methanolic and hexanoic extracts showed MIC values of 1.25 and 2.5 mg/mL, respectively. However, these extracts were not potent against *A. niger, P. citrinum*, *M. phaseoli*, and *R. nigricans* (Haripyaree et al., 2021). The hexanoic extract obtained from *V. trifolia* leaves showed remarkable efficacy against the fungal plant pathogen *Fusarium* sp. Within the first 2 days of the experiment, the hexanoic extract completely inhibited the growth of the *Fusarium* sp. However, its inhibitory activity dropped significantly to 15% on day six of the experiment. On the other hand, the dichloromethane extract displayed a significant growth inhibition of 54% against *Fusarium* sp. within 4 days of the experiment (Hernandez et al., 1999).

6.1.9 Antiviral activity

V.trifolia demonstrated significant antiviral activity against *Molluscum contagiosum* and *Herpes simplex*, with effective concentrations of approximately 0.25 μg/mL and 0.5 μg/mL, respectively, at a 0.4 μg/mL concentration in an *in vitro* assay. Importantly, this antiviral efficacy was achieved without causing notable toxicity. These findings highlight the potential of *V. trifolia* as a promising natural source for developing safe and effective antiviral agents. Further exploration into the specific bioactive metabolites and their mechanisms of action, as well as broader applications in clinical settings, would enhance our understanding of the therapeutic potential of *V. trifolia* in antiviral interventions (Vimalanathan et al., 2009).

6.1.10 Anti-HIV activity

In a research study, the impact of aqueous and 80% ethanol extracts from 20 medicinal plants of Thai on HIV type 1 reverse transcriptase activity was investigated. The results revealed that the water extracts of *Vitex glabrata* R. Br. (branch), *V. trifolia*. (aerial part), and *Vitex negundo* L. (aerial part) displayed a remarkably good inhibition ratio (% IR) higher than 90% at a concentration of 200 μg/mL in 1 h incubation. Doxorubicin hydrochloride, as a positive control, inhibited the HIV-1 RT activity at 1 mM by 98.3%. These findings suggest that these specific extracts from *V. glabrata*, *V. trifolia*, and *V. negundo* possess a strong potential as candidates for further investigation in the development of anti-HIV therapies due to their significant inhibitory effects on HIV-1 reverse transcriptase activity (Woradulayapinij et al., 2005).

6.1.11 Anti-malaria activity

In an investigation utilizing semi-structured questionnaires and informant interviews to gather knowledge about plants associated with malaria and related symptoms, the antimalarial potential of the extracts from 70 plant species, representing 62 genera and 34 families, was evaluated. The results highlighted Solanaceae as the most frequently cited family, with 7 species showing promising antimalarial properties. Noteworthy results were observed within the Lamiaceae family, specifically *Vitex negundo* L. and *Vitex trifolia* L., identified as antimalarial agents, with documentation from the Soon Valley region in Pakistan for the treatment of malaria. These findings underscore the ethnobotanical importance of certain plant

species within communities for their potential antimalarial properties (Shah and Rahim, 2017).

6.1.12 Respiratory disorder

In an *in vitro* study screening the inhibitory effect of alcoholic and hexanoic extracts of *V. trifolia* on histamine release from RBL-2H3 cells revealed that 0.5 mg/mL resulted in more than 80% inhibition of IgE-dependent histamine release from RBL-2H3 cells (Ikawati et al., 2001). A separate study demonstrated that combining *Curcuma xanthorrhiza* Roxb. rhizome (Zingiberaceae; *Curcumae xanthorrhizae* rhizoma), *V. trifolia* leaves, *Zingiber officinale* Roscoe. rhizome (Zingiberaceae; *Zingiberis* rhizoma) and *Echinacea purpurea* (L.) Moench herb (Asteraceae) exhibited synergistic immunomodulatory effects. The combination, administered at 490 mg/kg and 980 mg/kg, significantly enhanced the macrophage phagocytic index, reaching 15.29 μgr/mL and 26.78 μgr/mL, respectively. These values were compared to *E. purpurea* as a positive control, with a phagocytic index of 12.53 ± 1 μgr/mL at 750 mg. Moreover, the combination increased the production of IgG antibodies, with concentrations reaching 35 μgr/mL and 38 μgr/mL at doses of 490 mg/kg and 980 mg/kg, respectively. Again, these values were compared to *E. purpurea* as a positive control, which showed a 35 μgr/mL concentration at 750 mg. This study highlights the potential synergistic immunomodulatory effects of combining these botanical drugs (Ikawati et al., 2019).

6.1.13 Wound healing effect

In a comparative analysis of wound healing potential, the ethanol leaf extract of *V. trifolia* demonstrated superior activity compared to *Vitex altissima* L. f.. The incision wound tissue tensile strength for the positive control was 600.00, while it was 578.20 for *V. trifolia* and 529.08 for *V. altissima*. Hydroxyproline levels, indicative of collagen formation, were higher in the ethanol leaf extract of *V. trifolia* (2,567 μg/100 mg) compared to *V. altissima* (2012 μg/100 mg), with a negative control registering at 1943 μg/100 mg. Granuloma dry weight, a measure of tissue healing, was notably higher in *V. trifolia* (157.30 mg/100 g) compared to *V. altissima* (136.50 mg/100 g), with the control at 33 mg/100 g. Additionally, in dead space wound tissue tensile strength, *V. trifolia* demonstrated enhanced strength (491.20 g) compared to *V. altissima* (430.50 g), surpassing the positive control (181 g). These findings indicate that *V. trifolia* significantly improves the quality of wound healing and scar formation, outperforming *V. altissima* in various wound healing parameters (Manjunatha et al., 2007).

6.2 Bioactivities of pure metabolites

Labdane-type diterpenes isolated from *V. trifolia*, namely, vitexilactone (**30**), (5S,6R,8R,9R, 10S)-6-acetoxy-9-hydroxy-13 (14)-labden-16,15-olide (**39**), rotundifuran (**26**), vitetrifolin D (**76**), and vitetrifolin E (**77**), exhibited remarkable effects on cellular processes. In an *in vitro* study, at higher concentrations (100.0 μg/mL), these metabolites demonstrated a significant induction of apoptosis in both tsFT210 and K562 cells. Conversely, at lower concentrations, they impeded the cell cycle progression of both tsFT210 and K562 cells, specifically at the G0/G1 phase (Li et al., 2005b).

Six flavonoids, persicogenin (**143**), artemetin (**138**), luteolin (**127**), penduletin (**141**), vitexicarpin (**135**) and chrysosplenol-D (**140**), were isolated from *V. trifolia* inhibited the proliferation of sFT210 cancer cells with the IC_{50}s > 100 μg/mL (inhibition rate at 100 mg/mL was 21,47.9%) for persicogenin > 100 μg/mL (inhibition rate at 100 mg/mL was 49.6%) for artemetin, 10.7 μg/mL for luteolin, 19.8 μg/mL for penduletin, 0.3 μg/mL for vitexicarpin, and 3.5 μg/mL for chrysosplenol-D in 17 h treatment. It was shown that the mentioned metabolites exerted their anti-proliferative effect on tsFT210 cells *via* inhibiting the cell cycle and inducing apoptosis (Li et al., 2005c).

A variety of diterpenoids, including vitetrifolin I, D, E, F, H, (**75–79**), vitexoid (**15**), as well as vitexilactone (**30**), 6-acetoxy-9-hydroxy-13 (14)-labdane-16,15-olide (**39**), previtexilactone (**41**), and 6-acetoxy-9,13; 15,16-diepoxy-15-methoxylabdane (**53**), were isolated from the fruits of *V. trifolia*. These metabolites demonstrated inhibitory effects on the proliferation of Hela cells, with IC_{50} values ranging from 4 to 28 μM. Vitetrifolin I exhibited the strongest potency, inducing cell cycle arrest at the G0/G1 phase and promoting apoptosis in Hela cells (Wu et al., 2009a). On the other hand, seven labdane-type diterpenoids, namely, vitextrifolins A–G (**18–19**, **21–25**), derived from the fruits of *V. trifolia*, did not exhibit significant toxicity (IC_{50} < 5 μg/mL) against various cell lines, including human colon carcinoma (HCT116) in an *in vitro* study, human lung adenocarcinoma (A-549), human promyelocytic leukaemia (HL-60), and human breast carcinoma (ZR-75–30) (Zheng et al., 2013a). Twenty-seven diterpenoids derived from *V. trifolia* were examined for their inhibitory activity against DNA topoisomerase I. Among these metabolites, vitextrifloxide G (**72**) and vitextrifloxide I (**83**) demonstrated remarkable potency by exhibiting more than 81% inhibition at a concentration of 100 μM. To further assess the effectiveness of vitextrifloxide G, Luo et al.evaluated it using the MTT method in human colorectal carcinoma cells (HCT116), resulting in an IC_{50} value of 20.3 μM (Luo et al., 2017c).

Diaporxanthone A (**161**) and diaporxanthone F (**166**) displayed notable antifungal properties against *Nectria sp*. and *C. musae* (ACCC 31244), in the *in vitro* study. Diaporxanthone A exhibited antifungal activity at a minimum dosage of 10.0 μg/scrip, while diaporxanthone F demonstrated effectiveness at a lower dosage of 2.5 μg/scrip (Peng et al., 2021).

In vitro minimum lethal concentrations of the isolated metabolites of *V. trifolia* against epimastigotes of *Trypanosoma cruzi* were 11 mM for 9,13-epoxy-16-norlabda-13*E*-en-15-aL (**61**), 36 mM for 6*β*-acetoxy-9*α*,13-epoxy-16-norlabd-13*E*-en-15-aL (**63**), 34 for mM vitexifolin E (**60**), 34 mM of vitexifolin F (**78**), 66 mM of vitexilactone (**30**), 66 mM of (6-acetoxy-9-hydroxy-13 (14)-labden-16,15-olide) (**39**), and 265 mM of previtexilactone (**41**) (Kiuchi et al., 2004). Besides the bioactivities of the isolated metabolites from *V. trifolia*, the following metabolites can be highlighted as the major known ones.

6.2.1 Casticin

Casticin (**135**), a flavonoid isolated from *V. trifolia*, has demonstrated anti-inflammatory and antitumor effects on ADTC5 cells. However, it exhibited significant toxicity at a concentration of 40 μM after 2 h of treatment. In a dose-dependent manner at concentrations of 10, 20, and 30 μM, in

2 h incubation time, casticin reduced proinflammatory cytokines such as IL-6, TNF-α, and prostaglandin E2 (PGE2), as well as oxidative stress markers including MDA and inducible nitric oxide synthase (iNOS) expression. Specifically, at 30 μM, casticin downregulated IL-6 protein expression in IL-1β-stimulated ADTC5 to 40 pgr/mL compared to 67 pgr/mL in the control group. It also downregulated TNF-α protein expression to 50 pgr/mL from 110 pgr/mL in the control group and reduced PGE2 protein expression to 600 pgr/mL from 1,150 pgr/mL in the control group. These findings highlight the potential of casticin in modulating inflammatory responses and oxidative stress (Chu et al., 2020).

In a murine asthma model, administering casticin at doses of 5 or 10 mg/kg b. w. effectively mitigated airway hyperresponsiveness, airway inflammation, and oxidative stress in the lungs. These beneficial effects were attributed to regulating Th2 cytokine and chemokine gene expression within the lung. Casticin also demonstrated significant suppression of proinflammatory cytokine levels and eotaxin. Specifically, casticin reduced the IL-6 levels compared to the ovalbumin (OVA)-induced asthma group (70.4 ± 4.4 pg/mL in the control group vs. 37.4 ± 4.1 pg/mL in the prednisolone positive control; casticin at doses of 5: 57.7 ± 5.9 pg/mL, and casticin at doses of 10: 42.1 ± 7.1 pg/mL). Furthermore, casticin increased the INF-γ levels compared to the OVA group (93.6 ± 11.7 pg/mL in the control group vs 49.6 ± 9.5 pg/mL in the prednisolone positive control; casticin at doses of 5: 109.5 ± 9.5 pg/mL, and casticin at doses of 10: 121.9 ± 23.7 pg/mL). Additionally, it successfully reduced the adherence of THP-1 monocyte cells to BEAS-2B cells by suppressing ICAM-1 expression, with ICAM-1 level decreasing from 1,300 pgr/mL in control to approximately 600 pgr/mL with 20 μM casticin. These findings underscore the potential of casticin as a therapeutic intervention for asthma-related inflammation (Liou et al., 2018).

6.2.2 Rotundifuran

Rotundifuran (**26**) demonstrates notable suppression of cervical cancer cell lines, particularly HeLa and SiHa cells, with an IC_{50} of less than 10 μM in 24 and 48 h treatments, indicating its potent anti-proliferative activity. This suppression is attributed to the induction of apoptosis *in vitro*, underscoring its potential as an effective antitumor agent. Antitumor properties of rotundifuranare associated with its capability to target ROS-induced mitochondrial-dependent apoptosis involving the MAPK and PI3K/Akt signalling pathways. *In vivo* studies further validate the antitumor effects of rotundifuran, showcasing a reduction in tumor size to around 190 mm^3 at 40 mg/kg, in comparison to cis-platinum treatment as a positive control at 3 mg/kg/3 days, resulting in tumor size of 200 mm^3. These findings highlight the promising potential of rotundifuran as a therapeutic agent against cervical cancer (Gong et al., 2021).

6.2.3 Artemetin

In an *in vitro* study, artemetin (**138**) demonstrated suppressive effects on TNF-α and IL-1β at concentrations of 50 μg/mL and 100 μg/mL, leading to a reduction of TNF-α levels to 20% and IL-1β levels to 30% of their original concentrations after 48 h treatment. Dexamethasone at 64.4 ng/mL was the positive control, exhibiting a 0.25 and 40-fold change, respectively. Moreover, after 48 h of incubation, artemetin displayed inhibitory effects on the cell growth in U937 macrophages, with IC_{50} values of 125.6 ± 15.3 μg/mL (323.4 ± 39.3 μM). These results suggest the potential of artemetin in modulating inflammatory responses and inhibiting the growth of U937 macrophages (Wee et al., 2020).

6.2.4 Methyl-p-hydroxybenzoate

In an *in vivo* study, the larvicidal activities of methyl-*p*-hydroxybenzoate (**182**), isolated from the methanol extract of *V. trifolia* leaves, were evaluated against early 4th instar larvae of *C. quinquefasciatus* and *A. aegypti* mosquitoes. Remarkably, the metabolite exhibited complete mortality of the larvae for both mosquito species at a concentration of 20 ppm. The LC_{50} values were 5.77 ppm for *C. quinquefasciatus* and 4.74 ppm for *A. aegypti*. These findings underscore the potent larvicidal activity of methyl-phydroxybenzoate against the larvae of these disease-carrying mosquito species, suggesting its potential application as an effective and eco-friendly agent in mosquito control programs (Kannathasan et al., 2011).

6.2.5 Vitepyrroloid A

The metabolite vitepyrroloid A (**64**), when evaluated on the human nasopharyngeal carcinoma cell line CNE1, exhibited cytotoxic activity with an IC_{50} value of 8.7 μM after 3 days. This is compared to cisplatin as positive control, which had an IC_{50} value of 4.6 ± 0.1 μM. These findings suggest the potential cytotoxic efficacy of the metabolite against CNE1 cells, although cisplatin demonstrated a slightly lower IC_{50} value in this context (Luo et al., 2017c).

6.2.6 Vitextrifloxide G

Vitextrifloxide G (**72**) exhibited an IC_{50} value of 20.3 μM against HCT 116 human colorectal carcinoma cells in an *in vitro* study (Luo et al., 2017c).

6.2.7 Vitexilactone

In an *in vitro* experiment, vitexilactone (**30**) demonstrated the ability to enhance lipid accumulation and promote the expression of adiponectin and GLUT4 on the membrane of 3T3-L1 cells. Moreover, it effectively reduced the size of adipocytes and suppressed the phosphorylation of IRS-1, ERK1/2, and JNK in 3T3-L1 cells through PPARγ mediation. These findings suggest that vitexilactone holds promise as a potential candidate for developing improved antidiabetic agents (Nishina et al., 2017).

6.2.8 (-)-O-Methylcubebin

In an *in vitro* study, (-)-*O*-methylcubebin (**149**) exhibited significant antidiabetic properties at doses ranging from 1.5 to 50 μM. (-)-*O*-Methylcubebin showed no toxicity at 50 μM or less after 48 h of incubation in 3T3-L1 cells. It reduced the size of the adipocyte and facilitated the expression of proteins associated with adipogenesis, including adiponectin. Molecular analysis revealed that methylcubebin acted as an agonist for PPARγ, thereby promoting adipogenesis by inhibiting the phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2) and p38MAPK (Ukiya et al., 2019a).

6.2.9 Vitexicarpin

Vitexicarpin (**135**) exhibited substantial inhibitory effects on the growth of human cancer cells. In *an in vitro* study, it displayed IC_{50} values of 0.44 μM and 0.28 μM against HT-1080 and K562 cells, respectively, after 24 h and IC_{50} values of 19 μM and 0.66 μM against A2780 and HCT-15 cells after 48 h of treatment. This metabolite also led to distinct morphological changes indicative of apoptosis, and flow cytometric analysis revealed a dose-dependent sub-G0/G1 peak (Wang et al., 2005).

6.2.10 9-Hydroxy-13 (14)-labden-15,16-olide

9-hydroxy-13 (14)-labden-15,16-olide (**36**) displayed noteworthy efficacy against *Mycobacterium tuberculosis* H37Rv, with a MIC of 100 μg/mL in an *in vitro* study, compared to streptomycin, a reference antibiotic, exhibited a lower MIC value of 2.0 μg/mL. These results highlight the potential antimycobacterial activity of 9-hydroxy-13 (14)-labden-15,16-olide, despite its MIC being higher than the reference streptomycin (Tiwari et al., 2013).

6.2.11 Isoambreinolide

In the BACTEC-460 assay, isoambreinolide (**55**) displayed notable antimycobacterial properties, demonstrating significant antitubercular activity against *M. tuberculosis* H37Rv. The MIC of isoambreinolide was determined to be 25 μg/mL, while streptomycin, a reference antibiotic, showed a lower MIC value of 2.0 μg/mL. These results indicate the potential of isoambreinolide as an antimycobacterial agent, albeit with a slightly higher MIC than the reference (Tiwari et al., 2013).

6.2.12 Diaporxanthone D

In an *in vitro* experiment, diaporxanthone D (**164**) exhibited notable cytotoxic effects on EC109, A2870, HepG2, PC3, A549, and HBE cell lines, with IC_{50} values of 1.88, 8.11, 4.49, 1.66, 8.4 and 6.5 μM, respectively (Peng et al., 2021).

6.2.13 Miscellaneous

In an *in vitro* study, Bao et al. evaluated diterpenoid glucoside, (3S,5S,6S,8R,9R, 10S)-3,6,9-trihydroxy-13 (14)-labdean-16,15-olide 3-*O*-*β*-D-glucopyranoside (**69**), and an iridoid glucoside, (1S, 5S,6R, 9R)-10-*O*-*p*-hydroxybenzoyl-5,6*β*-dihydroxy iridoid 1-*O*-*β*-D-glucopyranoside (**3**) along with viteagnuside A (**70**), 10-*O*-vanilloylaucubin (**2**), agnusoside (**6**), nishindaside (**7**), 3-normal-butyl-nishindaside (**8**) and 3-normal-butyl-isonishindaside (**9**) isolated from *V. trifolia* on nitric oxide production in LPS-induced RAW 264.7 macrophages. Among the tested metabolites, (1S,5S,6R, 9R)-10-*O*-*p*-hydroxybenzoyl-5,6*β*-dihydroxy iridoid 1-*O*-*β*-D-glucopyranoside, 10-*O*-vanilloylaucubin, agnusoside, and 3-normal-butyl-nishindaside displayed moderate inhibitory activities, with IC_{50} values of 90.05 μM, 88.51 μM, 87.26 μM, and 76.06 μM, respectively. These values were compared to hydrocortisone as a positive control, which exhibited an IC_{50} value of 58.79 ± 3.32 μM (Bao et al., 2018).

7 Therapeutic development goals

Utilizing the identified bioactive metabolites, the initiation of a new phase of research focuses on the targeted design and experimental testing of pharmaceutical interventions. With this strategic approach, the intention is to position *V. trifolia* and its bioactive metabolites as potential sources for the development of effective treatments across a broader spectrum of diseases, including neurodegenerative disorders, cardiovascular diseases, metabolic disorders, and infectious diseases. This potential is attributed to their high concentrations of antioxidants, phenolic metabolites, and flavonoids. By broadening the focus beyond the initial therapeutic targets, efforts will be made to uncover new possibilities and contribute to the growing body of knowledge on the versatile pharmacological effects of *V. trifolia*.

Beyond the traditional approach to drug development, cutting-edge methodologies are incorporated to harness nanotechnology for enhanced drug delivery and improved bioavailability. The feasibility of nanoformulations derived from *V. trifolia* should be investigated, exploring their potential in targeted drug delivery systems designed to enhance efficacy, reduce side effects, and facilitate the penetration of bioactive metabolites into specific cells or tissues.

Finally, the intention was to present a roadmap for future research directions, emphasizing the translation of laboratory findings into clinical applications and the continued investigations into the therapeutic potential of *V. trifolia* and its bioactive metabolites. This comprehensive approach aims to bridge the gap between traditional knowledge and practical medical applications, with the boundaries of *V. trifolia* research intended to improve healthcare.

8 Conclusion

This comprehensive review has documented the intricate phytochemical profile of *V. trifolia*, particularly emphasizing its diverse ethnomedicinal applications. The identification of a myriad of bioactive compounds, encompassing a broad spectrum of terpenoids, flavonoids, lignans, phytosterols, phenylpropanoids, and xanthones, with a notable abundance of labdane diterpenoids, adds depth to our understanding.

Furthermore, the elucidation of the biosynthetic pathways for terpenoids, flavonoids, and iridoids provides a crucial foundation for exploring the molecular mechanisms underlying the synthesis of these bioactive constituents in *V. trifolia*.

Modern pharmacological investigations affirm the extensive therapeutic role of *V. trifolia* in addressing diverse health issues, ranging from tendon-and-bone-related conditions to infections and inflammations. Notably, the research underscores the potent biological activities of the plant, highlighting its antioxidant, anti-inflammatory, hepatoprotective, and anti-cancer properties. The confirmed antimicrobial, antiviral, anti-malarial, and anti-spasmodic effects further underscore the medicinal efficacy inherent in *V. trifolia*.

Moreover, advancements in analytical techniques offer deeper insights into the phytochemistry of *V. trifolia*, paving the way for identifying and characterizing novel secondary metabolites. Integrating highly precise analytical methods with bioassay-guided fractionation enriches our understanding of the plant's phytochemistry and establishes a framework for future pharmacological explorations. This review, thus, contributes to the evolving landscape of *V. trifolia* research, providing a platform for continued investigation into its therapeutic potential.

Furthermore, due to the lack of clinical trials, our study emphasizes the necessity to conduct such trials to test the plant products' efficacy. However, it is essential to highlight that further toxicological investigations are required to explore safety aspects comprehensively. Moreover, investigating the bioavailability of the plant's bioactive chemicals provides valuable insights into their absorption and distribution, making a substantial contribution to a more profound understanding of their potential applications.

The investigation of medicinal plants, including *V. trifolia*, encounters challenges and limitations such as variability in bioactive compounds, complex synergistic effects, and a lack of standardization; asides from limited clinical evidence, potential adverse effects, regulatory issues, and ethical concerns in harvesting further constrain research. Our study proposes conducting rigorous scientific investigations and fostering collaboration to bridge the gap between traditional and modern methodologies, using *V. trifolia* as a focal point.

9 Perspectives

The exploration of the compounds within *V. trifolia* is recommended, with a specific focus on integrating advanced bioinformatics tools, particularly machine learning algorithms, and simulated modelling applications such as Swiss ADME, Pkcsm, and Qikprop. The objective is to anticipate the potential of predicting the interactions and characteristics of the bioactive compounds present in *V. trifolia*. The application of predictive modelling holds promise in the pathway from initial compound discovery to therapeutic application. Moreover, recognizing the imperative need for translational research, the call for clinical trials within this category is underscored. Including clinical trials is anticipated to bridge the gap between experimental findings and real-world therapeutic applications. This crucial step validates the efficacy of *V. trifolia*-derived compounds and generates essential data for potential future pharmaceutical advancements. Integrating bioinformatics and clinical trials will synergistically propel *V. trifolia* research into a transformative phase, offering novel therapeutic possibilities for global health improvement.

Author contributions

JM: Conceptualization, Supervision, Validation, Visualization, Writing–review and editing. MK: Data curation, Formal Analysis, Investigation, Methodology, Software, Writing–original draft. AD: Formal Analysis, Investigation, Methodology, Validation, Writing–original draft. MN: Data curation, Formal Analysis, Methodology, Visualization, Writing–review and editing. FR: Data curation, Formal Analysis, Funding acquisition, Software, Writing–original draft. MH: Conceptualization, Project administration, Supervision, Validation, Writing–review and editing. AI: Investigation, Methodology, Project administration, Validation, Writing–original draft, Writing–review and editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

The authors thank the Vice-Chancellor for Research of Shiraz University of Medical Sciences.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2024.1322083/full#supplementary-material

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frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 12 April 2024
DOI 10.3389/fphar.2024.1372456

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OPEN ACCESS

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RECEIVED 18 January 2024
ACCEPTED 04 April 2024
PUBLISHED 12 April 2024

CITATION
Zhang W, Pan X, Fu J, Cheng W, Lin H, Zhang W and Huang Z (2024), Phytochemicals derived from *Nicotiana tabacum* L. plant contribute to pharmaceutical development.
Front. Pharmacol. 15:1372456.
doi: 10.3389/fphar.2024.1372456

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Phytochemicals derived from *Nicotiana tabacum* L. plant contribute to pharmaceutical development

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The *Nicotiana tabacum* L. plant, a medicinal resource, holds significant potential for benefiting human health, as evidenced by its use in Native American and ancient Chinese cultures. Modern medical and pharmaceutical studies have investigated that the abundant and distinctive function metabolites in tobacco including nicotine, solanesol, cembranoid diterpenes, essential oil, seed oil and other tobacco extracts, avoiding the toxic components of smoke, mainly have the anti-oxidation, anti-lipid production, pro-lipid oxidation, pro-insulin sensitivity, anti-inflammation, anti-apoptosis and antimicrobial activities. They showed potential pharmaceutical value mainly as supplements or substitutes for treating neurodegenerative diseases including Alzheimer's and Parkinson's disease, inflammatory diseases including colitis, arthritis, sepsis, multiple sclerosis, and myocarditis, and metabolic syndrome including Obesity and fatty liver. This review comprehensively presents the research status and the molecular mechanisms of tobacco and its metabolites basing on almost all the English and Chinese literature in recent 20 years in the field of medicine and pharmacology. This review serves as a foundation for future research on the medicinal potential of tobacco plants.

KEYWORDS

***Nicotiana*, neurodegeneration, inflammation, metabolic syndrome, nicotine, solaneso, cembranoid diterpenes, tobacco extracts**

1 Introduction

Tobacco (*Nicotiana tabacum* L.), of the genus *Nicotiana* in the Solanaceae family, is an important economic crop and scientific research model plant. In recent years, beyond the cigarette industry, the innovative use of tobacco plants has inspired much research into organic fertilizers, biological pesticides, polymer biomaterials, food, feed, daily chemicals, and most notably, the medical field. Tobacco was first documented in human use at the end of the 15th century because of its medicinal value. Native Americans regard tobacco as a "holy herb" or "God's remedy" used to treat a variety of diseases such as bronchitis, toothache, sore throat, pleuritis, jaundice, epilepsy, rhinitis, gastroenteritis, diarrhea, headache, otitis, whooping cough, syphilis, arthritis, dermatitis, colds, burns, abscess

festering, and mosquito bites, and also applied for stopping bleeding, reducing fever, anti-fatigue, and whitening teeth (Sanchez-Ramos, 2020). In China, tobacco plants are also recorded as a characteristic medicinal resource. The Chinese Materia Medica documents the medicinal role of tobacco, including promoting "Qi" (vital energy) and relieving pain, eliminating dampness, detumescence, detoxicating and killing insects. It is mainly used to treat fullness with food stagnation, Qi stagnation with pain, arthralgia, carbuncle, furuncle, scabies, eczema, snakebite, sprain and contusion. The Compilation of National Chinese Herbal Medicine describes tobacco's efficacy as warming, sweet, and toxic, with effects related to detumescence, detoxification, and insecticidal. It is primarily employed in treating furuncle, tinea capitis, psoriasis, alopecia, and snakebites, as well as diseases such as neck carbuncle, back carbuncle, wind phlegm, and crane knee, including bone tuberculosis and chronic suppurative knee arthritis.

The global smoking-banning campaign in the modern era also restricted investigation into the medicinal value of tobacco plants to a great extent. Only in the late 20th century, researchers turned their attention to diseases affecting brain and nerve development, especially neurodegenerative diseases with a high incidence among the elderly, such as Alzheimer's disease and Parkinson's disease. The medical value of tobacco was increasingly recognized and efforts to promote it are growing. As proposed by Professor Anne Charlton of Manchester University, we should put aside the prejudice caused by the adverse effects of smoking and, instead, systematically analyze tobacco plants to explore substances with medical treatment value (Charlton, 2004).

With advancements in pharmacology, the biological activity mechanisms of many medicinal plants and their metabolites have been clarified. In many cases, individual constituents are used for treatment after extraction and purification. About a quarter of the prescription drugs originate from plants (Goldstein and Thomas, 2004), such as the antimalarial drug artemisinin (Price, 2000), anticancer drug paclitaxel (Zhu and Chen, 2019), and cardiovascular drug ginkgolide (Tian et al., 2017). At present, the main medicinal constituents brought into focus in tobacco plants include the alkaloid nicotine, phenolic substances such as rutin, apigenin and quercetin, phenolic acids such as chlorogenic acid, ferulic acid and caffeic acid, organic acids such as citric acid and malic acid, terpenoids such as solanesol and cembranoid diterpenes, and tobacco essential oils such as α- and β-ionone. In recent years, with the popularization of metabolomics, other metabolites with pharmacological activity in tobacco plants, such as vitamin E, vitamin K1, scopolamine, mesmine, and ferruloyltyramine, have also been identified and isolated (Xia et al., 2014). In addition, it is worth mentioning that polysaccharides, polypeptides and sterols from plants also have important biological functions in the medical field. For example, plant polysaccharides can effectively regulate the balance of blood lipids and blood sugar in the human body, mediate immune activity, exert anti-cancer effects, and activate cell antioxidant activity (Jiao R. et al., 2016). Plant polypeptides exhibit antioxidant, antiviral and metabolic syndrome regulatory effects, especially blood pressure lowering effects (Chai et al., 2021). Phytosterols are effective in reducing cholesterol levels (Yuan, 2020). Besides, tobacco is rich in sugars (constituting 25%–50% of dry weight) and proteins (26%–29% of dry weight at the seedling stage and 12%–15% of dry weight at the mature stage), as well as phytosterols such as β-sitosterol, stigmasterol, and rape sterols. Therefore, tobacco will be an important plant resource for drug development in the future.

This review will focus on the status of the pharmacological research on the main and abundant functional substances of tobacco plants in recent 20 years. These include the alkaloid nicotine, terpenes (including solanesol and cembranoid diterpenes), as well as extracts from tobacco leaves and seed.

2 Nicotine

Nicotine ($C_{10}H_{14}N_2$) is the main addictive substance in tobacco smoke, and belongs to the pyridine and pyrrole alkaloids, accounting for about 5% of the weight of tobacco and 90%–95% of the total alkaloids in tobacco. It mainly exists in tobacco, but is also found in a small amount in other solanaceous species. Alkaloids are nitrogen-containing compounds with remarkable anti-tumor, anti-microbial, anti-inflammatory and analgesic effects (Jiang et al., 2016). Nicotine, as an agonist of nicotinic acetylcholine receptor (nAChR), can act on the vagal pathway and its collateral branch and the anti-inflammatory pathway mediated by the nicotinic acetylcholine receptor, which is the main mechanism of nicotine's pharmacological activity (Author Anonymous, 2014; Onor et al., 2017). The complex communication between the nervous system and immune system provides nicotine with a wide range of pharmacological activities and great potential for disease treatment. However, nicotine can be a double-edged sword. High-dose nicotine is toxic, while low-dose nicotine may have different positive effects, just like most clinical drugs. The negative effects of low-dose nicotine can be counteracted by other drugs (Barreto et al., 2014). Hereby, we introduce the application value of nicotine in the medical field, mainly focusing on neurodegenerative diseases, inflammation, and obesity metabolic disorders (Figure 1).

2.1 Positive effect of nicotine on neurodegenerative diseases

Neurodegenerative diseases are diseases of the nervous system caused by the selective degeneration and loss of neurons. Patients with these diseases will experience a decline in normal viability, resulting in problems with independent mobility, as well as memory and cognitive decline. Alzheimer's disease and Parkinson's disease are the two most common neurodegenerative diseases, which increases steadily with age and affect tens of millions of people worldwide (Erkkinen et al., 2018). Alzheimer's is the most prevalent dementia disease worldwide, causing 60%–80% of all dementia cases. The pathogenesis of Alzheimer's is complex. The neuronal damage and neurotoxicity caused by intracellular neurofibrillary tangles formed by β-amyloid protein (Aβ) deposition and abnormal phosphorylation of tau protein are the most important pathological features of Alzheimer's (Alhowail, 2021). The pathogenesis of Parkinson's mainly involves the loss or degeneration of dopamine neurons or dopamine-producing neurons in the substantia nigra of the midbrain (Barber et al., 2001). Nicotine administration can improve the cognitive impairment in

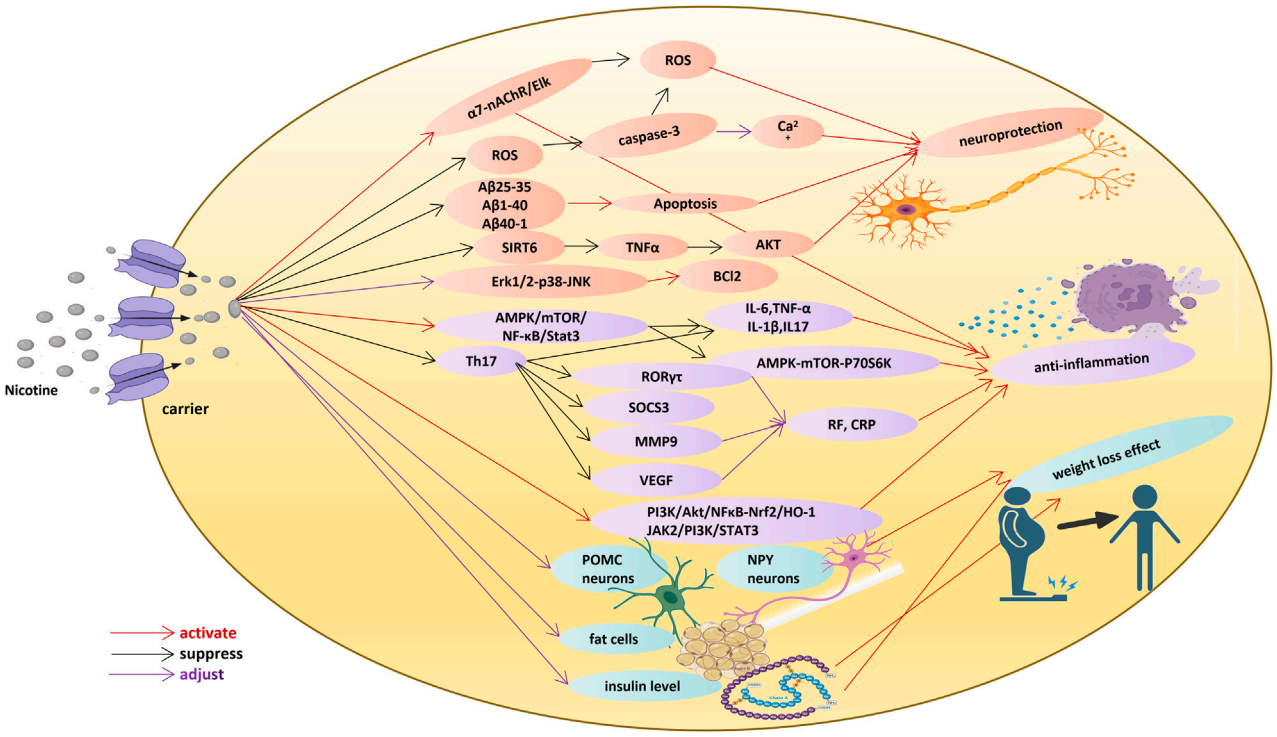

FIGURE 1
Nicotine function in neuroprotection, anti-inflammatory effect, and weight loss effect.

Alzheimer's, and the motor impairment and memory impairment in Parkinson's, thus protecting nerve cells.

At the cellular level, nicotine activates the α7-nAChR/Elk signaling pathway to reduce the oxidative damage caused by hydrogen peroxide, improve damaged mitochondrial membrane potential, and inhibit the damage to hippocampal neurons induced by excessive accumulation of reactive oxygen species (ROS) (Dong et al., 2020). Nicotine weakens the apoptosis of primary hippocampal cells induced by $A\beta_{25\text{-}35}$, $A\beta_{1\text{-}40}$ or $A\beta_{40\text{-}1}$ in Sprague-Dawley rats and reduces neurotoxicity by decreasing caspase-3 activity, clearing ROS and maintaining Ca^{2+} homeostasis (Liu and Zhao, 2004). In the Parkinson's model of neuroblastoma SH-SY5Y cells induced by picoline ion (MPP^{+}), nicotine pretreatment can activate α7 nAChR and further inhibit the cleavage of PARP-1 and caspase-3, resulting in a significant inhibition of cell death (Lu et al., 2017). In the Alzheimer's model of SH-SY5Y cells, nicotine can inhibit the activity of caspase-3 of apoptotic protein and upregulate the expression of anti-apoptotic protein BCl2 through the Erk1/2-p38-JNK signaling pathway, thus protecting them from the neurotoxicity induced by $A\beta_{25\text{-}35}$ (Xue et al., 2014). Nicotine can also promote the processing of AβPP non-amyloid protein mediated by α-secretase (ADAM10) and reduce the accumulation of neurotoxins via activating Rack1 protein-dependent PKC phosphorylation (He et al., 2020).

Nicotine has played a positive role in several animal models of Alzheimer's disease. In $A\beta_{25\text{-}35}$-induced Alzheimer's mice, nicotine treatment increased the upregulation of anti-apoptotic protein BCl_2 in the hippocampus of mice, while the Morris water maze navigation test proved that nicotine improved spatial working memory disorder (Xue et al., 2014). In a rat Alzheimer's model induced by chloroindoleamine (CHL), Y maze and radial arm maze tests confirmed that nicotine significantly improved the spatial memory deficit, and restored the antioxidant capacity of superoxide dismutase in the rat hippocampus, showing potential properties for memory improvement (Hritcu et al., 2017). Through Y maze, new object recognition and radial arm maze assessment, nicotine metabolites cotinine and 6-hydroxy-L-nicotine, also showed cognitive improvement effects, positively regulating the expression of brain-derived neurotrophic factor (BDNF), neuronal specificity and postsynaptic memory consolidation related protein (arc) and proinflammatory factor interleukin one beta (IL-1β) in Alzheimer's rats induced by Aβ, exhibiting higher affinity with α4β2 nAChR and α7 compared to nicotine. Hence, these metabolites may have greater potential as drug candidates for promoting cognition, antioxidation and anti-acetylcholinesterase (Boiangiu et al., 2020).

Nicotine also plays a neuroprotective role in several animal models with Parkinson's disease. In 6-hydroxydopamine-induced Parkinson's mouse. Nicotine can activate α7 nAChR receptor to play beneficial roles in motor deficiency, dopaminergic neuron loss, activation of astrocytes and microglia, and the decrease of dopamine in striatum via Wnt/β-Catenin signal transduction (Liu et al., 2017). In 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) Parkinson's mouse model, nicotine inhibits the activity of deacetylase SIRT6 or prevents the accumulation of SIRT6 molecules by mediating the degradation of proteasome,

thus reducing the accumulation of inflammatory factor TNF-α, increasing the expression of phosphokinase AKT and signal transduction, and improving the survival rate of neurons (Nicholatos et al., 2018). Nicotine pretreatment has a neuroprotective effect on nigrostriatal injury in PD models, while the repair after injury has poor effects. This effect involves the mediation of various nAChR subtypes, including α4β2 nAChR, α6β2 nAChR and α7 nAChR, and may also involve changes in different downstream kinases, such as AKT, PI3K, and FGF2, thereby exerting a preprotective effect on injury (Quik et al., 2009). Therefore, we posit that the sustained release of a nicotine transdermal patch may avoid the desensitization readily caused by nicotine, and may be a healthcare product that can be promoted for preventing neural degeneration and improving life quality in the elderly in the future.

2.2 Anti-inflammatory effect of nicotine

The cholinergic anti-inflammatory pathway has been extensively studied in terms of its immune regulation function and protective effects on a variety of inflammation-related diseases, including ulcerative colitis, arthritis, sepsis, skin inflammation, multiple sclerosis, and myocarditis. The activation of the cholinergic anti-inflammatory pathway by nicotine verifies its extensive anti-inflammatory effects.

Ulcerative colitis is a non-transmural colitis disease, characterized by intermittent attack and remission, abdominal pain, diarrhea and rectal bleeding. Due to the complex pathogenesis of ulcerative colitis, available treatment protocols are limited and often fail to achieve satisfactory efficacy (Baumgart, 2009). According to epidemiological analysis, smokers have lower possibility of suffering from ulcerative colitis than non-smokers. The combination of nicotine patches for percutaneous treatment of cholinergic anti-inflammatory pathways with conventional treatment has shown better clinical effect than the single treatment (Kannichamy et al., 2020). However, the clinical sample size is small and the data is not sufficient. In animal models, subcutaneous injection, intraperitoneal injection and dietary nicotine can stimulate nAChR receptors, mainly the α7-subunit, but also the α3-, α5-, β2-and β4-subunits, inhibit proinflammatory cytokines such as IL-6, TNF-α, IL-1β, IL17 and IFN-γ produced by immune cells through the AMPK/mTOR/NF-κB/Stat3 inflammatory signaling pathway and AMPK-mTOR-P70S6K autophagy pathway, to alleviate ulcerative colitis induced by different inducers such as dextran sodium sulfate (Snoek et al., 2010; Lakhan and Kirchgessner, 2011; Gao et al., 2020). In addition, nicotine improves the integrity of intestinal wall cells (Costantini et al., 2012) by regulating cell tight junction proteins MAdCAM-1, occludin and ZO-1, thus accelerating wound healing. Due to the lack of specificity of nicotine in ulcerative colitis, nicotine clinical trials were halted. Hence, more preclinical research data on nicotine in ulcerative colitis is needed to determine the target and path of action so as to carry out multi-combination interventions.

Rheumatoid arthritis is characterized by chronic inflammation and synovial intima hyperplasia, which leads to cartilage deterioration and bone destruction, often lasting for several months (Disease et al., 2016). Under normal circumstances, there is a certain proportion of helper T cells 1 (Th1) and helper T cells 2 (Th2) in the body. The coordination between the two types of cells maintains the balance of the human immune system. When the Th1/Th2 ratio increases, indicating a shift towards Th1 immunity, the immune response produces a series of proinflammatory factors such as IL-1β, IL-6, IL-17, TNF-α, IFN-γ and IL-8, while the expression of anti-inflammatory factors such as IL-4, IL-5 and IL-10 declines, leading to the infiltration of inflammatory cells in synovial tissue (Gomes et al., 2018). Nicotine can induce the production of Th2 cells, reduce the level of GATA3, and block the progression of arthritis in mice after 33 days of collagen induction. In addition, nicotine can inhibit the immune response of Th17 cells, the cytokine IL-17 secreted by Th17 and its transcription factor RORγτ (Wu et al., 2014; Yang et al., 2014), and suppress cytokine signaling protein SOCS3 (Li et al., 2018), matrix metalloproteinase MMP9 and angiogenesis factor VEGF (Arshadi et al., 2020), to control rheumatoid factor (RF) and C-reactive protein (CRP), and reduce pannus formation and joint destruction in rheumatoid arthritis. The NF-κB and STAT-mediated intracellular anti-inflammatory signaling pathway and the vagus nerve play a key mediating role in the improvement of rheumatoid arthritis by nicotine (Li et al., 2010; Zhou et al., 2012).

Sepsis is a life-threatening organ dysfunction caused by the host's maladjusted response to infection. If it is not identified or treated in a timely manner, it may lead to septic shock, multiple organ failure and even death (Singer et al., 2016). Through *in vivo* research, Wang et al. (Wang et al., 2004) first found that nicotine can reduce the mortality rate of mice with multimicrobial peritonitis from 84% to 51%, even when drug is administered to the mice after the occurrence of clinical disease. Nicotine could inhibit the activation of the NF-κB anti-inflammatory pathway through stimulating α7 nAChR and suppressing the secretion of high mobility group protein B (HMGB1), thereby inhibiting the production of proinflammatory cytokine plasminogen activator inhibitor-1 (PAI-1) as well as the microcirculation damage caused by sepsis. In septic mice infected by *Escherichia coli* or lipopolysaccharide injection, nicotine simultaneously reduced the proinflammatory factors of TNF-α, IL-6 and IL-1β in peritoneal lavage fluid and plasma and maintained the activities of serum enzymes AST and ALT of hepatic function so as to prevent liver failure and improve the survival rate by 40% (van Westerloo et al., 2005; Zhou et al., 2011). In acute sepsis induced by cecal ligation and perforation combined with lipopolysaccharide, the intraperitoneal injection of nicotine also actively reduces mortality in a short time through increasing intracellular calcium concentration by stimulating a7 nAChR and activating the anti-inflammatory signal pathways of PI3K/Akt/NFκB-Nrf2/HO-1 and JAK2/PI3K/STAT3. Nicotine has the capacity to reduce the oxidative damage of organs and tissues and it also prevents systemic inflammation and organ failure by regulating the oxidative stress system (Ozdemir-Kumral et al., 2017). In the treatment with nicotine, the splenic branch of the vagus nerve plays an indispensable role in the anti-inflammatory pathway (Huston et al., 2006; Rosas-Ballina et al., 2008). Microsomal prostaglandin E synthetase-1 gene (mPGES-1) and the PGE2 produced in the spleen are the key factors connecting the immune and nervous systems affected by nicotine (Revathikumar et al., 2018). Because sepsis, especially acute sepsis, only needs short-term treatment, concern that the long-

term use of nicotine will adversely affect the nervous system is less significant here.

Skin inflammation is a general term that encompasses skin inflammatory diseases that are caused by various internal and external infections or non-infectious factors, and is not an independent disease. Due to its complex etiology and diverse clinical manifestations with repeated attacks, its clinical treatment is more difficult. The use of ultraviolet radiation exposure is a common and characteristic method to induce skin inflammation. An early intervention study in 1997 showed that a nicotine patch inhibited the skin's inflammatory response to sodium dodecyl sulfate and ultraviolet radiation (Mills et al., 1997). By regulating α7 nAChR and SOCS3, oral administration of nicotine for 6 weeks significantly reduces the production of IL-1β in the skin of female rats exposed to ultraviolet light and skin redness and swelling were alleviated (Osborne-Hereford et al., 2008). Subcutaneous or intraperitoneal injection of nicotine in male Wistar rats increased the level of serum corticosterone and inhibited the increase of nitric oxide produced by polymorphonuclear leukocytes (mainly TNF-α), thus inhibiting the plasma exudation caused by passive skin reaction (Kubo et al., 2003; Kubo et al., 2004). Nicotine significantly reduced IL-8, IL-6 and VEGF levels in keratinocytes and endothelial cells co-cultured with the serum of Bechet patients (Kalayciyan et al., 2007), indicating that nicotine has therapeutic potential in reducing systemic vascular inflammation.

Multiple sclerosis is an autoimmune demyelinating disease characterized by inflammation of the central nervous system, demyelination and neurodegeneration, which is manifested by vertigo and weakness. The etiology of multiple sclerosis is still unknown and there is no method of complete cure. In a Swedish population-based study (7,883 cases, 9,437 controls), it was found that nicotine reduced the risk of multiple sclerosis in different subjects who received snuff, suggesting that nicotine plays an anti-inflammatory and immunomodulatory role in multiple sclerosis. Nicotine has a protective effect in the experimental autoimmune encephalomyelitis animal model of multiple sclerosis. Nicotine patch allows ependymal cells to proliferate in the inflammatory region by reducing the expression of nestin protein in neuroepithelial stem cells. It also has an obvious influence on the activity, activation and function of microglial cells, and can increase the number of mature anti-inflammatory M2 microglial cells ($NG2^+$ and $CC1^+$ subtype) so as to promote disease recovery (Gao et al., 2014; Gao et al., 2015). Nicotine also reduces the levels of pro-inflammatory factors IL-1, TNF-α and IFNγ and increases the levels of anti-inflammatory factor IL-10 in spleen cells, thereby improving the neurophysiological indexes. When nicotine is administered in combination with mesenchymal stem cells in rats, the efficacy is better than with the single treatment without nicotine (Khezri et al., 2018). It is worth noting that nAChRα7, α9 and β2 may play different roles under the action of nicotine. In clinical applications, it may be necessary to set specific inhibitors to make nAChR play a specific role, and the detailed mechanism needs to be further verified (Simard et al., 2013; Liu et al., 2017).

Myocarditis may cause symptoms from mild dyspnea or chest pain to cardiogenic shock and sudden death, and it can also be relieved without specific treatment. Myocarditis is usually caused by viral infection and, to date, there is no special treatment available (Cooper, 2009). In the myocarditis mouse model infected by coxsackie B3 virus, the injection of high concentration nicotine (1.2 mg/kg, i. p.) downregulated the expression levels of inflammatory factors IL-1β, IL-17A, TNF-α, IL-1 and IL-6 based on STAT3 activation, improved the damage to left ventricular function, and alleviated myocardial injury, increasing the survival rate by 35% (Cheng et al., 2014; Li-Sha et al., 2015; Li-Sha et al., 2016). Nicotine can also reduce the release of proinflammatory factors in the heart by regulating the proportion of immune cells Th1 and Th17 in the spleen (De-Pu et al., 2018). The nicotine-mediated reduction of coxsackie B3 virus replication and the anti-apoptosis effect on myocardial cells involve α7 and α3β4 nAChR, which further enhance cardiac function through upregulating the expression of survivin via the α3β4 nAChR/PI3K/Akt signaling pathway (Li et al., 2019). It is necessary to clarify the role of the vagus nerve's integrity in this process.

Apart from the conditions mentioned above, nicotine plays an active role in many other inflammatory diseases, including pancreatitis, allergic inflammation, nasal eosinophilic inflammation, muscle inflammation, uveitis, anorexia/cachexia syndrome, and systemic lupus erythematosus. The effect of nicotine is more significant in inflammatory diseases with complex pathogenesis, which are difficult to cure and with recurrent attacks. Its advantage in multi targets may enhance its efficacy and improve individual survival rate, but it also suffers the problem of poor specificity. Therefore, the treatment of inflammatory diseases with nicotine depends on detailed studies on the acetylcholinergic anti-inflammatory pathway, the vagus nerve, the central nervous system regulation and the interaction with immune cells. Considering the addictive properties of nicotine, it is also critical to control the dosage. According to the approximate dosage range of nicotine tartrate (0.4–1.2 mg/kg per day) for intraperitoneal injection in mice, we calculated that the safe dosage range of nicotine salt for subcutaneous injection in humans is about 7.2–28.8 mg/kg per day in our previous report (Zhang et al., 2022). Currently, there are three concentrations of nicotine patches for smoking cessation treatment, namely, a 24-h patch with dosages of 21, 14, and 7 mg. Nicotine patches may be a potential choice for chronic recurrent inflammation (Zhang et al., 2022).

2.3 Weight loss effect of nicotine

Obesity has become a global public health problem. It is characterized by chronic low-grade inflammation, which can readily lead to nonalcoholic fatty liver disease, insulin resistance, cardiovascular disease, depression and other complications. Epidemiological studies have shown that there is a strong relationship between smoking and weight. In different ages, the weight of non-smokers is often higher than that of smokers. Quitting smoking may result in weight gain (Audrain-McGovern and Benowitz, 2011), which highlights the weight loss effect of nicotine from another perspective.

Nicotine has been shown to improve obesity mainly through four mechanisms. Firstly, nicotine regulates energy intake and reduces appetite by acting on hypothalamic neurons and the neuropeptide system related to food intake. Low-concentration

nicotine (100–1,000 nM) has a regulatory effect on anorexic POMC neurons, NPY neurons, and secretin/orexin neurons in the hypothalamus, and can mediate neuropeptide Y, leptin, orexin and its receptors and uncoupling proteins, as well as the level and activity of neurotransmitters such as dopamine and monoamine, with the effect of controlling food intake and reducing obesity (Li et al., 2000; Huang et al., 2011). However, different nicotine intake methods will complicate its effectiveness.

Secondly, nicotine can reduce the size of fat cells and fat content by regulating fat decomposition and synthesis, and it can also raise the heat production of brown fat and transfer fat storage from adipose tissue to muscle utilization. In male Sprague-Dawley rats, after subcutaneous micro-pump injection of nicotine for 1 week, the body weight of rats was reduced by 37%, while fat pad reduction was 21%, and fat decomposition increased by 78%. Nicotine reduces the activity of lipoprotein lipase (LPL) in adipose tissue and increases the activity of LPL in muscle. This means that the intake of triglycerides in adipose tissue is reduced, the decomposition is increased, and the cells become smaller, while the consumption of triglycerides in myocardium and skeletal muscle is increased (Sztalryd et al., 1996). When CD-1® IGS male mice were fed with feed containing nicotine, the mice exhibited body weight loss (19.7%), increased physical stamina and decreased respiratory exchange rate under the condition of no change in food intake, which also induced the decomposition of triglycerides in adipose tissue and weight loss. The most obvious change is that the weight of epididymal fat pad and the size of fat cells have significantly decreased while the heat production of brown fat has increased (Liu et al., 2018). The high expression of UCP1 protein and the increased binding of guanine nucleoside 5′-hydrogen phosphate in brown fat indicate that the metabolic rate of fat increases after nicotine injection (Arai et al., 2001), which confirms that nicotine acts directly on weight loss without the side effects of two-way regulation.

Thirdly, nicotine regulates insulin levels induced by obesity and glucose production (Seoane-Collazo et al., 2021). Nicotine fed with the diet increases the insulin level of mice 4.3-fold and improves the blood glucose level, without affecting insulin sensitivity (Liu et al., 2018). The plasma glucose level of male obese Zucker rats also decreased significantly after long-term oral administration of nicotine with drinking water, and the glycogen content, glycogen synthase activity and gluconeogenesis in the liver were significantly lower than those in the control group, indicating that nicotine can reduce insulin resistance in obese diabetic rats by reducing the release of glucose in the liver, thus reducing the level of blood sugar to some extent (Liu et al., 2003). Nicotine also significantly reduced the hyperglycemia level and incidence of type 1 diabetes in a mouse model, and prevented the insulin level from falling, which is closely related to nicotine's effect on the expression profile from Th1 to Th2 cytokine and the amelioration of pancreatitis (Mabley et al., 2002).

Fourthly, nicotine alleviates steatohepatitis induced by adipose tissue inflammation and obesity. Nicotine can not only reduce body weight by stimulating triglyceride decomposition in adipose tissue and heat production in brown adipose tissue (Seoane-Collazo et al., 2014; Liu et al., 2018), but also significantly inhibits adipose tissue inflammation, and ameliorates the steatohepatitis of obese mice induced by genetic obesity and high-fat diet (Lakhan and Kirchgessner, 2011; Wang et al., 2011). In C57BL/6J (B6) obese mice induced by genetic obesity (db/db) and high-fat and high-sugar diet, nicotine inhibited the increase of F4/80 in obesity-induced inflammation and the levels of proinflammatory cytokines such as TNF-α, IL-6, IL-1β and iNOS in serum (Wang et al., 2011). Furthermore, in isolated Kupffer cells of the liver, nicotine could alleviate steatohepatitis by inhibiting the ERK/NF-κB/iκB signaling pathway by interacting with α7 nAChR (Zhou et al., 2015a; Zhou et al., 2015b; Chen et al., 2016; Li et al., 2016). As for Sprague-Dawley obese rats fed a high-fat diet, nicotine ameliorated liver damage and reduced lipid inflammatory indicators in the liver such as PPARγ, TNF-α and IL-6 by reducing endoplasmic reticulum stress (Seoane-Collazo et al., 2014). The regulatory effect of α7 nAChR on the action of immune factors MCP-1 and KC has been confirmed in stearic acid (C18:0) or TNF-α-induced 3T3-L1 adipocytes (Jiao et al., 2016) and α7 nAChR$^{-/-}$ deficient mice (Wang et al., 2011). Nicotine osmotic pump was effective in alleviating steatohepatitis in Wistar rats induced by L-amino acid. Apart from the proinflammatory factors of TNF-α, IL-6, IL-1β, nicotine inhibits hepatocyte apoptosis by regulating apoptosis proteins Bax and caspase-3. In addition, the acetylcholine anti-inflammatory pathway involved in the hepatic branch of the vagus nerve is one of the essential links for nicotine to alleviate nonalcoholic steatohepatitis (Kanamori et al., 2017).

3 Solanesol

Solanesol ($C_{45}H_{74}O$) is a long-chain triple sesquiterpenoid primary alcohol composed of nine isoprene units. Its special all-trans chain structure has lipid antioxidant activities and strong free radical absorption abilities. Additionally, solanesol also has antibacterial, anti-inflammatory and anti-ulcer biological activities (Figure 2). It is the raw material for the preparation of ubiquinone drugs such as vitamin K2, coenzyme Q10 (CoQ10) and N-solanesyl-N, N′-bis(3,4-dimethoxybenzyl) ethylenediamine, an anticancer agent synergiser. Tobacco is the most abundant source of plant solanesol, accounting for about 1%–4% of the dry weight. Due to the complex synthesis of solanesol, it is currently mostly extracted from tobacco (Yan et al., 2019a).

3.1 Antioxidant and anti-inflammatory activities of solanesol

Solanesol has many non-conjugated double bonds in its structure, which possess strong free radical absorption ability and antioxidant activity. Free radicals are products of respiration and metabolism in the human body. Under normal circumstances, the generation and elimination of free radicals are in a state of dynamic balance. Free radicals play an indispensable role in the normal growth of cells, energy metabolism and internal environment stability. However, when excessive free radicals are generated in the human body, they cause oxidative stress damage to cells, tissues and organs, induce various diseases, and accelerate the aging of the body. Under pathological conditions, excessive free radicals further aggravate diseases through oxidative stress. More than 95% of free radicals in the human body are oxygen free radicals including $O_2^{\bullet -}$,

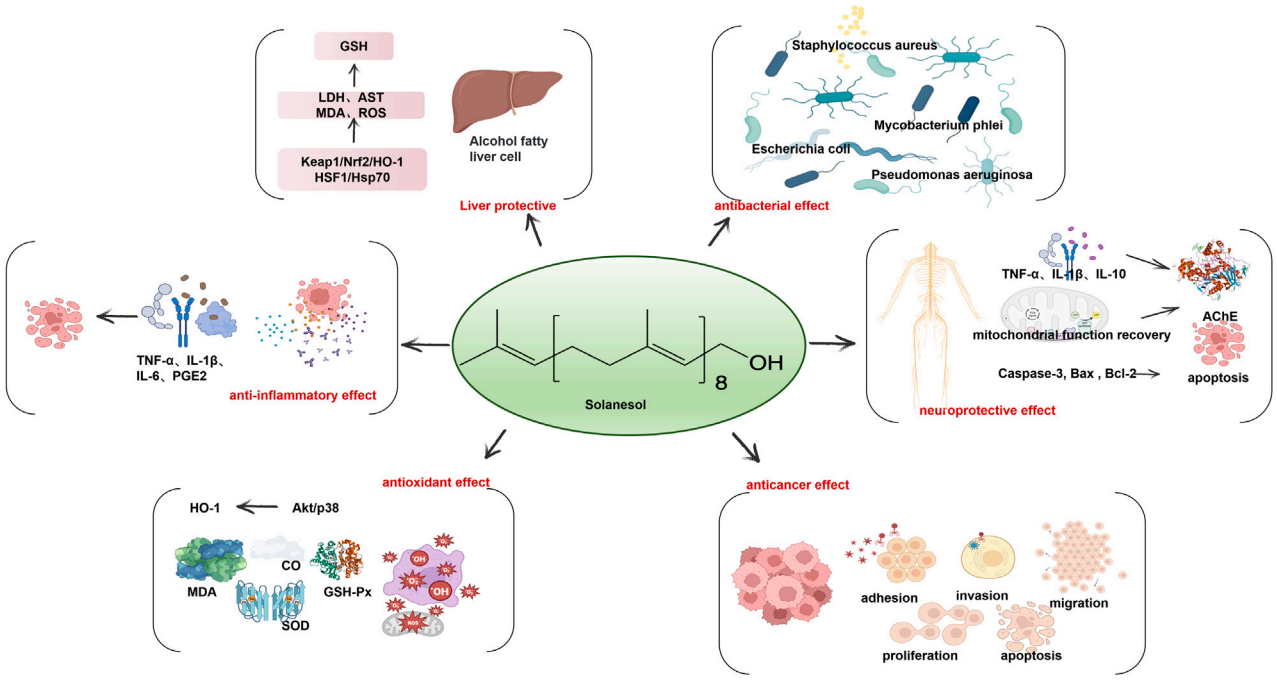

FIGURE 2
Solanesol has a wide range of biological activity including liver protection, anti-inflammatory effect, antioxidant effect, anticancer effect, neuroprotective effect and antibacterial effect.

$HO_2^{\bullet}$, $LO^{\bullet}$, $LOO^{\bullet}$, $NO^{\bullet}$ and $HO^{\bullet}$. Of these, the hydroxyl free radical ($HO^{\bullet}$) displays the strongest activity (Droge, 2002).

In vitro studies show that in the pyrogallol autoxidation system and Fenton reaction system, solanesol scavenges $O_2^{\bullet-}$ and $HO^{\bullet}$, with an equivalent scavenging effect to vitamin C (Vc) and antioxidant Trolox (Ma et al., 2011; Bai et al., 2014). The Vc/Fe^{2+} excitation system and microsome model of lipid peroxidation *in vitro* have shown that lipid peroxidation can be inhibited by solanesol (IC_{50} = 2.5 mM) (Ma et al., 2011). In lipopolysaccharide (LPS)-induced inflammatory injury in RAW264.7 macrophages of mice, solanesol enhances the transcriptional level of Nrf2 by activating Akt and p38 signaling pathways, which further upregulates the expression level and the activity of heme oxygenase-1 (HO-1), and improves the antioxidant capacity of cells. It can also trigger the anti-inflammatory pathway and reduce the expression of inflammatory factors TNF-α, IL-6 and IL-1β, thus inducing autophagy and alleviating cell inflammation (Yao et al., 2017).

In *vivo* antioxidant models, solanesol has a protective role in aging mice and inflammatory mice based on its antioxidant effect. In the D-galactose-induced aging mouse model, solanesol increases the activity of superoxide dismutase (SOD) in brain tissue and serum, and decreases the content of malondialdehyde (MDA) and nitric oxide in serum. This shows that solanesol protects the brain, which is particularly vulnerable to oxygen free radicals, and has a certain ability to remove nitrogen free radicals (Ma et al., 2011). In the periodontitis rat model established by ligation of bilateral maxillary second M by silk thread combined with viscous high-sugar diet for 4 weeks, solanesol shows no obvious effect on reducing alveolar bone loss or inhibiting the activation of osteoclasts, but it significantly increases the activities of SOD and glutathione peroxidase (GSH-Px) in plasma and reduces the content of MDA. Furthermore, it significantly lowers the expression level of inflammatory factors TNF-α, IL- 1β and PGE2 in plasma (Zhang, 2016).

In addition, the fat solubility of solanesol is more conducive to its exerting free radical scavenging ability in the lipid part of the skin. Excessive production of free radicals in the skin can cause pigmentation, resulting in reduced skin elasticity, skin aging and even cancer. Under the external stimulator of ultraviolet light, solanesol exhibits an inhibitory rate of more than 90%. By applying solanesol to fresh pig skin, solanesol alleviates the degree of skin atrophy and browning, and has whitening and anti-aging effects by inhibiting the activity of tyrosine kinase that induces skin melanin production (Bai et al., 2014).

3.2 Liver protective activity of solanesol

The induction of HO-1 by solanesol in different cells may be a universal mechanism. In human liver LO2 cells with ethanol-induced oxidative damage, solanesol alleviated hepatotoxicity by activating Keap1/Nrf2/HO-1 and HSF1/Hsp70 signaling pathways, significantly inhibiting the activities of lactate dehydrogenase (LDH) and AST in injured hepatocytes, and blocking ethanol-induced upregulation of MDA and ROS levels and downregulation of

glutathione (GSH) levels. Solanesol could further inhibit cell apoptosis by inhibiting the morphological damage of the nucleus and the mature shear of apoptotic protein caspase-3 and PARP, suggesting that solanesol may have liver protective effects in alcoholic fatty liver (Yao et al., 2015). However, it is worth noting that LO2 cells are contaminated with Hela tumor cells, and the results obtained as an induction model are questionable (Shao and Chen, 2023).

3.3 Neuroprotective effect of solanesol

Solanesol has a neuroprotective role by regulating neuroinflammatory factors TNF-α, IL-1β and IL-10, and the activity of mitochondrial complexes such as NADPH dehydrogenase, succinate dehydrogenase (SDH), ATP, and CoQ10. It also inhibits brain oxidative damage through enhancing SOD and GSH and reducing MDA and nitrite, restoring the levels of neurotransmitters acetylcholine and acetylcholinesterase (AChE), and regulating brain diseases by mediating biochemical indicators *in vivo* such as dopamine (Mehan et al., 2018; Sharma et al., 2019; Rajdev et al., 2020). In the combined rat model of intracerebral and ventricular hemorrhage, solanesol significantly ameliorated behavior and movement disorders, oxidative damage and neuroinflammation, and restored the activity of mitochondrial complexes (I, II, and V) in hemorrhagic rats after 35 days of treatment, indicating that solanesol has multiple-target activities in various cellular and molecular cascade reactions. When solanesol is combined with standard drug treatments (donepezil, memantine, celecoxib, and pregabalin) to treat symptoms after intracerebral hemorrhage in rats, it has a synergistic effect (Rajdev et al., 2020). In an experimental model of autism induced by propionic acid injection into the lateral ventricle, solanesol restored the complex enzyme levels in the mitochondrial electron transport chain. Long-term treatment can benefit the recovery of memory and the decrease in the level of neuroproinflammatory factors, and restore neurotransmitter levels, slow down oxidative stress, improve dopamine levels, enhance exercise ability and exhibit antidepressant activity (Sharma et al., 2019). In the experimental model of Huntington's disease induced by 3-nitropropionic acid, low-concentration solanesol improved the function of mitochondria and acted on the striatum, cortex and hippocampus of the brain to promote the recovery of brain energy, enhance the level of acetylcholine, improve memory and motor ability, strengthen the antioxidant defense system of the brain, and alleviate Huntington's disease (Mehan et al., 2018). A recent study also shows that solanesol can ameliorate ouabain-induced bipolar disorder by activating the SIRT-1 signaling pathway (Rajkhowa et al., 2022). Data demonstrate that solanesol can increase the level of SIRT-1 in cerebrospinal fluid, plasma and brain homogenates samples and regulate apoptosis markers (Caspase-3, Bax and Bcl-2), mitochondrial complexes I, II, IV, V and CoQ10. It can also reduce inflammatory cytokines TNF-α and IL-1β to simultaneously restore the level of neurotransmitters, including serotonin, dopamine, glutamate and acetylcholine, and reduce the markers of oxidative stress, thereby limiting the severity of this chronic mental disease. Hence, solanesol has the effect of alleviating neuroinflammation and improving neuronal memory and motor dysfunction. It can also change neurochemical reactions and enhance the cerebral antioxidant defense in a neuroprotective role.

3.4 Antibacterial activity of solanesol

Based on *in vitro* bacteriostasis experiments using the agar diffusion method and double dilution method, solanesol exhibits significant inhibitory effects on *E. coli*, *Mycobacterium phlei*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. However, it has a poor inhibitory effect on *Bacillus circulans* and *Bacillus subtilis* (Chen et al., 2007).

3.5 Pharmacological activities of solanesol derivatives

3.5.1 Anticancer effects of solanesol derivatives

Many natural products enjoy a reputation as promising anticancer agents. A number of compounds based on dietary isoprenoids have attracted attention due to their chemopreventive and anti-proliferative properties. Some little-known isoprenoid derivatives have gradually been applied in mainstream chemotherapy (Newman and Cragg, 2016). As an isoprenoid compound, solanesol was recognized by molecular dynamics analysis to be an inhibitor of focal adhesion kinase (FAK) protein to block its binding to ATP. The FAK protein participates in cell adhesion, invasion, migration, proliferation and apoptosis and is involved in tumor occurrence, development and prognosis (Daneial et al., 2017).

A derivative of solanesol, *N, N′*-bis(3,4-dimethoxybenzyl)-*N*-solanesyl ethylenediamine has been screened with multiple tumor cell lines. It is not only toxic to tumor cells, but can also mediate the multidrug resistance protein p-gp glycoprotein to restore sensitivity of tumor cell lines to the anticancer drugs vinblastine and doxorubicin (Sidorova et al., 2002). *Trans-N, N′*-bis(3,4-dimethoxybenzyl)-*N*-solanyl 1,2-diaminocyclohexane (N-5228) can also completely reverse the effect of human bladder cancer drug-resistant cell line KK47/TX30 on paclitaxel by mediating p-gp (Enokida et al., 2002).

Solanesol can also be utilized to modify traditional anti-cancer drugs to enhance anti-tumor activity, improve the bioavailability and precision targeting ability of insoluble drugs, and reduce the toxicity of anti-cancer drugs to normal cells. The 12 synthesized derivatives of the dibasic acid solanesol alkyl 5-fluorouracil diester inhibited the proliferation of human lung cancer cell A549 and colon cancer cell HCT1169 to varying degrees, and solanesol itself presents certain inhibitory effects on tumor cell proliferation (Xiao et al., 2012). Recently, the research team of Henan University reported amphiphilic solanesol derivatives of methyl poly (ethylene glycol) (mPEG) with solanesyl thiosalicylate (mPEG-STS), solanesyl thiosalicylate hydrazone (mPEG-HZ-STS), solanesyl dithiodipropionate (SPDP), solanesyl succinate (SPGS), and the solanesyl thiosalicylic acid (STS) polymer (HA-STS) designed with hyaluronic acid (HA) as the basic skeleton, which exhibit inhibitory effects on breast cancer and liver cancer cells. The micelles loaded with doxorubicin, which were more active than those loaded with blank micelles, displayed significant

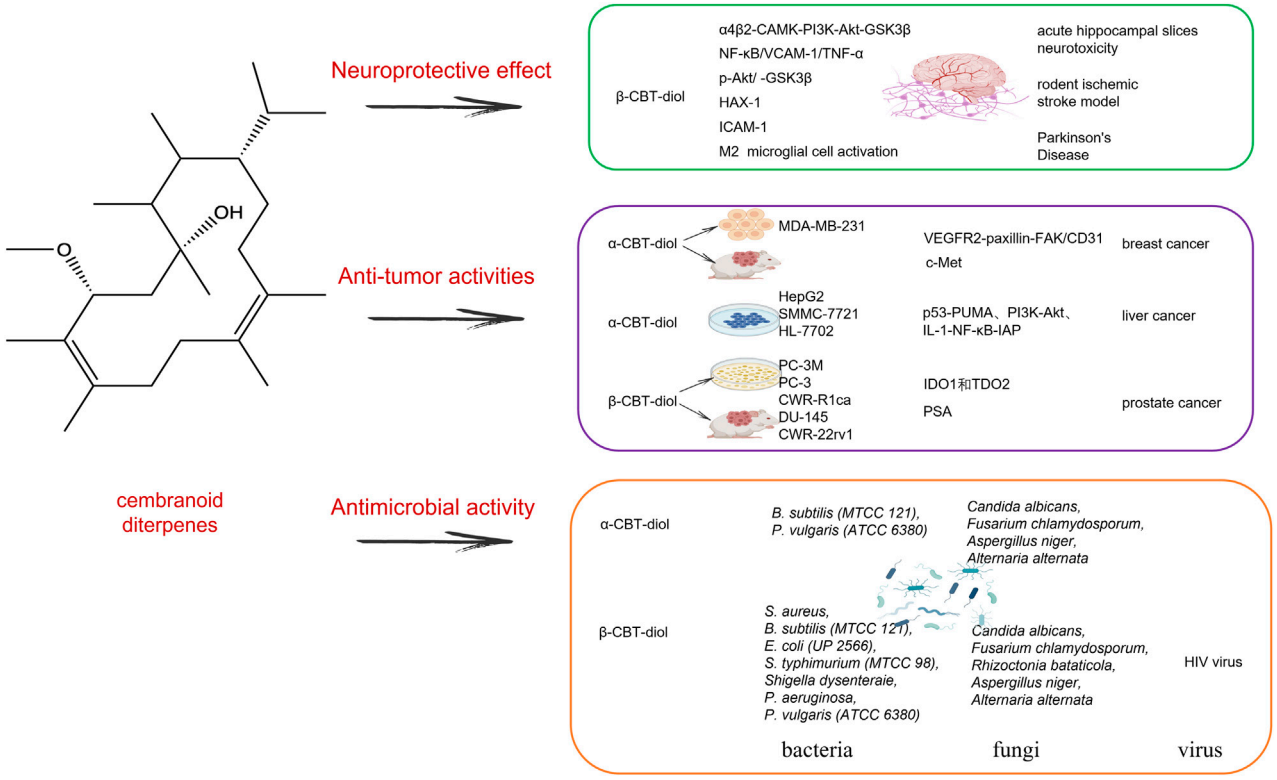

FIGURE 3
The mechanism effect of cembranoid diterpenes mainly including α-CBT-diol and β-CBT-diol in neuroprotective effect, anti-tumor activities and antimicrobial activities.

antihepatoma activity in H22 tumor-bearing mice. Among them, SPDP, mPEG-HZ-STS and HA-STS loaded with doxorubicin present the highest effects (Qin et al., 2017; Xiong et al., 2019; Yu, 2019).

3.5.2 Solanesol as raw material for drug synthesis

Solanesol is the key raw material for the synthesis of ubiquinone drugs including CoQ10 and vitamin K2. CoQ10 plays a pivotal role in mitochondrial oxidative phosphorylation and acts as a lipid-soluble antioxidant. It is involved in fatty acid, pyrimidine and lysosomal metabolism and directly mediates the expression of many genes, including those involved in inflammation. CoQ10 can be used to treat hypertension, and neurodegenerative, cardiovascular and other diseases, and is applied as a dietary supplement for patients with type 2 diabetes, heart failure, chronic kidney disease and liver disease (Hargreaves et al., 2020). Vitamin K2 is a lipid-soluble vitamin implicated in bone metabolism, which plays an active role in the prevention and treatment of osteoporosis. In addition, it can promote blood coagulation and prevent vascular calcification (Schwalfenberg, 2017).

4 Tobacco cembranoid diterpenes

Cembranoids are natural diterpenes possessing 14-membered macrocyclic rings substituted by an isopropyl residue at C-1 and three symmetrically disposed methyl groups at positions C-4, C-8, and C-12 (El Sayed and Sylvester, 2007). The earliest discovered natural cembranoid was the (+)-cembrene from pine oleoresin, later it was found that the leaf and flower cuticular wax of most Nicotiana species afforded high amounts of the cembranoids, and they are the main source of tobacco aroma substances (Mudhish et al., 2022). Most useful bioactive cembranoids are intentionally broken down during commercial tobacco fermentation to produce some unique flavor, in fact, these natural cembranoids have shown several biological potentials, including antitumor, neuroprotective and antibacterial activity. Here, we mainly reviewed two major valuable substances that have been continuously updated (1S,2E,4R,6R,7E,11E)-2,7,11-cembratriene-4,6-diol (β-CBT-diol) and (1S,2E,4S,6R,7E,11E)-2,7,11-cembratriene-4,6-diol (α-CBT-diol) (Figure 3).

4.1 Anti-tumor activities

α-CBT-diol and β-CBT-diol mainly played a role in inhibiting the growth, invasion and recurrence of tumor cells in breast cancer, liver cancer and prostate cancer. α-CBT-diol could control invasive and metastatic breast malignancies mainly through angiogenesis inhibition (Ebrahim et al., 2016; Hailat et al., 2017).

In one breast cancer research, α-CBT-diol showed a strong affinity with VEGFR2 at its ATP binding pocket *in silico*. Both

in vitro in TNBC MDA-MB-231 cells and *in vivo* in the implanted orthotopic MDA-MB-231 cells xenograft in athymic nude mice, α-CBT-diol significantly downregulated the activated VEGFR2-paxillin-FAK axis. Also, *in vivo,* it showed statistically significant reduction in tumor size, CD31 value, and markedly more active than *in vitro* because of the longer half-life, stability and the more active metabolites with allylic hydroxylation at C-19 and/or C-20 methyl carbons. Furthermore, the formation of blood vessels in matrigel injected in mice showed α-CBT-diol significantly reduced hemoglobin concentration (about 80% reduction) values when compared with vehicle-treated control, indicating α-CBT-diol is an effective angiogenesis inhibitor and would be useful to control of VEGF-dependent breast malignancies in the future (Hailat et al., 2017). The same group also found that α-CBT-diol has the antiproliferative, anti-migratory and anti-invasive effects against multiple breast cancer cell lines which the c-Met overexpressing MDA-MB-231 breast cancer cells are the most sensitive, indicating that α-CBT-diol may be a novel c-Met inhibitors for the c-Met dependent malignancies therapy (Ebrahim et al., 2016).

In liver cancer, α-CBT-diol inhibited the proliferation of HepG2, SMMC-7721 and HL-7702 tumor cells, reduced the formation of carcinoma cell clones, disrupted the cell cycle by significantly increasing S phase, induced cell apoptosis by regulating p53-PUMA, PI3K-Akt, and IL-1-NF-κB-IAP pathways (Yuan et al., 2019). But the genes involved in the pathways in the study were identified using Illumina sequencing in HepG2 cells, the differentially expressed genes need further verification *in vivo*.

In prostate cancer, β-CBT-diol showed its advantage in several pathways for the treatment of prostate cancer, especially for its higher recurrence. Earlier in the 2008, in PC-3M prostate cancer cells, β-CBT-diol has been proven to inhibit cell invasion in Matrigel assay, through reducing transepithelial resistance, enhancing paracellular permeability, producing a tighter intercellular barrier and enhancing the cell-cell adhesion (El Sayed et al., 2008). A recent study in 2022 further verified anti-prostate cancer activity of β-CBT-diol in multiple cell lines, tumor model, and biomarkers analysis (Mudhish et al., 2022). β-CBT-diol inhibited the viability of five prostate cancer cell lines PC-3M、PC-3、CWR-R1ca、DU-145 and CWR-22rv1, reduced their migration and colony formation. β-CBT-diol reduced heme-containing enzymes IDO1 and TDO2 expression in the PC-3M cell line which has a higher metastatic potential compared to other prostate cancer cell lines, indicating its important roles in cancer cell motility and immune escape. *In vivo*, β-CBT-diol inhibited PC-3M cells locoregional recurrences after primary tumor surgical excision and tumor distant recurrence, and reduced the levels of plasma kynurenine and the recurrence protein marker PSA in mice (Mudhish et al., 2022). β-CBT-diol, as a small-molecule natural product suitable for the control of hormone-independent prostate cancer recurrence, may have a broader application scope.

4.2 Neuroprotective effect

In the past 20 years from the year 2001–2022, the study on the neuroprotective effects of cembranoids has not stopped (Ferchmin et al., 2001; Ferchmin et al., 2005; Ferchmin et al., 2014; Martins et al., 2015; Hu et al., 2017; Fu et al., 2022). Since tobacco cembranoids were firstly discovered that they can be as the noncompetitive inhibitors to block the sensitization of nicotine by blocking agonist-induced ion current mediated by three distinct human AChR subtypes α4β2, α3β4, α1β1γδ-AChR (Ferchmin et al., 2001), β-CBT-diol not α-CBT-diol was later found to play a major role in neuroprotection by protecting acute hippocampal slices against neurotoxicity induced by N-methyl-D-aspartate and against the toxic organophosphorus compounds paraoxon and diisopropylfluorophosphate (Ferchmin et al., 2005; Eterović et al., 2013; Ferchmin et al., 2014). β-CBT-diol protected hippocampal sections from excitatory neurotoxicity induced by N-methyl-D-aspartate by modulating α4β2-CAMK-PI3K-Akt-GSK3β pathway, different from the cell-signaling pathways α4β2-PI3K-Erk-PKC underlying the neuroprotection provided by nicotine (Ferchmin et al., 2005). β-CBT-diol also ameliorated the damage caused by diisopropylfluorophosphate in the hippocampal area CA1 in rats, as a concrete manifestation of the decreased number of dead neurons by half when injected 1 h before or 24 h after diisopropylfluorophosphate and the significantly decreased number of activated astrocytes, by Fluoro-Jade B and amino cupric silver staining and nestin expression measurement (Ferchmin et al., 2014).

β-CBT-diol also decreased brain damage in rodent ischemic stroke models (Martins et al., 2015). β-CBT-diol treatment significantly reduced infarction size by more than half in brain ischemic stroke in mouse 10 min before permanent middle cerebral artery occlusion and rats after at 1 h after reperfusion, indicating β-CBT-diol may play a huge role in treating and preventing ischemic stroke. This has something to do with the β-CBT-diol, which can cross the blood-brain barrier and accumulate in the brain. It was further found that β-CBT-diol inhibited the expression of ICAM-1 in brain-derived endothelial cells and restored the phosphorylation of Akt that was stimulated by oxygen-glucose deprivation, thereby protecting neurons from inflammatory injury and death in ischemic stroke.

β-CBT-diol improved Parkinson's disease both *in vitro* neuro-2a cell models and *in vivo* rat models induced by 6-Hydroxydopamine challenge (Hu et al., 2017). β-CBT-diol treatment improved rat behavioral deficits by 4 weeks after injection of 6-Hydroxydopamine by decreasing the depletion of tyrosine hydroxylase in the striatum and substantia nigra, as indicated by comparing forelimb asymmetry scores and corner test scores. β-CBT-diol also protected the viability of neuro-2a cells by activating anti-apoptosis protein p-Akt and HAX-1, inhibiting pro-apoptosis protein caspase-3, and regulating the NF-κB/VCAM-1/TNF-α pathway to resist endothelial inflammation in murine brain-derived endothelial bEND5 cells. Thus, β-CBT-diol may be a promising therapeutic agent for Parkinson's disease.

The most recent study in the year of 2022 reported that β-CBT-diol protected neuronal cells from oxygen-glucose deprivation by modulating microglial cell activation (Fu et al., 2022), further provided data support for the study of β-CBT-diol as a drug for the treatment of ischemic stroke and neurodegenerative diseases. It has been found that β-CBT-diol exerted anti-inflammatory effects on microglia by regulating the M1/M2 phenotype to protect neurons from ischemia damage or inflammation. In LPS and oxygen-glucose deprivation -induced inflammation models of microglia N9 cells, β-CBT-diol promoted a neuroprotective tilt of microglia activation by down-regulating levels of NF-κB/iNOS, a marker of

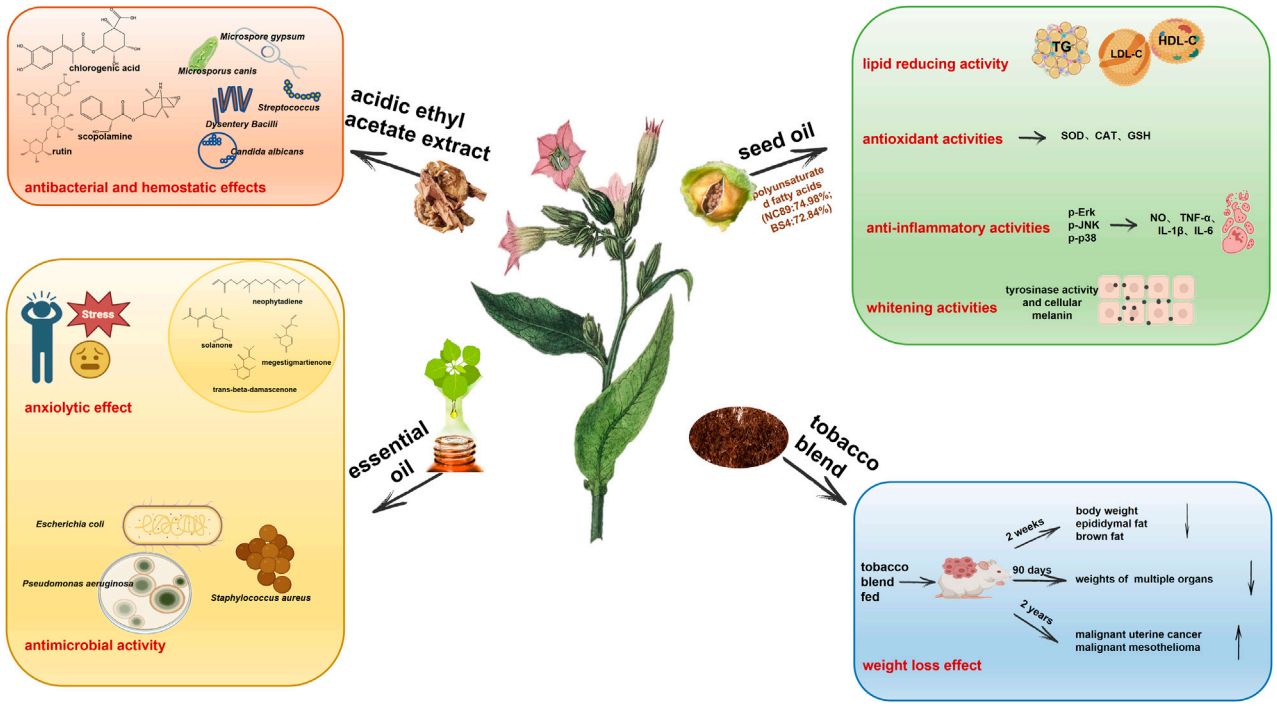

FIGURE 4
Tobacco plant extracts including acidic ethyl acetate extract, seed oil, essential oil, and tobacco blend have biological activities in antibacterial and hemostatic effects, anxiolytic effect, antimicrobial activity, lipid reducing activity, antioxidant activities, anti-inflammatory activities, whitening activities, and weight loss effect.

M1 inflammatory response, and up-regulating levels of Arg-1 and IL-10, markers of M2 anti-inflammatory activation. At the same time, β-CBT-diol produced beneficial factors in N9 medium, which further had a protective effect on oxygen glucose deprivation -induced Neuro2a cell damage, when the conditioned medium of β-CBT-diol-treated N9 cells was added to Neuro2a cells and incubated for 24 h. This study only detected a few markers at the cellular level, and future neuroprotective studies on β-CBT-diol require more in-depth exploration of molecular mechanisms at *in vivo*, so as to make adequate preparation for clinical application.

4.3 Antimicrobial activity

β-CBT-diol is very promising and has a broader spectrum of antimicrobial activities than α-CBT-diol (Aqil et al., 2011). β-CBT-diol showed good activity against the bacteria including *S. aureus, B. subtilis (MTCC 121), E. coli (UP 2566), Staphylococcus typhimurium (MTCC 98), Shigella dysenteraie, P. aeruginosa, P. vulgaris (ATCC 6380)*, while α-CBT-diol only inhibited the growth of *S. aureus, B. subtilis (MTCC 121),* and *P. vulgaris (ATCC 6380)* and was weaker than β-CBT-diol. β-CBT-diol also demonstrated broad-spectrum antifungal activity, inhibiting the tested fungi including *Candida albicans, Fusarium chlamydosporum, Rhizoctonia bataticola, Aspergillus niger,* and *Alternaria alternata*, while α-CBT-diol had a weaker inhibition on *C. albicans, F. chlamydosporum, A. niger,* and *A. alternata.* These findings highlight β-CBT-diol's potential as promising antimicrobial scaffolds.

Some reports showed that β-CBT-diol attenuated HIV neurotoxicity by reducing glutamate release independently of viral replication and inflammation. Furthermore, β-CBT-diol could be used to resist HIV virus replication, HIV-associated neurocognitive disorders and HIV virus-induced inflammation (Andrew Ferchmin et al., 2012; Yan et al., 2019b). Due to the lack of more detailed information, the application of HIV and HIV- related diseases needs to be carefully verified.

5 Tobacco extracts

There are few studies in modern pharmacology on tobacco plant extracts, and most focus on the extraction and purification of the active substances such as nicotine and solanesol. In fact, apart from inhaled tobacco smoke, the early external use of tobacco leaves and their juice in disease treatment has been the inspiration for the application of tobacco extracts in modern medicine in anti-inflammatory, analgesic, bacteriostatic and hemostatic applications. For example, chewing green tobacco leaves in your mouth can help with oral mucositis. A heated mixture of tobacco and salt was crushed and applied to the root of the cervical gland to treat bacteriostasis, hemostasis and inflammation. Tobacco powder can also be applied to locally cure cuts or burns. The external application of tobacco can also treat the bites of toxic reptiles and insects, skin molds and ulcers (Charlton, 2004; Sanchez-Ramos, 2020). The medical significance of tobacco extract in modern medical research is mainly manifested in the continuation and

in-depth proof of early records, as demonstrated in the following sections (Figure 4).

5.1 Antibacterial and hemostatic effects of tobacco acidic ethyl acetate extract

The acidic ethyl acetate extract of tobacco has strong broad-spectrum antibacterial and rapid hemostatic effects (Chen, 2017). *In vitro* bacteriostasis experiments based on the filter paper method, 96 well plate micro broth dilution method and minimum bactericidal concentration determination show that acidic ethyl acetate tobacco extracts have inhibitory effects, in the order from strong to weak, on *Dysentery Bacilli*, *Streptococcus*, *C. albicans*, *Microsporus canis*, *Trichophyton rubrum*, *Microspore gypsum* and *A. niger*, with the minimum inhibitory concentration and minimum bactericidal concentration of 1.56 mg/mL and 3.12 mg/mL, respectively. After the extract was intragastrically administered to mice for 1 week, the coagulation time of mice was observed after tail cutting. The coagulation time of the mice in the acidic ethyl acetate extract (0.4007 g/kg) group was about 154 s, which was 37.4% shorter than that of the blank control group, and close to that of the Yunnan Baiyao positive group. Its effective constituents include β-sitosterol, 3-O-β-D-glucopyranosyl-stigmasterol, anisodamine, quercetin, 7-hydroxy-6,6′-dimethoxy-3,7′-O-bis-coumarin, gallic acid, caffeic acid, chlorogenic acid, kaempferol 3-O-rutinoside, p-hydroxybenzoic acid and rutin after analysis by NMR and mass spectrometry. Of these constituents, chlorogenic acid, scopolamine and rutin may play a major role.

5.2 Tobacco seed oil

5.2.1 Lipid reducing activity of tobacco seed oil

Tobacco seed oil is an excellent vegetable oil resource. The average oil content of tobacco seeds is about 39.4% and the content of unsaturated fatty acids is high (87.8%–89.4%), which is 7.5–8.5 times higher than that of saturated fatty acids. The content of linoleic acid is high (71.6%–75.36%), and its fatty acid composition is close to that of grape seed oil. The content of essential amino acids is higher than that of olive oil (Su, 2016; Yang et al., 2019). Su et al. (Su, 2016) systematically studied the toxicology and lipid-lowering activity of tobacco seed oil and found that the LD50 of tobacco oil for mice was more than 21,500 mg/kg, and no genotoxicity was found in a *Salmonella Typhimurium* test, bone marrow cell micronucleus test or chromosome aberration test. When tobacco oil (5, 10 and 20 g/kg) is added to the feed, there is no observed adverse effect on rats in the long-term feeding experiment (30 days), suggesting that tobacco oil is a vegetable oil without acute or chronic toxicity or mutagenicity to animals. Further research has been conducted on hyperlipidemia in Sprague-Dawley male rats models. The intragastric administration of tobacco seed oil (2, 4 and 6 mL/kg per day) has the ability to reduce triglycerides, low-density lipoprotein cholesterol and increase high-density lipoprotein cholesterol in serum, as well as decrease atherosclerosis index and ameliorate steatosis of liver cells. Hence, tobacco seed oil is expected to become an oil product with health benefits, especially for patients with hyperlipidemia, or cardiovascular disease.

5.2.2 Antioxidant, anti-inflammatory, and whitening activities of tobacco seed oil

Seed oil of tobacco strain NC89 and BS4 are rich in polyunsaturated fatty acids (NC89: 74.98%; BS4:72.84%) that much higher than the values reported for other important food oils such as olive oils (25%), soybean oils (50.59%), and sesame oils (46%). Linoleic acid is the most abundant polyunsaturated fatty acid in NC89 and BS4. These two kinds of oil exerted the 1) antioxidant activity by scavenging ABTS, OH-, O2- radical, inhibiting ROS accumulation and enhancing SOD, CAT activities and GSH in H_2O_2-induced HepG2 cells 2) anti-inflammatory activity by inhibiting the expressions of NO, TNF-α, IL-1β, and IL-6 in LPS-induced RAW.264.7 cells through down-regulating the p-ERK, p-JNK, p-p38, and 3) whitening activity by inhibiting tyrosinase activity and cellular melanin production in melanoma B16 cells (Gu et al., 2022). These indicate that tobacco seed oil is a valuable and advantageous oil resource in food and cosmetic applications.

5.3 Weight loss effect of tobacco blend

Tobacco blend feeding can alleviate obesity and obesity-linked metabolic disorder in mice. When 27.46 g/kg of tobacco blend was added to the basic diet, after 2 weeks of feeding, the weight of mice was decreased by 16.3%, and the weight of epididymal fat and brown fat decreased by 67.6% and 42%, respectively, without inflammation. The increase in physical activity and the decrease of respiratory exchange rate in mice suggest that tobacco feeding can induce triglyceride lipolysis of adipose tissue to provide fatty acids. Without changing insulin sensitivity, the insulin level in plasma increases 3.6-fold and the blood sugar content decreases (Liu et al., 2018). Theophilus *et al.* (Theophilus et al., 2012; Theophilus et al., 2015) carried out feeding experiments lasting for 90 days and 2 years, with feeds including basic diet, tobacco blend mixed feed, tobacco blend water extract mixed feed and nicotine. Results indicate that plasma nicotine concentration in the middle and low dose groups is about 10–37 ng/mL, and that in the high dose group reaches 89 ng/mL, which is close to that in the plasma of smokers (10–50 ng/mL). The long-term tracking results show that the feed utilization rate of rats and mice is also decreased and, compared with obese mice, their weight is also significantly reduced (13%–28%), with decreased weights of organs such as brain, testis, salivary glands, adrenal glands, epididymis, pituitary gland and liver. After clinical observation, ophthalmological examination, toxicity dynamic analysis, clinical pathology, gross pathology and histopathological analysis, there was no increase in toxicity or carcinogenicity. During the 2-year feeding process, in addition to spontaneous and concomitant diseases, the incidence of malignant uterine cancer in female mice and malignant mesothelioma of the epididymis in male mice increases significantly. However, a decreasing tendency was observed in benign breast adenoma (female mice fed with tobacco), malignant skin basal cell carcinoma (female mice fed with water extract of tobacco blend), and benign thyroid follicular cell adenoma (male rats fed with water extract of tobacco blend). The incidence of tumors is also related to the genetic background and age of the mice, which require additional investigation.

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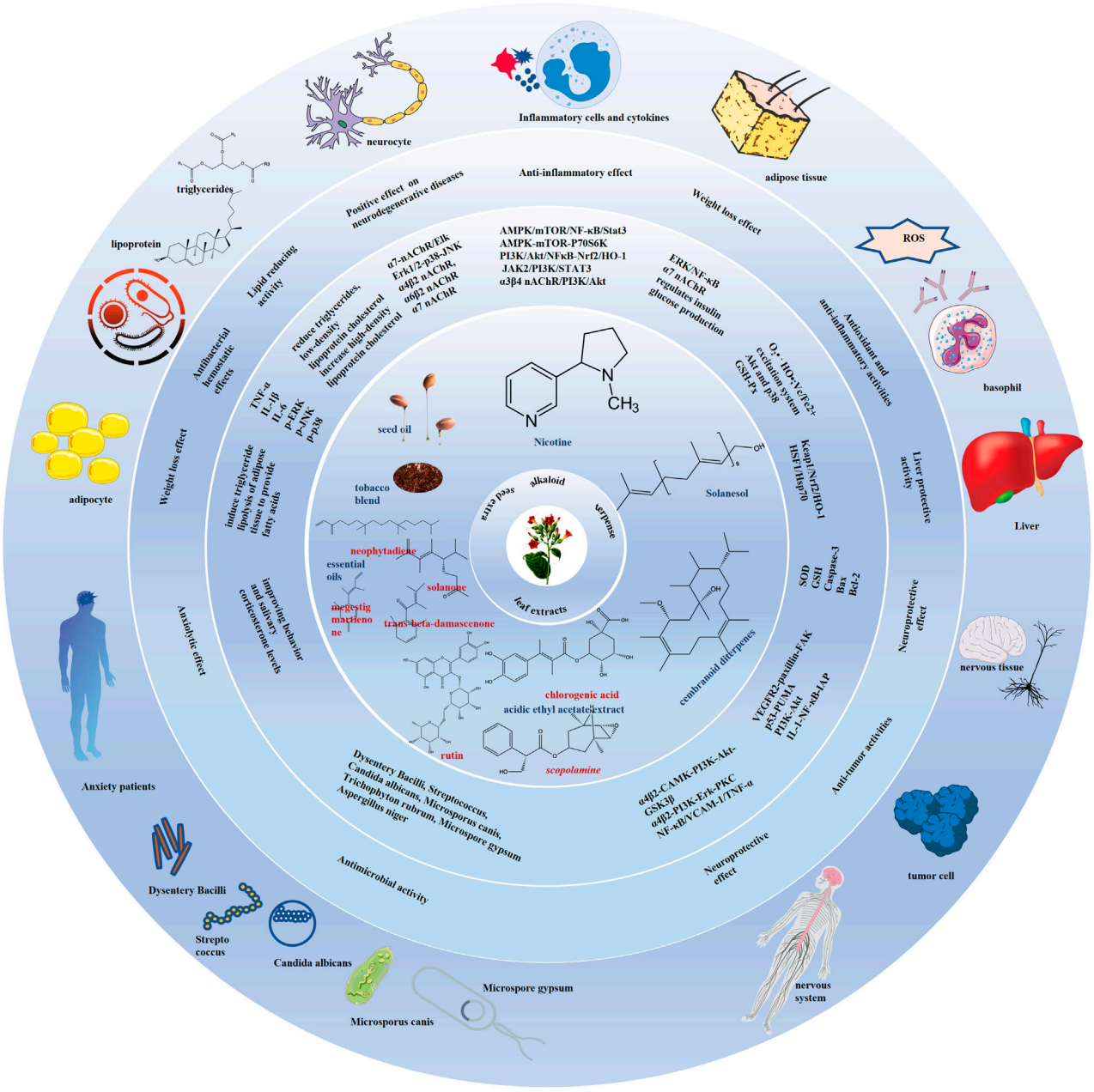

FIGURE 5
Summary of medical and pharmaceutical value of tobacco plant and its functional components. The tobacco and its components including nicotine, solanesol, seed oil, leaf extracts have neuroprotective, anti-inflammatory diseases and metabolic syndrome regulation effects through anti-oxidation, anti-lipid production, pro-lipid oxidation, pro-insulin sensitivity, anti-inflammation, anti-apoptosis and antibacterial activities. The main pathways of AMPK/mTOR/NF-κB/Stat3, PI3K/Akt/NFκB-Nrf2/HO-1, α4β2, α6β2, α7 nAChR/AKT/PI3K, α7 nAChR/ERK/NF-κB/iκB, Akt-p38/Nrf2/HO-1 and target proteins including the inflammatory factors IL-6, TNF-α, IL-1β, IL17, IFN-γ, IL-8, IL-4, IL-5, IL-1, IL-10, and PGE2, and the apoptosis markers bax, caspase-3, Bcl-2, and PARP are involved in their roles in regulating diseases.

Regarding tobacco plant extracts, on the premise of avoiding the toxic and carcinogenic substances ingested by smoking, the abundant medicinal substances in tobacco could exert safe and effective function in anti-inflammation, anti-bacteria, and fat and weight reduction. In the future, this smokeless tobacco product has the potential to play an important role in human healthcare and could also serve as a viable strategy to quit smoking.

5.4 Tobacco essential oils

As an important aromatic plant, tobacco contains more than 3,000 compounds and much more than other natural products (Mookherjee and Wilson, 1990). Tobacco essential oils may be the constituents that responsible for treating mental health problems such as anxiety and depression in the clinic (Berlowitz

et al., 2020). Recent research has also found that it has antimicrobial effects (Stojanovic et al., 2000; Palic et al., 2002; Popova et al., 2015).

5.4.1 Anxiolytic effect

Anxiety disorder is a prevalent and highly disabling mental health condition; however, there is still a lack of desirable therapeutic outcome, affecting up to 6% of the population during their lifetime (Maron and Nutt, 2017). According to the first report, the unique aroma of tobacco essential oils mainly contained neophytadiene and solanone, followed by megastigmatrienone and trans-beta-damascenone, which have an anxiolytic effect. Both Yunnan tobacco essential oil and Zimbabwe tobacco essential oil on male ICR mice in the light-dark box test and the maze test via inhalation and transdermal administration showed the anxiolytic effect through improving behavior and salivary corticosterone levels. Moreover, oral toxicity evaluation demonstrated that the concentrations of these oils were considered safe (Xie et al., 2021). However, we should carefully analyze the composition of essential oils to ensure that no toxic constituents are present, especially excessive nicotine residues which may have potential harmful effects. Purifying the main constituent for the future medical applications will be the most basic requirement.

Neophytadiene is the most noteworthy active metabolite in tobacco essential oil. In addition to tobacco, neophytadiene is also a major constituent of many plant essential oils, including *Jatropha curcas L (*Euphorbiaceae*), Acalypha segetalis, Zea mays* (Konstantopoulou et al., 2004; Aboaba et al., 2010; Adeosun et al., 2017). The anxiolytic-like, antidepressant-like, anticonvulsant, sedative effects and the anti-inflammation of neophytadiene (Bhardwaj et al., 2020; Gonzalez-Rivera et al., 2023) in the neuropharmacological actions may be the key point for tobacco essential oil, which has the effect not only on anxiolytic effect but also on the other mental diseases.

5.4.2 Antimicrobial activity

Early reports showed that tobacco essential oil mainly containing neophytadiene and solanone in the middle leaves of Otlja and Prilep showed greater activity against the microorganisms *E. coli*, *S. aureus* and *P. aeruginosa* than that in the upper leaves or than the CO2 extracts (Stojanovic et al., 2000; Palic et al., 2002).

6 Conclusion

Worth noting, this study is not intended to promote commercial tobacco smoking because it will never change the fact that tobacco smoke and its combustion products possess tobacco-specific carcinogens, which induce several malignancies. What we really advocate is that recognizing fresh tobacco bioactive metabolites is adding pharmaceutical value to this economically significant agricultural crop. The development of modern medicine and pharmacology, advances in compound separation, extraction and identification technology, and cross-disciplinary communication have been in step with research into and changing attitudes towards the tobacco plants. Effective functional metabolites of tobacco including nicotine, solanesol, seed oil containing high unsaturated fatty acids and linoleic acid, and leaf extracts mainly containing chlorogenic acid, kaempferol 3-O-rutinoside, p-hydroxybenzoic acid and rutin have emerged in many disease fields, especially in some difficult diseases: cranial nerve diseases including Alzheimer's disease, Parkinson's disease, Huntington's disease, intracerebral and ventricular hemorrhage, autism, and bipolar disorder; inflammatory diseases including rheumatoid arthritis, ulcerative colitis, septicemia, multiple sclerosis, myocarditis, and periodontitis; antibacterial activity including the elimination of *E. coli, M. phlei, P. aeruginosa, S. aureus, Dysentery Bacilli, Streptococcus, C. albicans, M. canis, T. rubrum, M. gypsum, and A. niger*. They exert the function of anti-oxidation, anti-lipid production, pro-lipid oxidation, pro-insulin sensitivity, anti-inflammation, anti-apoptosis and antibacterial activities by regulating the main pathways of AMPK/mTOR/NF-κB/Stat3, PI3K/Akt/NFκB-Nrf2/HO-1, α4β2, α6β2, α7 nAChR/AKT/PI3K, α7 nAChR/ERK/NF-κB/iκB, Akt-p38/Nrf2/HO-1 and target proteins of inflammatory factors IL-6, TNF-α, IL-1β, IL17, IFN-γ, IL-8, IL-4, IL-5, IL-1, IL-10, PGE2 (Figure 5). Thus, tobacco has become a medicine and treatment choice to improve quality of life, and even prolong life span.

The tobacco plant should be a good resource for drug development. However, biomedical research data on the efficacy of tobacco is limited, probably due to the following reasons. At present, the development and use of tobacco are mainly focused on the cigarette industry. Government funding and support are insufficient for scientific research institutions working in this new field. Furthermore, public attitudes towards tobacco are still dictated by the harmful effects of smoking. Acceptance of the potential role of the tobacco plant in healthcare will require positive publicity. Considering the current development direction of the cigarette industry, tobacco is not attractive to talents in the biomedical and multi-disciplinary fields, and the vitality of scientific and technological innovation is relatively low. In this emerging field, reports on the structural identification of bioactive tobacco plant metabolites, and efficacy and safety studies, are scarce. Furthermore, extract separation and purification are challenging, resulting in a high cost for highly pure products. Pharmacological studies are mostly conducted on a limited number of metabolites, such as nicotine and solanesol, and specific diseases. Insufficient investigation on molecular mechanisms also leads to the termination of clinical experiments due to lack of specificity.

In the future, research should focus on drug analysis, separation and identification to build a molecular library of drugs from tobacco plant resources. Purification technologies and the purification output of pharmaceutical compounds should be improved and refined to lay a solid foundation for drug research and development. Moreover, the molecular mechanisms should be studied in more depth to thoroughly understand the pharmacological and toxicological mechanisms and pharmacokinetics trait of tobacco and its metabolites so that the tobacco plant can play a positive therapeutic role in the treatment of human diseases.

Author contributions

WZ: Methodology, Writing–original draft, Writing–review and editing. XP: Funding acquisition, Writing–review and editing. JF: Data curation, Writing–review and editing. WC: Software, Writing–review and editing. HL: Supervision, Validation,

Writing–review and editing. WZ: Project administration, Writing–review and editing. ZH: Funding acquisition, Project administration, Writing–review and editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This work was supported by the Guangdong Basic and Applied Basic Research Foundation (grant number 2023A1515010353 and 2021A1515011220); Administration of Traditional Chinese Medicine of Guangdong Province of China (grant number 20211008); Special fund for scientific innovation strategy-construction of high-level Academy of Agriculture Science (grant number R2022PY-QY004, R2018YJ-YB3002); the foundation of director of Crops Research Institute, Guangdong Academy of Agricultural Sciences (grant number 202303); The Science and Technology Plan Project of Guangdong Province (2023B1212060038).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Glossary

AMPK	AMP-activated protein kinase
Th2	helper T cell 2
mTOR	mammalian target of rapamycin
GATA3	GATA binding protein 3
NF-κB	nuclear factor kappa B
RORγτ	RAR-related orphan receptor gamma
Stat3	signal transducers and activators of transcription 3
SOCS3	suppressor of cytokine signaling 3
PI3K	phosphoinositide 3-kinase
MMP9	matrix metallopeptidase 9
Akt	protein kinase B
VEGF	vascular endothelial growth factor
Nrf2	nuclear factor erythroid 2–related factor 2
CRP	C-reactive protein
HO-1	heme oxygenase 1
HMGB1	high mobility group protein B
nAChR	nicotinic acetylcholine receptor
PAI-1	plasminogen activator inhibitor-1
ERK	extracellular signal-regulated kinase
ROS	reactive oxygen species
iκB	ikappaB kinase
AST	glutamic oxalacetic transaminase
IL-6	interleukin 6
ALT	glutamate pyruvate transaminase
TNF-α	tumor necrosis factor-alpha
IL-1β	interleukin 1β
LPL	lipoprotein lipase
IL17	interleukin 17
PPARγ	peroxisome proliferator activated receptor gamma
IFN-γ	interferon gamma
MCP-1	monocyte chemoattractant protein-1
IL-8	interleukin 8
Bax	BCL2 associated X apoptosis regulator
IL-4	interleukin 4
LPS	lipopolysaccharide
IL-5	interleukin 5
SOD	superoxide dismutase
IL-1	interleukin 1
GSH-Px	glutathione peroxidase
IL-10	interleukin 10
MDA	malondialdehyde
PGE2	prostaglandin E2
Vc	vitamin C
JNK	c-Jun N-terminal kinase
GSH	glutathione
SIRT6	Sirtuin 6
LDH	lactate dehydrogenase
FGF2	fibroblast growth factor 2
SIRT1	Sirtuin 1
P70S6K	ribosomal protein S6 kinase beta-1
Bcl-2	B-cell lymphoma 2
MAdCAM-1	mucosal vascular addressin cell adhesion molecule 1
FAK	focal adhesion kinase
ZO-1	zonula occluden-1
ATP	adenosine triphosphate
Th1	helper T cell 1
p-gp	glycoprotein

frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 13 May 2024
DOI 10.3389/fphar.2024.1360179

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OPEN ACCESS

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RECEIVED 15 January 2024
ACCEPTED 29 April 2024
PUBLISHED 13 May 2024

CITATION
Ma L, Li J, Zhang X, Zhang W, Jiang C, Yang B and Yang H (2024), Chinese botanical drugs targeting mitophagy to alleviate diabetic kidney disease, a comprehensive review.
Front. Pharmacol. 15:1360179.
doi: 10.3389/fphar.2024.1360179

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Chinese botanical drugs targeting mitophagy to alleviate diabetic kidney disease, a comprehensive review

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Diabetic kidney disease (DKD) is one of the chronic microvascular complications caused by diabetes, which is characterized by persistent albuminuria and/or progressive decline of estimated glomerular filtration rate (eGFR), and has been the major cause of dialysis around the world. At present, although the treatments for DKD including lifestyle modification, glycemic control and even using of Sodium-glucose cotransporter 2 (SGLT2) inhibitors can relieve kidney damage caused to a certain extent, there is still a lack of effective treatment schemes that can prevent DKD progressing to ESRD. It is urgent to find new complementary and effective therapeutic agents. Growing animal researches have shown that mitophagy makes a great difference to the pathogenesis of DKD, therefore, exploration of new drugs that target the restoration of mitophagy maybe a potential perspective treatment for DKD. The use of Chinese botanical drugs (CBD) has been identified to be an effective treatment option for DKD. There is growing concern on the molecular mechanism of CBD for treatment of DKD by regulating mitophagy. In this review, we highlight the current findings regarding the function of mitophagy in the pathological damages and progression of DKD and summarize the contributions of CBD that ameliorate renal injuries in DKD by interfering with mitophagy, which will help us further explain the mechanism of CBD in treatment for DKD and explore potential therapeutic strategies for DKD.

KEYWORDS

mitochondria, mitophagy, diabetic kidney disease, Chinese botanical drugs, treatment

1 Introduction

Diabetic kidney disease (DKD) is one of the serious microvascular complication in diabetic mellitus (DM), and approximately 40% of T2DM patients will develop to DKD (Stephens et al., 2020). The global prevalence of diabetes in the 20-79 age group is projected to rise to 12.2% by 2045, affecting an estimated 783 million people (Sun et al., 2022). DKD is a progressive disease characterized by the microalbuminuria in early stage, persistent massive proteinuria and increased creatinine levels in middle stage, and culminating in ESRD eventually. The renal pathology of DKD is mainly characterized by glomerular mesangial cell (GMCs) proliferation, glomerular basement membrane (GBM) thickening, and extracellular matrix accumulation, which eventually leads to renal fibrosis (Jiang et al., 2019; Wang et al., 2021). To date, few effective therapeutic strategies can inhibit the

deterioration of DKD. The present strategies for treating DKD are still limited to strictly management of hyperglycemia, lipids, blood pressure, and the use of RASS blockers. Although more and more clinical trials have shown that the SGLT2 inhibitors will be a potential oral agents for prevent DKD (Mosenzon et al., 2019; Heerspink et al., 2020; Giglio et al., 2023). The non-steroidal selective mineralocorticoid receptor antagonist (MRA) finerenone has also been gradually proved to have great potential in the treatment of DKD (Filippatos et al., 2021; Filippatos et al., 2022; Agarwal et al., 2022). Renal replacement treatment (RRT) or kidney transplantation are still be the ultimate choice for DKD patients with ESRD. Therefore, it is necessary to further get a thorough understanding of the pathogenesis of DKD to explore new drugs including complementary and alternative medicine to delay the progression of DKD.

As we know, the kidney is the second highest oxygen consumption organ in our body. The kidney not only contains different cell types but also performs a variety of physiological functions such as endocrine functions, regulating blood pressure and intraglomerular hemodynamics, transporting solutes and water, maintaining acid-base balance, reabsorbing nutrients, and eliminating fuel or drug metabolites. The normal physiological function of kidney cells depends on adequate energy supply from mitochondria (Murphy and Hartley, 2018). However, more and more researches have shown that mitochodrial damages and dysfunction played an essential role in the pathophysiology of different kidney diseases, as well as in DKD (Ratliff et al., 2016; Duann and Lin, 2017). Numerous researches have shown that the dysfunction of mitochondria was involved in the accelerated progression of DKD (Wang et al., 2012; Coughlan et al., 2016; Long et al., 2016; Mise et al., 2020). Therefore, appropriate and timely removal of aged or abnormal mitochondria to maintain mitochondrial homeostasis is crucial to relieve the damage of DKD. Mitophagy is a highly conserved mechanism for selective removal of dysfunctional and fragmented mitochondria via the autophagic machinery, which has been recognized as a pivotal mechanism for regulating mitochondrial quality and quantity control (Lemasters, 2005; Palikaras et al., 2018; Pickles et al., 2018) A growing body of studies have proven that mitophagy is impaired *in vivo* and *in vitro*, and the signaling pathways regulating mitophagy are inhibited (Higgins and Coughlan, 2014; Czajka et al., 2015; Chen et al., 2018). Enhancing the level of intracellular mitophagy has a significant renoprotective effect in DKD and supplementation with MitoQ, a mitochondrial antioxidant, was confirmed to protect against DKD through upregulating the levels of mitophagy via Nrf2/PTEN-induced putative kinase protein 1 (PINK1) (Tagawa et al., 2016; Xiao et al., 2017; Yang et al., 2019b). Therefore, both basic and clinical studies aimed to modulate or restore impaired mitophagy may provide innovative therapeutic strategy for DKD.

CBD has a history of thousands of years and was used to treat various diseases during the long development of Chinese nation. Also, CBD is popular in the world and widely used in more than 100 countries owing to its safety clinical efficacy. Currently, more and more studies have confirmed that traditional CBD has unique advantages and good clinical efficacy in delaying the progression of DKD, which has gradually aroused the interest of nephrologists (Tang et al., 2021; Zhang et al., 2022; Shen et al., 2024).

Over the past decades, the potential molecular mechanisms of CBD for the treatment of DKD have been researched extensively. Multiple studies have revealed that CBD can exert renoprotective action through regulating autophagy (Xu et al., 2017; Wang et al., 2018; Zhan et al., 2019), and even mitophagy (Hang et al., 2018; Wang X. et al., 2020). This review will sum up the molecular mechanism of mitophagy during the occurrence and progression of DKD. Furthermore, recent advances in preventing DKD via regulating mitophagy from the perspective of CBD will also be discussed.

2 Regulatory signaling pathways of mitophagy

Once fails to be repaired, mitochondria are eliminated by mitophagy to prevent excessive production of ROS and slow down apoptosis caused by inflammatory response damage. Typically, mitophagy is divided into PINK1/Parkin-dependent and independent pathways. Moreover, there are three principal pathways that modulate mitophagy in mammals: the Pink1/Parkin pathway, the BNIP3L pathway, and the FUNDC1 pathway. Among them, activation of PINK1, which is thought to be the initiating event for the induction of mitophagy, works vitally during the process of mitophagy. According to the available research results, the signaling pathways of regulating mitophagy are broadly divided into three categories: ubiquitin (ub)-dependent mitophagy pathways, receptor-mediated pathways and membrane lipid-mediated signaling pathways (Chourasia et al., 2015) (Figure 1).

2.1 Ubiquitination-dependent mitophagy pathway

2.1.1 PINK1-Parkin pathway

At present, the PINK1-Parkin pathway has been most extensively studied. In the meantime, the most well-known protein components that control mitophagy for mitochondrial maintenance and quality control are PINK1 and Parkin (Pickles et al., 2018). PINK1 is a serine/threonine kinase located in the mitochondria whose function is an operator and sensor under the control of mitochondrial membrane potential (Zhou et al., 2008). In the wake of loss of mitochondrial membrane potential, PINK1 is stabilized and activated at the outer mitochondrial membrane (OMM). Located inside the cytoplasmic lysosome, parkin is an E3 ubiquitin ligase (Matsuda et al., 2010). Transfer of Parkin to mitochondria is essential for initiating mitophagy. When cells are stimulated, such as mitochondrial stress, proteotoxic stress and misfolded proteins, Parkin migrates to the OMM in large numbers, and works with ubiquitin-activating enzymes and ubiquitin molecules to ubiquitinize and modify damaged extramitochondrial membrane proteins, promoting the degradation of damaged mitochondria (Sarraf et al., 2013). Whereas, PINK1, located upstream of Parkin, exerts its functions to phosphorylate Parkin and ubiquitin, promoting the movement of Parkin from cytoplasm to the OMM (Narendra et al., 2010). Therefore, the activation of Parkin in response to

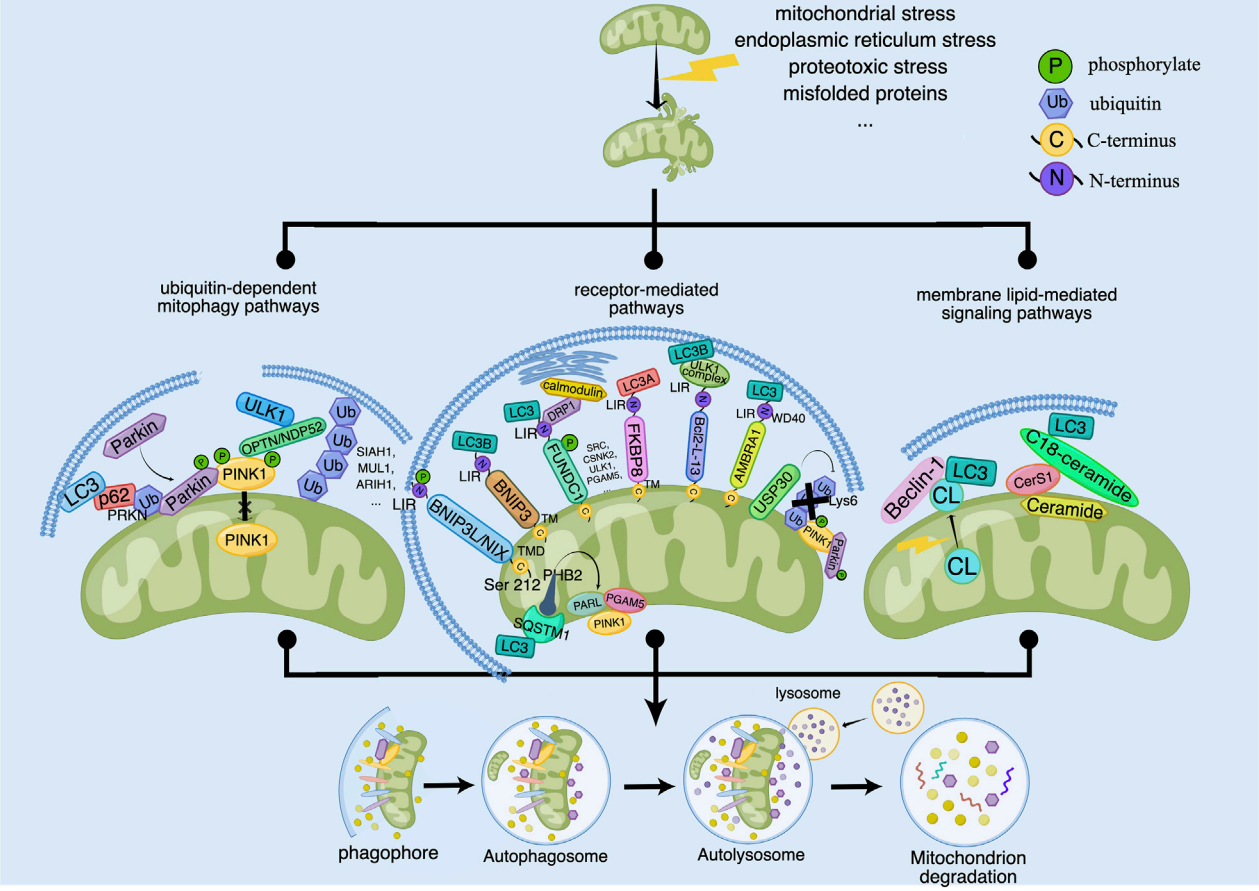

FIGURE 1
The signaling pathways for regulating of mitophagy. Some outer and inner risk factors such as Mitochondrial stress, endoplasmic reticulum stress, proteotoxic stress and misfolded proteins stimulate cells and lead to mitochondrial damage, which can trigger mitophagy to clear the damaged mitochondria. The main signaling pathways for regulation of mitophagy contain ubiquitin-dependent mitophagy pathways, receptor-mediated pathways and membrane lipid-mediated signaling pathways. Ub-dependent mitophagy involves the PINK1/Parkin dependent and non-PINK1/Parkin-dependent (receptor-mediated) pathways. PINK1 activates mitophagy by activating Parkin or recruiting OPTN/NDP52 and other ubiquitin ligases such as SIAH1, MUL1 and ARIH1. Receptor-mediated pathways involves the OMM and IMM proteins. OMM proteins include BNIP3L/NIX, BNIP3, FUNDC1, FKBP8, Bcl2-L-13, AMBRA1 and USP30, which regulate the coupling between OMM and the autophagosome membrane. IMM proteins include autophagy receptors such as PHB2. PHB2 can induce mitophagy by binding LC3 directly or forming a ternary protein complex with chelate 1 (SQSTM1) and LC3. In addition, PHB2 can regulate PINK1 by regulating the activity of mitochondrial protease PARL. Membrane lipid-mediated signaling pathways mainly include cardiolipin (CL) and ceramide. CL is located in the IMM in normal mitochondria. When mitochondria are damaged, CL is transferred to the OMM and interacts with LC3, which mediates mitophagy. And ceramide depends on CerS1 and C18-ceramide to promote the formation of LC3 and induce mitophagy.

mitochondrial damage is followed by ubiquitin phosphorylation of PINK1.

Normally, the PINK1 dimer remains stable until each kinase structural domain is phosphorylated (Gan et al., 2022). As the PINK1 protein translocates to the inner mitochondrial membrane (IMM), it is fragmented by PARL and then rapidly destroyed by ub-proteasome degradation (Sato and Sato, 2011). Once mitochondria are impaired or depolarized, PINK1 protein hydrolysis is inhibited and no longer degraded by cleavage, but rapidly accumulates in the OMM, where stable PINK1 is activated by autophosphorylation, and then recruits cytoplasmic Parkin to translocate to the surface of OMM, activating Parkin's E3 ubiquitin ligase activity. Subsequently, receptor proteins, such as p62, recognize the mitochondrial ubiquitination signal and trigger the phosphorylation of activated PINK1 by kinase activity, then initiates the autophagic elimination of damaged mitochondria through LC3 and eventually enters the autophagic lysosomal pathway for degradation (Rakovic et al., 2011). Parkin and PINK1 act as a pair in degrading OMM proteins via ubiquitin-dependent degradation, which is required for mitochondrial surface protein renewal (Chan et al., 2011; Yoshii et al., 2011).

2.1.2 PINK1 via Parkin non-dependent mechanism

According to general consensus, PINK1 stimulates mitochondrial protein ubiquitination by phosphorylating and activating Parkin (Koyano et al., 2014; Lazarou et al., 2015), but it can also promote mitochondrial ubiquitination independently of Parkin (Rojansky et al., 2016). Through its ubiquitin binding domain, PINK1 can recruit OPTN/NDP52 to mitochondria, where it induces ULK1 to trigger mitophagy (Lazarou et al., 2015). Moreover, it has been shown that during binding to PINK1, unmodified Ub can be converted to Ub-cr

88 conformation, stabilizing the complex and encouraging subsequent phosphorylation of Ser65 residues (Schubert et al., 2017). Additionally, other E3 ubiquitin ligases, such as SIAH1 and ARIH1, activate mitophagy in an independent manner from Parkin as well (Yao et al., 2021). They engage in the ubiquitination of mitochondrial surface proteins, followed by the recruitment of autophagic vesicles by OMM proteins to complete the phagocytosis and degradation of damaged mitochondria. In addition, TBK1 is capable of phosphorylating autophagy receptors, thereby enhancing the process.

2.2 Receptor-mediated mitophagy pathways

2.2.1 Proteins in OMM

2.2.1.1 Bcl-2/E1B- 19k-interacting protein 3-like receptor (BNIP3L/NIX)

BNIP3L/Nix, a uncharacteristic membership of the pro-apoptotic Bcl-2 subfamily of BH3 proteins, is also a protein embedded in the OMM through its c-terminal transmembrane structural domain (TMD) (Chinnadurai et al., 2008). It contains a WXXL modal structure (i.e.,LIR) next to its N-terminal end (Xie et al., 2020), which enables OMM to couple to autophagosomal membranes and also dialogue with autophagy regulatory mechanisms to induce mitophagy through its LIR motif (Hamacher-Brady and Brady, 2016). In contrast, its N-terminus can bind to the mTOR-activating protein Rheb, and thus the reduced effect of mTOR activation has an impact on autophagy enhancement (Li et al., 2007)). In mitophagy, the onset and development of BNIP3L requires a combined mechanism of LIR phosphorylation and BNIP3L dimerization (Marinković et al., 2021). BNIP3L dimerization is a potentially novel molecular mechanism. Serine 212 is the major amino acid residue at the C-terminus of BNIP3L that extends into the membrane space and is responsible for dimerization.

2.2.1.2 Bcl-2/adenovirus E1B 19-kDa interacting protein 3 (BNIP3)

BNIP3, identical to BNIP3L, is a mitochondrial protein harboring a BH3 structural domain. BNIP3 has previously been determined to be a pro-apoptotic factor that interacts with adenovirus E1B-19 kDa and BCL2 proteins (Yasuda et al., 1998). BNIP3 is composed of a complex n-terminal region and a characteristic c-terminal transmembrane structural domain (Ogretmen and Hannun, 2004). Both BNIP3 and BNIP3L have the same N-terminal LIR, and their amino acid sequences are somewhat similar. At the same time, similar to Nix, BNIP3 regulates both mitophagy and cell death (Tang et al., 2019). As a key regulator of Parkin non-dependent mitophagy, BNIP3 binds to LC3B on autophagosomes and promotes phagocytosis of damaged mitochondria (Villa et al., 2018). Under hypoxic conditions, autophagy can be observed through the accumulation of the LC3 lipidated form (LC3II). Under the absence of BNIP3, mitophagy can be upheld by NIX, and elimination of pro-apoptotic mitochondria could contribute to the inhibition of apoptosis regulation (Abdrakhmanov et al., 2021) Nevertheless, other studies have shown that knockdown of BNIP3 resulted in increased residual mitochondria and increased expression of Nix on the membrane, but the damage caused by hypoxia was not compensated. Therefore, in BNIP3 knockout mice, an increase in Nix expression could be found, but the results also indicated that the level of mitophagy remained low and was uncompensated by the increase in Nix (Chourasia et al., 2015).

2.2.1.3 FUN14 structural domain containing 1 receptor (FUNDC1)

FUNDC1 is a widely expressed protein localized to mitochondria and has been identified as a specific receptor for mitophagy under hypoxic conditions (Youle and Narendra, 2011). Moreover, research also revealed that FUNDC1 functions as a mitophagy receptor role in a carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP)-induced mitophagy (Liu et al., 2012). It covers three transmembrane regions of the OMM, one of which extending to the c-terminus of the mitochondrial intermembrane and one extending to the cytoplasmic n-terminus. FUNDC1 recruits LC3 via its LIR structure in the n-terminal region, and under normal conditions, FUNDC1 is unable to interact with LC3 due to Tyr18 and Ser13 phosphorylation. As a comparison, during hypoxia, Ser13 is dephosphorylated by the phosphatase PGAM5, assuring interaction between FUNDC1 and LC3 and thus starting mitophagy. Point mutations or deletions in the LIR structural domain results in impaired mitophagy mediated by FUNDC1 in HeLa cells. Interestingly, FUNDC1-mediated mitophagy is strongly influenced by FUNDC1 phosphorylation status (Lv et al., 2017). Analysis showed that after the interaction of Lys49 of LC3B with Ser17 of phosphorylated FUNDC1, the lateral chain of LC3B is subjected to a major rearrangement of structure to adapt to phosphorylated FUNDC1, thereby working as a sensor of the phosphorylation state of FUNDC1 (Kuang et al., 2016). The phosphorylation status of FUNDC1 is mediated by phosphatases like CSNK2 and SRC, which determines its interaction with Atg8 proteins to regulate mitochondrial turnover that is FUNDC1-induced (Terešak et al., 2022). Besides, FUNDC1 interacts not only with LC3B but also with DRP1 and calmodulin at the mitochondrial-endoplasmic reticulum junction, which seems to be required for the recruitment of DRP1, for the reason that mutant forms of FUNDC1 that cannot bind DRP1 unable to promote mitophagy.

2.2.1.4 FK506 binding protein 8 (FKBP8)

FKBP8 (referred to FKBP38 as well) is a novel OMM mitophagy receptor belonging to the family of FK506 binding protein. The structural domain of FKBP8 is composed of four structural domains containing the n-terminal Glurich structural domain behind the peptidyl proline cis-trans isomerase structural domain, three tetrapeptide repeat structural domains, the calmodulin binding structural domain and a TM structural domain (Shirane-Kitsuji and Nakayama, 2014). FKBP8 is anchored in the OMM through its TM structural domain with the n-terminal pointing to the cytoplasm. Unlike other receptors, FKBP8 has a high affinity for LC3A and is significantly higher than LC3B. FKBP8 located in the OMM reacts with lipidated LC3A preferentially, interacts with lipidated LC3A in a LIR-dependent manner, and recruits LC3A to damaged mitochondria, ultimately causing mitophagy (Yoo et al.,

2020; Aguilera et al., 2022). However, LC3A recruitment to mitochondria is not absolutely necessary, but the existence of FKBP8 does enhance LC3A recruitment to mitochondria, but particularly in response to mitochondrial stress (Bhujabal et al., 2017). What is interesting is that FKBP8 is not degraded by autophagic vesicles during this period (Vara-Perez et al., 2019). It has been found that FKBP8 escapes from the mitochondria to the endoplasmic reticulum in the course of CCCP-induced parkin-mediated mitophagy, where it binds to Bcl-2 to exert an anti-apoptotic effect and thus avoids mitochondrial autophagic degradation, which makes it essential for avoiding unnecessary apoptosis process of mitophagy (Saita et al., 2013). However, the mechanisms involved still deserve further investigation.

2.2.1.5 Bcl-2-like protein 13 (Bcl2-L-13)

Bcl2-L-13 is a pro-apoptotic member of the BCL2 protein family, which is integrated into the OMM through its c-terminal transmembrane structural domain, while the N-terminal end is exposed in the cytoplasm. Likewise, Bcl2-L-13 is designated as a congener of autophagy-associated protein 32 (Atg32) in mammalian cells. Like Atg32, BCL2-L-13 has a mitochondrial localization and LIR motif (Murakawa et al., 2015). It is a mitochondrial receptor, which can make up for Atg32 in yeast (Mao et al., 2011). In general, mitochondrial depolarization trigger the expression of Bcl2-L-13, and knocking out Bcl2-L-13 prevents mitochondrial uncoupler carbonyl cyanide 3 chlorophenylhydrazone (CCCP)-induced mitophagy, whereas its overexpression is responsible for inducing mitochondrial disruption and mitophagy, but acts independently of DRP-1 and Parkin (Murakawa et al., 2015). Previous studies have identified the ULK1 complex, a counterpart of the Atg1 complex, as being required for Bcl2-L-13-mediated mitophagy in mammalian cells. After recruitment of the ULK1 complex, Bcl2-L-13 triggers mitophagy by means of the interaction of the LIR motif in the Bcl2-L-13-ULK1 complex with LC3B (Murakawa et al., 2019).

2.2.1.6 Autophagy/beclin 1 regulatory factor 1 (AMBRA1)

AMBRA1 contains three motifs, including two PxP motifs, two TQT motifs and one LIR motif. AMBRA1 does not have an apparent structural domain, furthermore, only the WD40 structural domain, which contains about 40 amino acids and serves as a binding site for protein-protein or DNA interactions, exists at its N-termina (Maria Fimia et al., 2007). Thus, AMBRA1 can supply a framework for assembling protein complexes or mediating temporary interactions with other proteins (Jain and Pandey, 2018). As well, AMBRA1 has a significant role in apoptosis as an autophagy-associated protein and a direct substrate for cystathionin and calpain (Maria Fimia et al., 2007). During the stage of autophagy induction, AMBRA1 is capable of modulating ULK1 kinase activity, as well as interacting with BECLIN1 and VPS34 to manage relevant activities (Li X. et al., 2022). Under normal conditions, AMBRA1 has a preference to interact with mitochondrial BCL-2 (mito-BCL-2), and when mitophagy is activated, the interaction between AMBRA1 and mito-BCL-2 is broken (Strappazzon et al., 2011). It is found that AMBRA1 is a nonsubstrate interaction of Parkin, and the interaction between AMBRA1 and Parkin is strengthened after mitochondrial depolarization, resulting in mitochondrial removal in a Parkin-mediated way (Van Humbeeck et al., 2011). AMBRA1, a cofactor for E3 ubiquitin ligase HUWE1 activity, fosters the interaction of HUWE1 with MFN2, contributes to ubiquitination and degradation of MFN2, and impacts mitophagy eventually. On the other hand, HUWE1 promotes the interaction of the LIR motif of AMBRA1 with LC3 to induce mitophagy (Di Rita et al., 2018).

2.2.1.7 Ubiquitin-specific protease 30 (USP30)

Constitutively, USP30 is the active deubiquitinase (DUB) known to be anchored in the MOM. Due to its unique transmembrane structural domain, it is found to be located in the OMM and peroxisome, and prefers to adopts a peculiar catalytic three-element and molecular construction to decompose the Lys6-linked ubiquitin chain prefavourably (Sato et al., 2017). Functionally, USP30 is a modulator of mitochondrial morphology and mitophagy (Yue et al., 2014). Acting upstream of PINK1 to set the threshold for initiating mitophagy (Marcassa et al., 2018), loss or reduction of USP30 levels leads to increased mitochondrial turnover (Bingol et al., 2014; Cunningham et al., 2015). USP30 is the only DUB related to mitochondrial surface composition, and it has been suggested that it mainly functions as an antagonist of mitophagy, either by removing ubiquitinated OMM proteins from Parkin substrates on the mitochondrial surface to antagonize PINK1/Parkin-mediated mitophagy (Bingol et al., 2014), or by reacting directly with Parkin to inhibit mitophagy (Wang Y. et al., 2020; Rusilowicz-Jones et al., 2020). The entire Lys6 link chain is prevented from hydrolyzing USP30 by phosphorylating distal ubiquitin by PINK1 (Wang et al., 2022), and ubiquitination of USP30 by Parkin may also contribute to its degradation, so Parkin and PINK1 may be able to moderate USP30. On the other hand, inhibition of USP30 expression or activity could allow cells to overcome the defects of PINK1 and Parkin, and restore the clearance of impaired mitochondria (Bingol et al., 2014). However, it has also been proven that USP30 can exert a regulatory effect on mitophagy independently of PINK1/Parkin activity under basal conditions (Rusilowicz-Jones et al., 2020).

2.2.2 Proteins in IMM

2.2.2.1 PHB2

PHB2, a member of the PHB family, contains an N-terminal transmembrane region essential for mitochondrial localization and also binds to PHB1 through the C-terminal loop region, participating as a complex to maintain mitochondrial structure and respiratory chain function (Osman et al., 2009). Moreover, PHB2 is also a highly conserved membrane scaffolding protein, and the presence of PHB2 on the IMM contributes to the maintenance of normal mitochondrial morphology, as well as the level of resistance to oxidative stress and apoptosis, which affects mitochondrial function (Artal-Sanz and Tavernarakis, 2010). On the one hand, PHB2 binds to the autophagosomal membrane-associated protein LC3 on damaged mitochondria through the LIR structural domain, and on the other hand, PHB2 combines with chelator 1 (SQSTM1) and LC3 to form a ternary protein complex in the loading of LC3 onto damaged mitochondria, which stimulated mitophagy (Xiao et al., 2018). PHB2 depletion destabilizes PINK1 in the mitochondria, preventing PRKN/Parkin, ubiquitin, and OPTN from being recruited to the mitochondria by the mitochondria after mitochondrial membrane depolarization or misfolded protein aggregation. By stabilizing PINK1 and increasing PRKN's mitochondrial recruitment, PHB2 also encourages mitophagy

through the PINK1/PRKN pathway (Yan et al., 2020). Significantly, we found that PHB2 regulates PINK1 processing by modulating the activity of the mitochondrial protease PARL. Furthermore, when mitochondria are depolarized, PHB2 stabilizes PINK1 via the PARL-PGAM5 axis.

2.3 Membrane lipid-mediated signaling pathways

2.3.1 Cardiolipin (CL)

Cardiolipin (CL), also known as diphosphatidylglycerol, is produced by phosphatidylglycerol and cytidine diphosphate-diacylglycerol catalyzed by cardiolipin synthase (CLS) (Schlattner et al., 2014). Being a distinctive phospholipid of IMM, CL takes part in the cross-talk between lipid-protein and serves as part of the ingredients necessary for the maintenance of mitochondrial action (Khalifat et al., 2011). Redistribution of CL may also come into play in phagocytosis formation through binding to Beclin-1 and LC3 (Li X.-X. et al., 2015), and is likely to occur through autophagy protein interactions at sites of contact within and outside the membrane. It was reported that the distribution of CL could be interchangeable between the IMM and OMM in response to autophagic or apoptotic stimuli. In normal mitochondria, CL is located in the IMM. When mitochondria are damaged, a large portion of CL is transferred to the OMM and interacts with the autophagy protein LC3 to mediate mitophagy (Chu et al., 2014).

2.3.2 Ceramide

Ceramide is a bioactive sphingolipid with particular structure. It is made of sphingosine backbone, and is esterified into fatty acyl chains by an amide bond at carbon 3 (Ogretmen and Hannun, 2004). Variations in the length of the fatty acyl chain yield numerous distinct ceramides, such as C14- to C26- ceramides (Saddoughi and Ogretmen, 2013). Mitochondrial ceramide derives from neutral sphingomyelinase (N-SMase) in response to increased production of reactive oxygen species (ROS) (Ogretmen and Hannun, 2004). As a core molecule in sphingolipid metabolism, ceramide takes part in regulating autophagy on a wide range of levels, which includes the selection of targets for the autophagic process, the induction of lethal mitochondrial autophagy and the elimination of damaged mitochondria. Ceramide accumulation in mitochondria can induce ceramide stress-induced mitophagy and ATP production reduction. It has been reported that Ceramide-induced mitophagy is dependent on ceramide synthase 1 (CerS1) and its metabolite C18-ceramide. Moreover, CerS1 and C18-ceramide invokes non-apoptotic lethal mitophagy selectively. Ectopic expression of CerS1 or processing of C18-ceramide facilitates the formation of LC3-II and its direct integration of ceramide and membranes of mitochondrial, attracting the binding of autophagosomes to damaged mitochondria and the occurrence of mitophagy (Sentelle et al., 2012). Some studies have shown that ceramides play a crucial role in Pink1 related Parkinson's disease. Ceramides have been found to accumulate in mitochondria and have a negative impact on mitochondrial function (most notably, ETC.) (Vos et al., 2021). In addition, in the absence of PINK1, accumulation of ceramides can cause ceramide-induced mitophagy, to compensate for the loss of PINK1 dependent mitophagy. Therefore, reducing ceramide levels may be one of the treatment strategies for PINK1 related PD (Vos et al., 2022).

In summary, there are still many unknown fields regarding the specific molecular mechanisms of lipid mediated mitophagy. CL and ceramides are different molecules that can regulate mitochondrial dysfunction through signal transduction and recruitment of autophagy mechanisms. However, their regulatory mechanisms are still unclear, and more research is needed to explain the molecular process of lipid mediated mitophagy.

3 Mitophagy in kidney cells of DKD

As we know, the glomerulus is the basic structural unit of the kidney for filtration function, and is mainly composed of podocytes, endothelial cells and mesangial cells. These 3 cell types depend on each other through complex biological processes to maintain the normal physiological activity of glomerulus. In addition, as one of the cells highly enriched in mitochondria, renal tubular epithelial cells (RTECs) are also very important in the progression of DKD. In recent years, although some exciting results have been achieved about the specific mechanism of mitophagy in renal intrinsic cells during the progression of DKD, we still need great efforts to further confirm these results. Here is an overview of the relationship between the mitophagy in kidney cells and DKD currently (TABLE 1).

3.1 Mitophagy in podocytes

Podocytes are glomerular visceral epithelial cells. The dysfuntion of podocyte structure and function influence in the development of DKD, and podocyte homeostasis has been used as one of the therapeutic targets in DKD (Dai et al., 2017). Podocytes have a high degree of terminal differentiation, resulting in a poor ability to re-enter the cell cycle and an inability to re-proliferate usually, and therefore rely heavily on autophagy, which is also usually exhibited at high levels (Hartleben et al., 2010). Autophagy is one of the fundamental self-healing mechanisms that maintain podocyte function, and its self-healing properties are essential for cell differentiation and proliferation (Wang and Choi, 2014). Podocytes are rich in mitochondria, which are essential components of the cytoplasm of podocytes and are the main energy-supplying organelles. Mitophagy maintains cell and tissue metabolism and homeostasis by removing damaged organelles (Mizushima and Komatsu, 2011; Feng et al., 2014; Xiong and Zhou, 2019), therefore, enhancing the activity of podocyte autophagy is of major importance for maintaining podocyte homeostasis (Liu et al., 2018).

High Glucose (HG) has been proven to promote mitochondrial dysfunction and podocyte apoptosis by suppressing mitochondrial autophagic activity (Li et al., 2016). Additionally, HG, hypoxia and abnormal immune response induce imbalance of mitochondrial homeostasis, deficient autophagy, inflammation and oxidative stress in podocytes (Clark and Parikh, 2021). Sirtuins (SIRTs) have been found to function synergistically in promoting mitophagy in podocytes and can ameliorate podocyte injury and proteinuria (Aventaggiato et al., 2021). SIRTs are class III histone deacetylases that rely on nicotinamide adenine dinucleotides

TABLE 1 Regulation of mitophagy in renal cells of DKD.

Renal cells	Upstream gene proteins	Mitophagy receptor	Effects on mitophagy	Effects on DKD	References
Podocytes	FOXO1	PINK1	FOXO1 overexpression prevented the HG-induced downregulation of PINK1 mRNA levels	FOXO1 overexpression, to some degree, promoted the recovery of injured podocytes	Li et al. (2016)
	Sirt1	BNIP3	Sirt1 positively regulates CR-mediated enhancement of hypoxia-induced autophagy upstream of Bnip3	Sirt1 mRNA expression levels correlated negatively with serum cystatin C levels	Kume et al. (2010)
	Sirt6	AMPK	Sirt6 overexpression could effectively reduce podocyte apoptosis accompanied by AMPK phosphorylation.; upregulation of Sirt6 was demonstrated to significantly reduce mitochondrial superoxide content and cellular ROS production	Sirt6 overexpression attenuates HG-induced mitochondrial dysfunction and apoptosis via promoting AMPK phosphorylation	Fan et al. (2019)
Renal tubular epithelial cells	TIPE1	PHB2	The deficiency of TIPE1 could promote its proteasomal degradation by regulating the expression of PHB2, which could propel the mitophagy of RTECs	The deficiency of TIPE1 couldultimately slow down renal tubular cell injury and EMT	Liu et al. (2022)
	-	OPTN	OPTN silencing significantly inhibited HG-induced mitophagosome formation, and overexpression of OPTN relieved cellular senescence through promoting mitophagy	In clinical specimens, renal OPTN expression was gradually decreased with increased tubulointerstitial injury scores.OPTN expression also negatively correlated with serum creatinine levels, and positively correlated with eGFR	Chen et al. (2018)
	DUSP1	Parkin	Overexpression of DUSP1 reverses the decreased Parkin protein in mitochondria	DUSP1 plays a defensive role in the pathogenesis of DN by restoring Parkin-mediated mitophagy	Lu et al. (2021)
	TXNIP	mTOR	TXNIP siRNA restored tubular mitophagy through inhibition of the mTOR signaling pathway	Blockade of TXNIP could suppress the production of interstitial collagens and reduce renal interstitial fibrosis in DKD	Huang et al. (2016)
	Nrf2	PINK	Nrf2-mediate regulation of PINK transcription and ameliorating mitochondrial oxidative stress and aberrant mitochondrial dynamics	Transfection with Nrf2 siRNA or PINK siRNA in HK-2 cells exposed to HG conditions partially blocked the effects of mitoQ on mitophagy and tubular damage	Xiao et al. (2017)
	STING1	PINK1	Activation of STING1 can upregulate the expression of PINK1 in HK-2 cells	Activation of STING1/PINK1 pathway can alleviate the injuries of kidney tissues of HFD/STZ-induced diabetic mice and HK-2 cells cultured in HG.	Zhu et al. (2023)
Glomerular mesangial cells	EPO	PINK1/Parkin	EPO can promote the expression of PINK1/Parkin-mediated mitophagy-related genes	EPO could attenuate renal injury and reduce oxidative stress	Yi et al. (2022)
Glomerular endothelial cells	FGF13	Parkin	The bifunctional role of FGF13 deficiency in promoting mitophagy and inhibiting apoptosis through Parkin can shape mitochondrial homeostasis regulation	Endothelial-specific deletion of FGF13 potentially alleviates T2DN damage, while FGF13 overexpression has the opposite effects	Sun et al. (2023)
	Nrf2/ARE	PINK	The specific Nrf2 inhibitor ML385 could inhibit mitophagy, as revealed by the decreased protein levels of PINK and Parkin	Activation of Nrf2/ARE signaling may restore diabetic nephropathy induced mitochondrial dysfunction and impaired renal function	Sun et al. (2019)

Annotation: FOXO1, Forkhead transcription factor O1; SIRT1, sirtuin 1; Sirt6:sirtuin 6; TIPE1, tumor necrosis factor alpha-induced protein 8-like 1; DUSP1, dual-specificity protein phosphatase 1; TXNIP, siRNA:Thioredoxin interacting protein siRNA; Nrf2, NF-E2-related factor 2; STING1, stimulator of interferon genes; EPO, Erythropoietin; FGF13, Fibroblast growth factor 13; PINK1, PTEN-induced putative kinase 1; BNIP3, Bcl-2, 19-kDa interacting protein 3; AMPK, AMP-activated protein kinase; mTOR, Mechanistic target of rapamycin; PHB2, prohibitin 2; OPTN, optineurin; ARE:antioxidant response elements.

(NAD+) and are relevant to various cellular signaling pathways, including mitochondrial function as well as autophagy (Kume et al., 2010). Among them, in glomerular disease models in humans and animals with DKD, the expression of SIRT1 is inclined to go down in renal cells (Yacoub et al., 2014). In DKD, hyperglycemia exacerbates podocyte apoptosis by raising the production of advanced glycosylation end products (AGEs), which increases FOXO4 acetylation and inhibits SIRT1 expression (Chuang et al., 2011). The mice podocyte with Sirt1 knocked out appeared severe proteinuria and renal fibrosis with mitochondrial dysfunction (Chuang et al., 2014; Liu et al., 2014). SIRT3 also inhibits permeability transition pore opening in mitochondria and improves mitochondrial function and dynamics, thereby mediating mitophagy (Zhao W. et al., 2021). What's more, overexpressed SIRT6 protected mitochondria via phosphorylation of AMPK in podocytes (Fan et al., 2019).

There are also many receptors participate in the mitophagy of podocytes. Li et a (Li J. et al., 2020) proved that Smad4 was increased in both diabitics and mouse podocytes, and Smad4 located to mitochondria affect glycolysis and oxidative phosphate in podocytes induced by HG, thus causing podocyte damage. Overexpressed FOXO1 could active PINK1/Parkin-dependent mitophagy, thereby eliminating abnormal mitochondria and ameliorating damage of podocyte in diabetic mice induced by streptozotocin (STZ), which suggested the vital role of FOXO1 in regulating podocyte mitophagy (Li et al., 2017). Moreover, it was shown that both hyperinsulinemia and hyperglycemia could inhibit the autophagic activity of podocytes by inducing excessive activation of the autophagy regulatory protein mTORC1 (Mizushima and Komatsu, 2011). Chen et al. (Chen et al., 2021) found that HG can inhibit autophagy by activating the Janus kinase/signal transducer and transcription signaling pathway in mice and podocytes (Jakhar et al., 2016). At the same time, oxide accumulation, ubiquitinated proteins and endoplasmic reticulum stress may damage mice podocytes easily when the autophagy-associated protein 5 (podocyte-specific protein) is in absence, which eventually lead to proteinuria (Hartleben et al., 2010).

3.2 Mitophagy in renal tubular epithelial cells

The autophagy in RTECs could influence in kidney hypertrophy and tissue injury, which finally result in the development of DKD (Ma et al., 2020). And increasing evidences shown that the flawed mitochondrial dynamics and the excessive oxidative stress in mitochondria are principal factors of renal tubular injury in DKD (Higgins and Coughlan, 2014). Besides, more and more researches have also found that mitophagy in renal tubular is closely connected with tubulointerstitial injury in DKD (Chen et al., 2018). Han et al. (Han et al., 2021) have observed that the activation of mitophagy in RTECs from diabetic mice is downregulated significantly and they have testified that the AMPK agonist metformin could ameliorated oxidative stress and interstitial fibrosis in kidney by activating the p-AMPK-Pink1-Parkin pathway in diabetic mice induced by high fat diet (HFD) and STZ. The study has shown that Sirt3 prevents cell injury through AMPK-mediated autophagy (Zhao et al., 2018; Wang et al., 2019) Furthermore, Sirt3 also inhibits mitochondrial damage and cardiomyocyte apoptosis by activating autophagy and mitophagy in cardiomyocytes stimulated by HG (Yu et al., 2017). On this basis, Wang et al. (Wang Y. et al., 2021) further certified that HG environment could activite the Notch-1/Hes-1 pathway in RTECs, and the promotion of autophagy by Sirt3 was diminished after the activation of Notch-1/Hes-1 pathway. Therefore, it was suggested that Sirt3 may stimulate autophagy in RTECs by inhibiting the Notch-1/Hes-1 signaling pathway to finally achieve the therapeutic effect of DKD.

In addition, TIPE1, a novel partner of the tumor necrosis factor-α -induced protein 8 family, was initially supposed to be a potential molecule involved in cell necrosis and apoptosis, which may regulate the progression of cell death. Liu et al. (Liu et al., 2022) observed that TIPE1 was upregulated in RTECs from patients and mice with DKD, which in turn disrupted HG-induced mitochondrial homeostasis in RTECs via impairing the mitophagy mediated by PINK1/Parkin signaling pathway. Moreover, the deficiency of TIPE1 could promote its proteasomal degradation by regulating the expression of PHB2, which could propel the mitophagy of RTECs, ultimately slowing down renal tubular cell injury and EMT. Hence, we speculate that TIPE1 may take part in the underlying mechanisms of DKD and inhibit the progression of mitophagy in RTECs.

To remove damaged mitochondria, Opineurin (OPTN) is enrolled into mitochondria by PINK1 during the course of mitophagy. It has been found that, the regulators of mitochondrial autophagosome formation, PINK1 and OPTN, are significantly reduced after high glucose stimulation. Silencing OPTN notably inhibites HG-induced mitochondrial autophagosome formation, while overexpression of OPTN alleviates cellular senescence by promoting mitophagy. Moreover, according to the study of clinical specimens, with the increase of renal tubular interstitial injury score, renal OPTN expression gradually decreased. Therefore, OPTN-mediated mitophagy is crucial for regulating HG-induced senescence of RTECs in DKD (Chen et al., 2018).

Dual specific protein phosphatase 1 (DUSP1) is reduced in human proximal tubular epithelial (HK-2) cells under high-glucose conditions. In HK-2 cells stimulated by HG, overexpression of DUSP1 reverses the decreased parkin protein in mitochondria. Conversely, but the deletion of parkin reverses the efficacy of overexpressed DUSP1 on mitophagy and apoptosis (Lu et al., 2021). Hyperglycemia upregulates the expression of thioredoxin-interacting protein, which leads to proximal tubular cell injury and inhibition of mitophagy by inhibiting BNIP3 expression and activating the mTOR signaling pathway in DKD patients (Huang et al., 2016). In a previous study, HK-2 cells were induced to undergo defective mitophagy, mitochondrial dysfunction and apoptosis, and reduced expression of PINK and Parkin in a high-glucose environment. However, these changes were reversed by mitoQ, a mitochondria-targeted antioxidant which protect DKD tubular injury by regulating mitophagy. MitoQ partially blocked mitophagy and renal tubular injury when Nrf2 siRNA or PINK siRNA were transfected under HG conditions (Xiao et al., 2017).

Stimulator of interferon response cGAMP interactor 1(STING1) is an evolutionarily conserved transmembrane protein that localizes to the endoplasmic reticulum (ER) membrane in immune and non-immune cells. A growing body of research supports that STING1 is emerging as a key regulator of autophagy (Hopfner and Hornung,

2020; Zhang et al., 2021; Jiménez-Loygorri and Boya, 2024). A recent study reported a potential association between STING1 and PINK1. The activation of STING1 could promote mitophagy by up-regulating PINK1 in mouse cardiomyocytes (wang 2020c). The latest study showed that the expression of STING1 and PINK1 was downregulated in the kidney tissues of HFD/STZ-induced diabetic mice and in HK-2 cells cultured in HG, accompanied by decreased mitophagy activity. Treating HK-2 cells with the STING1 activator mtDNA and STING1 pcDNA could enhance the levels of PINK1 and parkin, which can reduce HK-2 cells damages exposed to HG (Zhu et al., 2023), which may provide an innovative therapeutic basis for DKD treatment.

3.3 Mitophagy in glomerular mesangial cells

As a major part of the intraglomerular mesangial region, glomerular mesangial cells (GMCs) play a crucial part in carrying on the normal structure of glomerular capillaries and maintaining the homeostasis of mesangial matrix in kidney (Abboud, 2012). One of the histological features of DKD is matrix accumulation due to hypertrophy of GMCs (Haneda et al., 2003). It has been found that autophagy's level is suppressed in GMCs under prolonged high concentration of glucose and the reduced autophagic activity is in connection with the expressive suppression of FBW7 in GMCs induced by HG (Kitada et al., 2011; Gao et al., 2019). FBW7, a substrate recognition component of SCF-type ubiquitin ligase complex, connects ubiquitin to the target proteins, which concerning cell growth, proliferation, differentiation and apoptosis. In addition, HG environment can also induce GMCs senescence directly by downregulating the expression of connexin43 (Zhang et al., 2006). Additionally, one of the PAQR family, AdipoQ receptor 3 (PAQR3), participate in various biological processes like autophagy, cholesterol homeostasis, tumorigenesis and energy metabolism. The downregulation of PAQR3 reversed HG-induced activated PI3K/AKT pathway significantly, which inhibited cell proliferation and ECM accumulation in human GMCs instead (Li H. et al., 2018). It has been proved that AGEs-induced ROS take a vital part in mitochondrial depolarization-mediated apoptosis of GMCs (Xu et al., 2016). In addition, endoplasmic reticulum (ER) stress is important in the regulation of autophagy (Chandrika et al., 2015; Jakhar et al., 2016). AGEs induce apoptosis and death in GMCs and ER stress is the upstream of autophagy in GMCs exposed to AGEs (Chiang et al., 2016). Autophagy may make positive contribution to the apoptosis induced by AGEs in GMCs.

Erythropoietin (EPO) is a glycoprotein hormone excreted largely by kidney and acts in regulating erythropoiesis. *In vitro* experiments, EPO could promote autophagic flow, attenuate mitochondrial dysfunction, increase mitochondrial ROS and reduce the level of apoptosis in GMCs treated with HG. Besides, EPO could also increase the level of mitophagy by upregulating the expression of PINK1 and Parkin proteins in GMCs. It was also demonstrated that EPO can attenuate renal injury by promoting the expression of PINK1/Parkin-mediated mitophagy-related genes in DKD mice (Yi et al., 2022). To sum up, EPO attenuated the damage of DKD via restoring mitophagy mediated by PINK1/Parkin pathway.

3.4 Mitophagy in glomerular endothelial cells

Glomerular endothelial cells (GECs) are well differentiated cells with window pores and charged luminal glycocalyx layer. They are conducive to the glomerular filtration barrier (GFB) functionally (Fogo and Kon, 2010; Haraldsson and Nyström, 2012). In type 2 diabetic patients, reduced window pores in GECs are associated with the increased level of proteinuria and reduced glomerular filtration function (Weil et al., 2012). Previous researches have proved that in glomerulosclerosis models and human with DKD, endothelial dysfunction holds the key to the course of glomerular disease and DKD (Sun et al., 2013).

Mitophagy maintains the integrity of GECs and the ultrastructure of podocytes to keep the endostasis of GFB. It has been discovered that the mitochondrial structure is abnormal in the GECs of DKD experimental mode (Qi et al., 2017). Lenoir et al. (Lenoir et al., 2015) assessed the features of autophagy in diabetic GECs by STZ-induced mice with the specific deletion of Atg5 in endothelial cell. Compared to controls group, the proteinuria in diabetic mice with endothelial-specific deletion of Atg5 is more severe. They found glomerular capillary dilation and endothelial damage in diabetic mice with deficient autophagy of endothelial cells. Furthermore, ultrastructural analysis revealed the presence of GECs cytoplasmic disorganization, vacuolization, and isolated cells (mostly endothelial cells) in the glomerular capillary lumen by the diabetic mice accompanied with endothelial-specific deletion of Atg5, which redouble confirmed that the autophagy of endothelial cells plays a renoprotective role in the course of DKD.

Previous researches have indicated that the GECs prolonged exposured to high glucose environment leading to an excess production of the superoxide derived by mitochondria and a persistent accumulation of oxide, which caused the cell dysfunction and promoted glomerular injury in turn. Further, it is the increased level of mitochondrial superoxide in GECs that leads to GECs' dysfunction, and interestingly, it also exerts pathological effects on the adjacent podocytes (Casalena et al., 2020). Furthermore, HG can cause mitophagy deficiency, mitochondrial dysfunction and apoptosis in GECs, accompanied by decreased expression of PINK and parkin. CoQ10 can restore the expression, activity and nuclear translocation of Nrf2 in GECs cultured with HG, thus upregulating the expression of PINK and parkin, which suggest that CoQ10 act as a potent mitochondrial antioxidant and play a beneficial role in DKD by restoring mitophagy via the Nrf2/ARE signaling pathway (Sun et al., 2019).

Fibroblast growth factor 13 (FGF13) is a participator of the FGF homologous factors (FHFs) subfamily and is also an ancestral gene of the FGF family (Itoh and Ornitz, 2008; Sun et al., 2023) found that the expression of FGF13 was increased in GECs in DKD and the specific deletion of FGF13 in endothelial cell ameliorated the apoptpsis of HFD + STZ-induced glomerular cell, which suggest that the expression of FGF13 has a positive relation with T2DN. In the course of the experiment, FGF13 deficiency was observed to activate mitophagy and prevent apoptosis, then maintaining intra-mitochondrial homeostasis. It was finally demonstrated that FGF13 may exert a dual regulation of both promoting mitophagy and inhibiting apoptosis by affecting Parkin expression, ultimately exerting a crucial link for regulating mitochondrial homeostasis in the progression of DKD.

TABLE 2 Chinese Botanical Drugs in regulating mitophagy for treatment of DKD.

Types of CHM	Names of herbal medicine	Models	Target	Pathways	Ref. and year
Chinese botanical drugs decoction	Tangshen formula	db/db mice	PINK1↑,Parkin↑	PINK-1/Parkin signaling pathway	Chen et al. (2024)
	Huangqi-Danshen decoction	db/db mice	PINK1↓, Parkin↓	PINK1/Parkin pathway	Liu et al. (2020)
	Astragalus aksuensis Bge and Panax notoginseng F. H. Chen formula	HFD + HSD + STZ-induced rats	p-mTOR↓, PINK1↑, Parkin↑	mTOR/PINK1/Parkin pathways	Wen et al. (2020)
		HG-induced RMCs			
Single or couples of Botanical drugs	San-Huang-Yi-Shen Capsule	HFD + STZ-induced rats	PINK1↑, Parkin↑	PINK1/Parkin pathway	Song et al. (2018)
	Ginseng-Sanqi-Chuanxiong extracts	HG/PA-induced HAEC	AMPK↑	AMPK pathway	Wang et al. (2020a)
	Huangkui capsule	HFD + STZ-induced rats	STING1↑,PINK1↑,Parkin↑	STING1/PINK1 signaling pathway	Zhu et al. (2023)
		HK-2cells induced by HG			
Natural chemical metabolites	Esculetin	Type 1 diabetic rats with AKI and NRK-52E cells grown in HG exposed to sodium azide	Nrf2↑,Keap1↑,PINK1↑	Nrf2/PINK1/Parkin signaling pathway	Dagar N,et al. (2024)
	Icariin	STZ-induced rats	Sesn2↑, Keap1↓, Nrf2↑, HO-1↑, NLRP3↓	Sesn2/Keap1-Nrf2/HO-1/ NLRP3 axis	Ding et al. (2022)
		HG-induced MPC-5 cells			
	Astragaloside II	STZ-induced rats	Keap1↓, Nrf2↑, PINK1↑, Parkin↑	Keap1/Nrf2-PINK1/Parkin pathway	Su et al. (2021)
	Dioscin	HFD + STZ-induced rats	PINK1↑, Parkin↑	PINK1/Parkin pathway	Tao et al. (2018)
	Jujuboside A	HFD + STZ-induced rats	CaMKK2↑, AMPK↑, p-mTOR↓, PINK1→, Parkin↑	CaMKK2-AMPK-p-mTOR and PINK1/Parkin pathways	Zhong et al. (2022b)
	Astragaloside IV	db/db mice	PINK1↓, Parkin↓, p-Parkin (Ser 65)↓, LC-3II↓	PINK1/Parkin pathway	Li et al. (2022a)

Annotation: PINK-1, PTEN-induced putative kinase 1; HFD, high-fat diet; HSD, high-sugar diet; STZ, streptozocin; RMCs, renal mesangial cells; p-mTOR, Phosphorylated mammalian target of rapamycin; HG/PA-induced HAEC, high glucose and palmitate-induced human aortic endothelial cell; AMPK, AMP-activated protein kinase; HK-2cells, Hexokinase 2; AKI, Acute Kidney Injury; NRK-52E, the kidney tubular epithelial cells; STING1, stimulator of interferon response cGAMP, interactor 1; Nrf2, NF-E2-related factor 2; Keap1, Kelch-like ECH-associated protein 1; HG, high glucose; MPC-5, cells, Mouse Podocyte Clone-5; Sesn2, Sestrin2; HO-1, Haem Oxygenase 1; NLRP3, Nucleotide-binding oligomerization domain (NOD)-like receptor protein-3; CaMKK2, calmodulin-dependent kinase 2; LC-3II:light chain 3.

4 Chinese botanical drugs for treating DKD by regulating mitophagy

There are thousands of years in China that CBD has been used to solve clinical problems. Previously, studies have indicated that CBD alleviates blood glucose levels (Zhao M.-M. et al., 2021), peripheral neuropathy (Yu et al., 2021), peripheral vascular disease (Zhao et al., 2019) and other complications in diabetics. Some botanical drugs also show their renoprotection by reducing proteinuria and serum creatinine in DKD. And one of the mechanism is that CBD decrease inflammation, renal fibrosis, apoptosis and other pathological processes via the regulation of mitophagy (TABLE 2).

4.1 Chinese botanical drugs decoction for regulating mitophagy in DKD

Tangshen formula (TSF) is a classic Chinese herbal formula used in China to treat DKD. It consists of Astragalus mongholicus Bunge [Fabaceae; astragali radix], *Centella asiatica* (L.) Urb. [Apiaceae; centellae herba], Stephania tetrandra S. Moore [Menispermaceae; stephaniae tetrandrae radix], Prunus persica (L.) Batsch [Rosaceae; persicae semen], Rheum palmatum L. [Polygonaceae; rhei radix et rhizoma]. Chen et al. found that TSF has the potential to inhibit DKD through the PINK-1/Parkin-mediated mitophagy process based on network pharmacology results. Subsequently the researchers conducted the vivo experiments to validate the pharmacology results. HFD-induced db/db mice were divided into three groups randomly: the model group, the low-dose TSF treatment group (6.79 g/kg/d), and the high-dose TSF treatment group (20.36 g/kg/d), which were fed continuously for 8 weeks. The results confirmed that TSF ameliorated kidney damage and restored mitochondrial structure in db/db mice and promote mitophagy by activating the PINK-1/Parkin signaling pathway (Chen et al., 2024).

Huangqi-Danshen decoction (HDD) is Chinese botanical drugs formula compound consisting of Astragalus mongholicus Bunge [Fabaceae; astragali radix] and Salvia miltiorrhiza Bunge [Lamiaceae; salviae miltiorrhizae radix et rhizoma], both of them

have been used to improve anti-inflammatory and antioxidant (Li G.-H. et al., 2018; Ye et al., 2020). Xinhui Liu administered HDD (6.8 g/kg/day by gastric irrigation) to db/db mice for 12 weeks, then the mice treated with HDD maintained lower blood glucose and urinary ACR level. The renal histopathology revealed that HDD could alleviate glomerular hypertrophy, reduce the increase of mesangial cells and mesangial matrix, and slow down the extensive fusion of the visceral epithelial cells. Besides, the autophagosome encapsulating mitochondria were observed in renal tissue in db/db mice with transmission electron microscopy, but did not be found in HDD treatment group. The fewer expression of PINK1 and Parkin protein in HDD group suggested that HDD improved renal function by inhibiting instead of activating mitophagy mediated by PINK1/Parkin in db/db mice (Liu et al., 2020).

Astragalus aksuensis Bge and Panax notoginseng F. H. Chen formula (APF) is Chinese botanical drugs formula compound which is made up of Astragalus mongholicus Bunge [Fabaceae; astragali radix], Panax notoginseng (Burkill) F.H.Chen [Araliaceae; notoginseng radix et rhizoma], Angelica sinensis (Oliv.) Diels [Apiaceae; angelicae sinensis radix], Achyranthes bidentata Blume [Amaranthaceae; achyranthis bidentatae radix], Laminaria japonica Aresch. [Laminariaceae; Laminariae Thallus Eckloniae Thallus]. Using Agilent High performance liquid chromatography (HPLC) system combined with LC solution software and UV spectrophotometer to determine the five main effective metabolites of APF, namely, Astragaloside I, Astragaloside IV, Ferulic Acid, Calycosin, and β-ecdysterone. APF was used to solve the clinical problems in kidney diseases for several years. According to the research form Wen et al., the renal pathology in DKD mice showed the expansion of mesangial matrix and ECM and the fibrosis of tubule-interstitial, which could be ameliorated by the treatment of APF. The studies in autophagy deficiency of DKD (C57BL/6 mice induced by STZ + HFD) and cell (RMCs induced with HG) modle proved that APF could suppress p-mTOR and induce the activation of PINK1/Parkin signaling pathway. When they inhibited the autophagy of renal mesangial cells (RMCs) induced by HG with 3-methyladenine (3-MA), the benefit of APF was reduced. The result suggested that APF could delay the progression of DKD via enhancing the mitophagy level, which is mediated by mTOR/PINK1/Parkin pathway in RMCs (Wen et al., 2020).

4.2 Single or couples of botanical drugs for regulating mitophagy in DKD

San-Huang-Yi-Shen Capsule (SHYS) is composed of Astragalus mongholicus Bunge [Fabaceae; astragali radix], Panax quinquefolius L. [Araliaceae; panacis quinquefolii radix], Dioscorea oppositifolia L. [Dioscoreaceae; dioscoreae rhizoma], Tetradium ruticarpum (A.Juss.) T.G.Hartley [Rutaceae; euodiae fructus], Cuscuta australis R. Br. [Convolvulaceae; cuscutae semen], Polygonatum sibiricum Redouté [Asparagaceae; polygonati rhizoma], Rehmannia glutinosa (Gaertn.) DC. [Orobanchaceae; rehmanniae radix], Pogostemon cablin (Blanco) Benth. [Lamiaceae; pogostemonis herba], Hamamelis mollis Oliv. [Hamamelidaceae; hamamelis mollis Oliv.], Leonurus japonicus Houtt. [Lamiaceae; leonuri fructus], Salvia miltiorrhiza Bunge [Lamiaceae; salviae miltiorrhizae radix et rhizoma],Conioselinum anthriscoides 'Chuanxiong' [Apiaceae; chuanxiong rhizoma],Atractylodes macrocephala Koidz. [Asteraceae; atractylodis macrocephalae rhizoma], which has been demonstrated to improve renal function in diabetic patients (Song et al., 2018). Li et al. given SHYS(intragastric administration 0.81 g/kg and 1.62 g/kg for SHYS low-dose group and the high-dose group, respectively) to the rats induced by HFD and STZ for 8 weeks, and found that SHYS could protect renal function and reduce histopathologic changes in DKD rats. SHYS could improve the expression on Parkin, PINK1 and LC3-II protein and reduce the expression of p62, NLRP3, VDAC1, Tom20, and COXIV protein, which suggests that SHYS enhances the mitophagy mediated by Pink1/Parkin pathway and avoids mitochondrial and inflammatory injury to protect renal function from DKD (Li H. et al., 2022).

Another research demonstrated Ginseng-Sanqi-Chuanxiong (GSC) extracts (ferulic acid, notoginsenoside R1, ginsenoside Rg1, ginsenoside Re, and ginsenoside Rb1), which were extracted from Panax quinquefolius L. [Araliaceae; panacis quinquefolii radix], Panax notoginseng (Burkill) F.H.Chen [Araliaceae; notoginseng radix et rhizoma],Conioselinum anthriscoides 'Chuanxiong' [Apiaceae; chuanxiong rhizoma], could improve the expression and activation on AMPK. Under high glucose and palmitate-stressed conditions, GSC enhanced mitophagy on human aortic endothelial cell via the regulation of AMPK pathway. To some extent, the research provides a new method of Chinese medicine to improve the damage of RTECs caused by DKD (Wang S. et al., 2020).

Huangkui capsule (HKC)is a traditional Chinese medicine prepared from the extract of Abelmoschus moschatus Medik. [Malvaceae; Abelmoschus moschatus Medicus], and its total flavonoids (TFA) are its main active chemical metabolites. In China, HKC was widely used to treat various types of kidney diseases, including DKD (Li P. et al., 2020; Zhao et al., 2022). HKC has been shown to improve the DKD of NOD mice by regulating the gut microbiota, and subsequently improved the levels of metabolites (Shi et al., 2023). Kim et al.demonstrated that HKC prevented the accumulation of renal pathogenic proteins and mitochondrial dysfunction by regulating autophagy and mitochondrial dynamics, thereby alleviating the progression of DKD (Kim et al., 2018). A recent study found that STING1/PINK1-mediated mitophagy is impaired in the kidney in a diabetes mouse model induced by HF-D combined with STZ. Treatment with a high dose of HKC (2.0 g/kg/day) ameliorated the renal tubular injury accompanied by upregulation of STING1/PINK1 signaling pathway mediated mitophagy and mitochondrial recovery. *In vitro*, HKC administration significantly protected the mitochondrial dynamics and function in HK2 cells. This effect was dependent on the activation of STING1/PINK1 signaling pathway by HKC to increase mitophagy (Zhu et al., 2023).

4.3 Natural chemical metabolites for regulating mitophagy in DKD

Esculetin, a naturally occurring hydroxycoumarin derivative found in plants and fruits of Citrus limonia, Cortex fraxini, and Fraxinus rhynchophylla. Some studies have shown that a range of pharmacological activities including ROS scavenging, anti-inflammatory, and antifibrotic action and protective effect of

diabetes-associated chronic complications (Kadakol A., et al., 2015a; Kadakol A. et al., 2015b; Kadakol A., et al., 2016; Kadakol A., et al., 2017). Dagar et al. explore the potential effect of esculetin on IRI-AKI associated with diabetes by conducting different experiments. *In vivo*, T1DM Wistar rats were treated with two doses of esculetin (50 and 100 mg/kg/day orally) for 5 days followed by AKI established by bilateral ischemic-reperfusion injury (IRI). *In vitro*, esculetin (50 μM) treatment for 24 h was given to the NRK-52E cells grown in HG before which were exposed to sodium azide (10 mM) for induction of hypoxia/reperfusion injury (HRI). The results showed that treatment with esculetin significantly reduced the expression of specific markers for renal injury and increased the expression of PINK1 and Parkin. Besides, esculetin reduced mitochondrial oxidative stress by increasing the expression of Nrf2 and Keap1. Esculetin may alleviate mitochondrial dysfunction by inducing PINK1/Parkin mediated mitophagy, suggesting that esculetin is expected to become an effective therapy to prevent AKI-diabetes comorbidity (Dagar N,et al., 2024).

Icariin (ICA) is an essential constituent of flavonoid extracted from *Epimedium brevicornu Maxim.*[*Berberidaceae; epimedii folium*] (Li C. et al., 2015), which is a traditional Chinese herbal. It possesses numerous pharmacological functions such as ameliorating inflammation (El-Shitany and Eid, 2019), delaying renal fibrosis (Wang M. et al., 2021) and improving mitochondrial dysfunction. Qiao C et al. (Qiao et al., 2018) administered ICA(10uM) to HG-treated MPC-5 cells for 48hs. Then, the relevant biochemical indices and the ultrastructure of the podocytes revealed that ICA could reduce renal injury. And the increase in LC3, Sesn2, PINK1, PARK2 and mitophagy dye show that ICA could promote mitophagy in cultured cell and animal models. To figure out the principles of ICA in mitophagy, they inhibited the expression of Sesn2, Nrf2 and HO-1 respectively. It finally proved that under HG conditions, ICA could degradate Keap1 and activate Nrf2, and induce the activation of Nrf2/HO-1 signalling pathway to downregulate inflammatory cascade in connection with NLRP3, which was dependent on Sesn2-induced mitophagy (Ding et al., 2022).

Astragaloside II (AS-II) is a kind of saponin extracted from the root of Astragalus mongholicus Bunge [Fabaceae; astragali radix praeparata cummelle](Peng et al., 2008), which has anti-inflammatory (Wan et al., 2013) and immunoregulation (Qiao et al., 2019) effects in various diseases. The diabetic rat model was established by intraperitoneal injection of streptozotocin (STZ) at 55 mg/kg and treated with AS-II (3.2, 6.4 mg/kg/d) for 9 weeks to explore the protective effects of AS II on podocyte injury in DKD. The results indicated that AS II ameliorated albuminuria, renal histopathology, and podocyte foot process effacement and podocyte apoptosis in diabetic rats. Moreover, treated with AS-II also downregulated Keap1 protein level, upregulated Nrf2 expression, and promoted the expression of mitophagy-related protein PINK1 and Parkin in diabetic rats. In consequence, AS II ameliorates renal injury to the diabetic rats induced by STZ through the enhancing mitophagy of podocyte and the ability resistance to oxidative stress through regulation of Nrf2 and PINK1 (Su et al., 2021).

Dioscin is a steroidal saponin extracted from various kinds of vegetables and herbs belonging to the family of Dioscoreaceae, which shows the effect of anticancer, anti-infection, immunoregulation and hypolipidemic (Yang et al., 2019a). Dioscin reduced hyperglycemic and pancreatic injuries, and alleviated the markers' level of renal function and the histopathology of kidney in HFD + STZ-induced rats by difference pathways (Tao et al., 2018). Zhong et al. found that dioscin (20 mg per kg bw) effectively reduced blood glucose, inflammatory factor expressions, pancreatic injury, renal function markers and renal pathological changes in DKD (induced by HFD and STZ) rat kidneys. Moreover, dioscin can also reverse the NOX4 expression and disorder of the mitochondrial respiratory chain. Mitophagy and mitochondrial fission/fusion were improved by dioscin via regulating Parkin, PINK1, DRP1, p-DRP1 and MFN2 expressions. The results suggested that the enhancement of mitophagy maybe one of the mechanisms of Dioscin in its renoprotective effect (Zhong et al., 2022a).

Jujuboside A (JuA) is a triterpenoid saponins extracted from Ziziphus jujuba Mill. [Rhamnaceae; ziziphi spinosae semen]and has a lot of beneficial functions, such as anticancer (Wang W. et al., 2021), oxidation resistance and anti-inflammatory (Wang et al., 2023). Zhong et al. studied the reno-protective effects of JuA on HFD and STZ induced DKD rats. JuA (20 mg/kg) and Metformin (Met, 300 mg/kg) were administrated to diabetic Sprague Dawley rat for 8 weeks daily. Compared with untreated group, fasting blood glucose and renal damage were significantly decreased after JuA treatment. Further studies certified that the renoprotection of JuA on DKD may be connected with the improvement in autophagy and mitophagy caused by the activation of CaMKK2-AMPK-p-mTOR pathways and Pink1/Parkin pathways (Zhong et al., 2022b).

Astragaloside IV (AS-IV) is one of the major and active metabolites of Astragalus membranaceus Bunge [Fabaceae; astragali radix praeparata cummelle]. Liu et al. (Liu et al., 2017) administered AS-IV(adding to standard feed at a dose of 1 g/kg) to the db/db mice for 12 weeks to explore the mechanism of AS-IV in the treatment of DKD. The result showed that AS-IV ameliorated renal injury evaluated by urinary albumin excretion (UAE) as well as renal pathology, which was independent of the decrease in blood glucose and body weight. Moreover, the expression of PINK1, Parkin, p-Parkin (Ser65) and LC-3II protein were abnormally increased in db/db mice and were downregulated by AS-IV. So they inferred that AS-IV retarded renal injury by inhibiting the mitophagy, which is mediated by PINK1/Parkin in db/db mice.

5 Conclusion and future perspective

Here, we have summarized the regulatory pathways of mitophagy and its potential role during the pathogenesis and progression of DKD, as well as the aspects of treating DKD by using CBD in the context of mitophagy regulations. Despite these encouraging findings, there are still many uncertain issues should be tackled in the further study. Firstly, in the physiology and pathology of DKD, moderate mitophagy can help to maintain mitochondrial homeostasis by removing senescent and necrotic mitochondria, but whether excessive activation or inhibition of mitophagy will further promote the progression of the disease remains unclear. Secondly, the kidney is a multicellular organ and different types of cells play specific roles in the progression of DKD. Therefore, the modulation

and role of mitophagy in the kidney may be different in several renal innate cells, but the accurate regulation mechanism of mitophagy in various types of renal cells still need to be further explored. Thirdly, many signaling pathways and mechanisms, like oxidative stress, AGEs, inflammation, participate in the deterioration of DKD. Whether there is signal crosstalk between selective mitophagy and other signaling pathways also need to be investigated. Finally, although current studies have shown that the regulation of mitophagy may delay the progression of DKD, more efforts should to make to validate the preclinical findings in human samples and test the potential therapeutic implications in clinical trials. Moreover, how to precisely target mitophagy to treat DKD is also a great challenge in future.

Substantial evidence supports a significant role of CBD for treating DKD via regulating mitophagy, which shows us a glimmer of light to explore a promising therapeutic approach for ameliorating DKD. Despite these exciting results, there are still many unanswered include but not limited to the following questions to be addressed. In the first place, the current researches are more prone to focus on single or blended bioactive components derived from botanical drugs, which is not appropriate considering the benefits of multi-component and multi-pathway of CBD prescription. In another, the existing mechanisms of CBD in improving DKD by interfering with mitophagy are mainly focused on ubiquitin-dependent mitophagy pathways, special attention should be paid to receptor-mediated and membrane lipid-mediated signaling pathways. What is more, whether different traditional CBD have the same modulation of mitophagy is still a mystery. Last but not least, more studies should be conducted to clearly elucidate the kidney injury and beneficial function during the process of CBD against DKD targeting mitophagy. For example, Li et al. established an efficient method for the isolation of natural parkin ligands by centrifugal ultrafiltration and liquid chromatography/mass spectrometry. They successfully identified potential parkin ligands in Chinese medicine Polygoni Cuspidati Rhizoma et Radix and Sophorae Flavescentis Radix, and confirmed that there were 5 (kurarinol I, kurarinol one, kurarinol flavone G, Apigenin and emodin) could activate parkin in vitro self-ubiquitination assay, which provides the possibility of discovering more precise active components of CBD targeting mitophagy for the treatment of DKD in the future (Li et al., 2022). We believe that more and more mechanism studies will provide new potential treatment methods for DKD based on the perspective of mitophagy.

Author contributions

LM: Writing–original draft, Writing–review and editing, Conceptualization, Funding acquisition. JL: Writing–original draft, Writing–review and editing. XZ: Visualization, Writing–original draft. WZ: Investigation, Resources, Writing–original draft. CJ: Validation, Writing–review and editing. BY: Validation, Writing–review and editing. HY: Project administration, Supervision, Writing–review and editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This study was supported by the National Natural Science Foundation of China (No. 82004316).

Acknowledgments

We wish to sincerely thank the tool of Figdraw (www.figdraw.com) for creating Figure 1.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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TYPE Review
PUBLISHED 17 May 2024
DOI 10.3389/fphar.2024.1410998

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RECEIVED 02 April 2024
ACCEPTED 30 April 2024
PUBLISHED 17 May 2024

CITATION
Yu Y, Wang G, Liu Y and Meng Z (2024), Potential application of traditional Chinese medicine in age-related macular degeneration—focusing on mitophagy.
Front. Pharmacol. 15:1410998.
doi: 10.3389/fphar.2024.1410998

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Potential application of traditional Chinese medicine in age-related macular degeneration—focusing on mitophagy

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Retinal pigment epithelial cell and neuroretinal damage in age-related macular degeneration (AMD) can lead to serious visual impairments and blindness. Studies have shown that mitophagy, a highly specialized cellular degradation system, is implicated in the pathogenesis of AMD. Mitophagy selectively eliminates impaired or non-functioning mitochondria via several pathways, such as the phosphatase and tensin homolog-induced kinase 1/Parkin, BCL2-interacting protein 3 and NIP3-like protein X, FUN14 domain-containing 1, and AMP-activated protein kinase pathways. This has a major impact on the maintenance of mitochondrial homeostasis. Therefore, the regulation of mitophagy could be a promising therapeutic strategy for AMD. Traditional Chinese medicine (TCM) uses natural products that could potentially prevent and treat various diseases, such as AMD. This review aims to summarize recent findings on mitophagy regulation pathways and the latest progress in AMD treatment targeting mitophagy, emphasizing methods involving TCM.

KEYWORDS

age-related macular degeneration, mitophagy, oxidative stress, traditional Chinese medicine, mechanism

1 Introduction

Autophagy is a multifunctional degradation system that helps cells maintain homeostasis by encapsulating cytoplasmic proteins, damaged organelles, and pathogens into vesicles (autophagosomes), which subsequently fuse with lysosomes to create autolysosomes, leading to the degradation of the encapsulated cargo and the generation of amino acids, nucleotides, sugars, fatty acids, and adenosine triphosphate (ATP). By assisting in the regulation of protein, nucleic acid, and lipid balances, the modulation of reactive oxidative stress and oxygen species (ROS), and the improvement of mitochondrial function, autophagy contributes to cellular metabolic needs and the renewal of intracellular organelles (Nita and Grzybowski, 2023). Depending on the intracellular lysosomal degradation mechanism, three distinct forms of autophagy—macroautophagy, microautophagy, and chaperone-mediated autophagy—have been distinguished (Mizushima, 2018; Yao and Shen, 2020) (Figure 1). Macroautophagy is the most predominant and conserved type of autophagy and proceeds via five stages—induction, nucleation, extension, fusion, and degradation (Nieto-Torres and Hansen, 2021). During chaperone-mediated autophagy, the chaperone Hsc70 and co-chaperones identify target

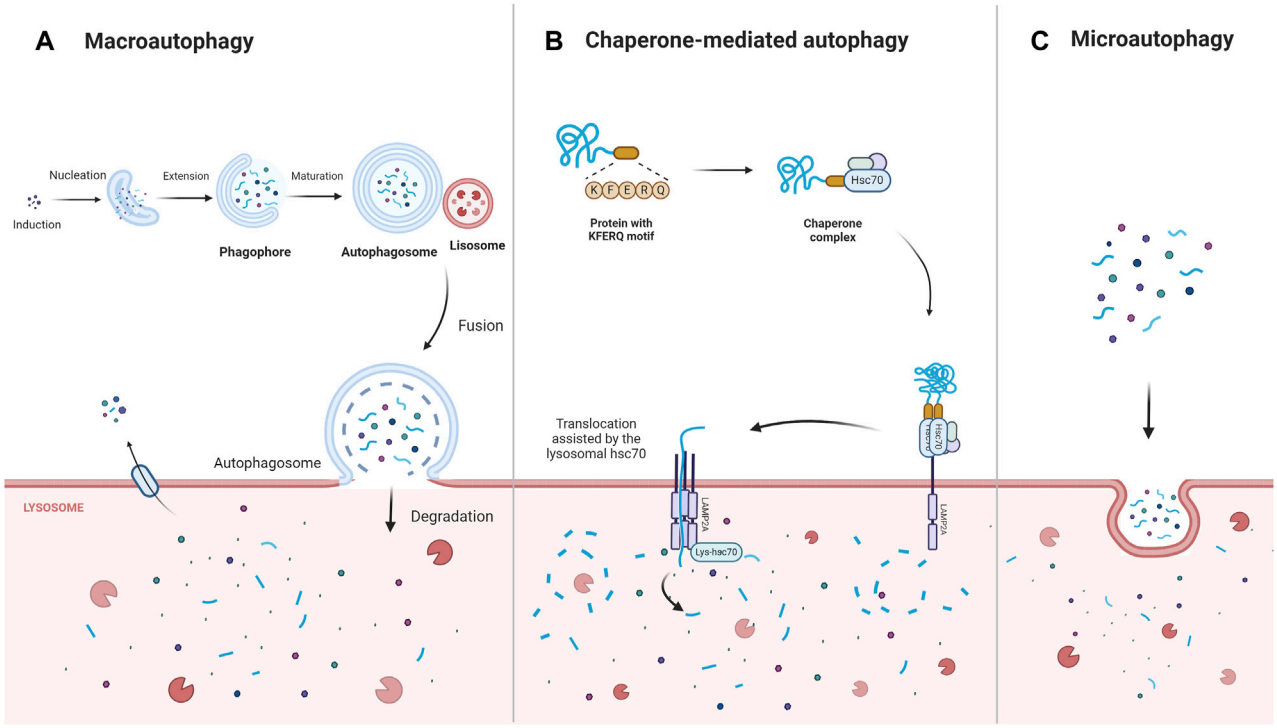

FIGURE 1
The main types of autophagy: macroautophagy, chaperone- mediated autophagy, microautophagy. Macroautophagy is induced, nucleated, and extended to form a double-membrane structure autophagosome, which wraps around the degraded material and ultimately fuses with the lysosome for degradation. Chaperone-mediated autophagy directly recognizes proteins with KFERQ-related motifs by means of the chaperone protein Hsc70, and the receptor protein LAMP2A on the lysosomal membrane recognizes the KFERQ motifs exposed by the binding protein and guides the target protein into the lysosome for degradation. In contrast, microautophagy directly engulfs the degraded material mainly through the invagination of the lysosomal membrane. Abbreviations: Lysosomal-associated membrane protein 2A (LAMP2A).

proteins containing KFERQ-like motifs and then transport these proteins to the lysosomal membrane to bind to the lysosomal receptor lysosomal-associated membrane protein 2A (LAMP2A), triggering receptor multimerization, cargo internalization, and degradation (Gómez-Sintes and Arias, 2021). In contrast, during microautophagy, the target proteins are directly engulfed by the lysosomal membrane through invagination (Fleming et al., 2022).

Mitophagy, a highly specialized type of autophagy, regulates mitochondrial fission and fusion, thereby eliminating malfunctioning and impaired mitochondria, promoting mitochondrial renewal, preventing ROS overproduction, inhibiting potential cellular oxidative damage, and ensuring mitochondrial quality (Wen et al., 2023). In response to ROS generation, nutrient deficiency, cellular senescence, and other factors, intracellular mitochondria are damaged and depolarized. During mitophagy, the impaired mitochondria are incorporated into autophagosomes, which then fuse with lysosomes, resulting in the breakdown of these organelles (Lu et al., 2023). Mitophagy is also involved in numerous physiological functions, such as delaying the ageing process and cell differentiation. Interference in these processes induces physiological senescence and several age-related diseases (Krantz et al., 2021; Banarase et al., 2023; Sanz et al., 2023). Recent evidence indicates that impaired mitochondrial energy metabolism and age-related diseases share pathological features, such as an increased mutation rate in mitochondrial DNA (mtDNA), impaired electron transport chain function, elevated ROS levels, and the enhanced release of pro-apoptotic factors (Fang et al., 2017). As a result, the dysregulation of mitophagy can induce mitochondrial dysfunction, leading to the progression of age-related diseases.

Age-related macular degeneration (AMD) is a lesion on the macula that primarily affects the retinal pigment epithelium (RPE), photoreceptor cells, Bruch's membrane, and choroidal multilayered tissue. Gradual degeneration of the outer retina and the formation of new blood vessels between the retina and Bruch's membrane can advance to geographic atrophic or choroidal neovascular AMD, commonly known as dry and wet AMD, respectively (Figure 2). It is estimated that the worldwide occurrence of AMD stands at 196 million, with projections suggesting an increase to 288 million by 2040 (Xiao et al., 2023). RPE cells are known for high oxygen consumption, continuous photostimulation, and susceptibility to lipid peroxidation product exposure (Kaarniranta et al., 2009). Consequently, oxidative stress and damage play a crucial role in the progression of RPE degeneration in AMD (Handa, 2012; Blasiak et al., 2013). Persistent oxidative stress on RPE cells may result in the accumulation of extracellular deposits and damaged organelles, nucleic acids, lipids, cellular proteins, and lipofuscin granules, thereby increasing the levels of ROS. These excessive levels of oxidized lipoproteins and ROS induce protein misfolding, aggregation, and persistent activation of the innate immune responses, leading to protein accumulation, mitochondrial dysfunction, and inflammasome activation in the

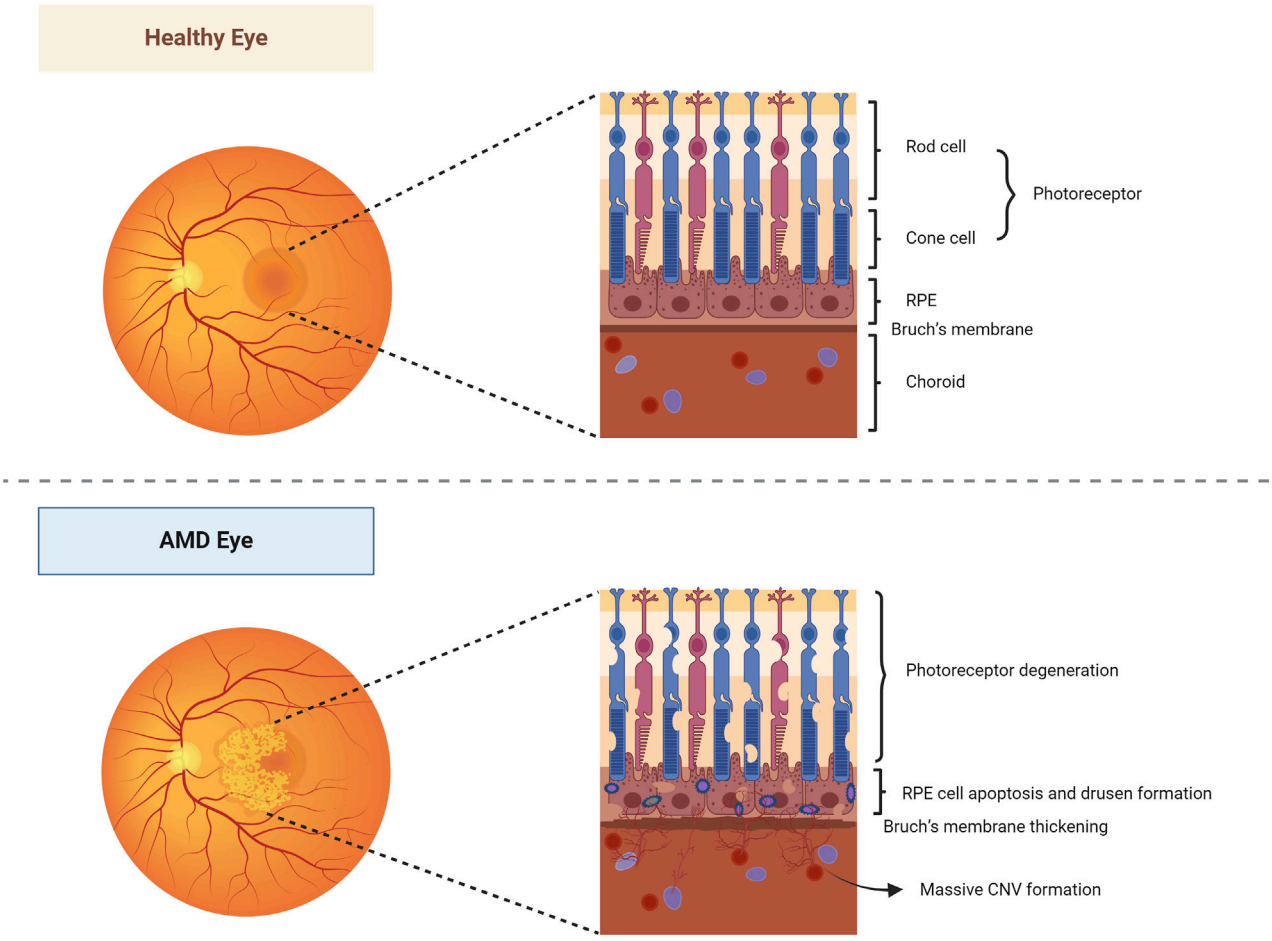

FIGURE 2
Healthy and AMD-affected eyes. In healthy eyes, the fundus is normal, and the morphology and structure of the photoreceptor cells, RPE layer, Bruch's membrane, and choroidal layer in the macula are normal. In AMD eyes, the pigmentation of the macular area was disorganized, and a slightly elevated neovascular membrane could be seen locally. Its photoreceptor cells, RPE, Bruch's membrane, and choroidal multilayer organization changed constantly, resulting in degeneration of photoreceptor cells, apoptosis of RPE cells, pigmentation production, thickening of Bruch's membrane, and formation of a large number of CNV. Abbreviations: AMD, age-related macular degeneration; RPE, retinal pigment epithelium; CNV, choroidal neovascularization.

RPE cells (Ferrington et al., 2016; Kauppinen et al., 2016; Piippo et al., 2018; Kaarniranta et al., 2019a; Kaarniranta et al., 2020). Mitochondria are the important sites of retinal oxidation and are mainly found within the RPE. Mitochondrial dysfunction reduces oxidative phosphorylation, results in excessive ROS production, increases mtDNA damage and mutations, and enhances the release of pro-inflammatory and pro-apoptotic factors, which induce oxidative stress, inflammatory response, and apoptosis (Murphy, 2009; Kageyama et al., 2012; Kaarniranta et al., 2020). Studies have shown that mitophagy regulates RPE cell damage and apoptosis by maintaining mitochondrial function and protein folding (Dhirachaikulpanich et al., 2022; Hyttinen et al., 2023).

Meanwhile, excessive accumulation of extracellular deposits inhibits mitophagy and promotes inflammation in AMD, whereas reduced accumulation restores mitophagy in RPE cells, alleviating AMD (Yu et al., 2023). Damage to mtDNA might be crucial in advancing AMD (Kaarniranta et al., 2019b). Recent reports have indicated that mtDNA damage increases with age; furthermore, elevated mtDNA damage, a higher number of mutations, and reduced DNA repair efficacy have been reported to be associated with the onset and staging of AMD (Piippo et al., 2018). Feher et al. (2006) reported a notable decline in the number of mitochondria and the absence of stromal density and cristae in the RPE of individuals with AMD compared to those in the control group. Zhao et al. (2011) stated that the suppression of oxidative phosphorylation in mouse RPE mitochondria led to the activation of the mechanistic target of the rapamycin (mTOR) pathway in an AMD mouse model. Proteomic analyses performed by Nordgaard et al. (2006) showed the differential expression of mitochondrial refolding and trafficking-related proteins in the RPE of AMD patients compared to that in the non-AMD controls. Subsequent research (Nordgaard et al., 2008) demonstrated that depending on the stage of AMD, the expression of mtHsp70, the mitochondrial translation factor Tu, mitofilin, subunit VIb of the cytochrome c oxidase complex, and α-, β-, and δ-subunits of the catalytic portion of ATP synthase was affected in the RPE. Using an *in vitro* model of AMD generated by fusing mitochondria-rich platelets from patients with AMD and mitochondrially-depleted retinal pigment epithelial

cells (ARPE-19), Dohl et al. (2022) reported increased concentrations of ROS, which could result in mtDNA damage, enhanced antioxidant responses, and increased expression levels of anti-inflammatory proteins. These results suggested that mtDNA damage response was fundamental in preventing AMD and slowing its progression. New findings indicate that boosting autophagy in RPE cells could be an innovative approach to tackle AMD.

Nevertheless, there are few approved mitophagy-modulating medications for AMD treatment. Current clinical AMD therapy includes lifestyle improvements (e.g., caloric restriction and moderate exercise) as well as pharmacological treatments [e.g., antioxidants (D Aloisio et al., 2022), tyrosine kinase inhibitors (Das et al., 2023), antidiabetic drugs (Thee et al., 2021; Mauschitz et al., 2022), anti-vascular endothelial growth factor (anti-VEGF), and other therapies (Francisco and Rowan, 2023; Servillo et al., 2023; Szigiato et al., 2023)]. Lately, traditional Chinese medicine (TCM) has garnered increasing attention for its antioxidant, anti-apoptotic, anti-inflammatory, and lipid-reducing activities, positioning it as a promising treatment option for AMD (Cao et al., 2022a; Yu et al., 2024). This review focuses on the molecular mechanisms governing mitophagy and innovative treatment approaches for employing TCM as a potential AMD therapy.

2 Mitophagy-specific pathways in AMD

2.1 Mitophagy mediated by PINK1/Parkin

Various mechanisms linked to mitophagy, which play a role in the onset of AMD, have been identified. The PTEN-induced putative kinase protein 1 (PINK1)/Parkin-mediated ubiquitination degradation pathway is the most characterized ubiquitin-dependent signaling pathway responsible for the control of mitochondrial structure and function (Bowling et al., 2019). Under normal conditions, PINK1 and Parkin are present at low levels in the mitochondrial outer membrane and cytoplasm, respectively. The cytoplasmic synthesis of PINK1 is followed by its relocation to the mitochondria, which is facilitated by the interaction between translocase of the outer membrane (TOM) and translocase of the inner membrane (TIM) (Kato et al., 2013; Sekine et al., 2019). Subsequently, PINK1 is cleaved by presenilin-associated rhomboid-like (PARL) (Meissner et al., 2015) and mitochondrial-processing protease (MPP) (Bayne and Trempe, 2019), leading to the degradation of PINK1 via the ubiquitin/proteasome pathway (Yamano and Youle, 2013). In response to stress, mitochondria are depolarized, and the level of ROS production increases, thereby inducing oxidative damage. During mitochondrial dysfunction, PINK1 degradation is blocked, causing accumulation at the mitochondrial outer membrane, the recruitment and phosphorylation of Parkin, and the activation of E3 ubiquitin ligase function. Subsequently, PINK1 and Parkin translocate from the cytoplasm to the mitochondrial outer membrane and ubiquitinate mitochondrial component proteins by forming polyubiquitin chains (Sauvé et al., 2022). Autophagy-associated proteins, such as nuclear dot protein 52 (NDP52), TAX1-binding protein-1 (TAX1BP1), optineurin (OPTN), and p62 (Shang et al., 2019; Zachari et al., 2020; Nguyen et al., 2023), recognize ubiquitinated mitochondria through their ubiquitin-binding domains (UBDs) and anchor their cargo to autophagic vesicle membranes via their LC3-interacting region (LIR) motifs, contributing to the formation of mitochondrial autophagosomes. Ultimately, autophagosomes fuse with lysosomes to form mature mitochondrial autolysosomes and trigger mitochondrial degradation (Callegari et al., 2017; Urbina-Varela et al., 2020) (Figure 3). Datta et al. (2023) reported that PINK1 levels were reduced in centro-concave RPE cells obtained from individuals with early AMD. Oxidative stress causes an increase in the concentration of heat shock protein Hsp70 in the lysosomes of RPE cells from patients with AMD, inhibiting the build-up of cytotoxic protein aggregates, alleviating lipofuscin-induced misfolding of intracellular proteins, and activating autophagy-mediated protein hydrolysis, thereby protecting RPE cells from oxidative stress (Kaarniranta et al., 2009; Subrizi et al., 2015). Hsp70 functions as a PINK1 degradation regulator, while Hsp70-interacting proteins, BCL2-associated athanogene 5 (BAG5) and BCL2-associated athanogene 2 (BAG2), play a significant role in regulating PINK1 stability by reducing its ubiquitination. Lowering Hsp70 levels downregulates PINK/Parkin-mediated mitophagy (Zheng et al., 2018a). Accordingly, the mechanism of Hsp70 action that leads to the protection of RPE cells via PINK1-mediated mitophagy could be significant in AMD progression. The regulation of antioxidant production and mitochondrial biosynthesis relies heavily on the involvement of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) and nuclear factor erythroid 2-related factor 2 (NFE2L2), respectively. These proteins upregulate antioxidant parameters and prevent the mitochondrial injury and apoptosis caused by ROS (Kaarniranta et al., 2020). Within the confines of the retina of NFE2L2/PGC-1α double knockout mice, a dry AMD model, high oxidative stress levels, protein aggregation, the significant upregulation of PINK1/Parkin expression, impaired mitochondrial injury, reduced mitophagy, and aberrant autophagic flux in RPE cells were observed. At the same time, the control group displayed minimal or no PINK1/Parkin activity (Sridevi Gurubaran et al., 2020). These results further demonstrate that controlling PINK1/Parkin-mediated mitophagy could be a viable approach to treating AMD.

2.2 Mitophagy mediated by BNIP3/NIX

Mitochondrial autophagy can also be activated via the BCL2-interacting protein 3 and NIP3-like protein X (BNIP3/NIX) pathway. BNIP3 and NIX, situated in the mitochondrial outer membrane, are hypoxia-inducible factors and are characterized by low expression in physiological states. The latest studies have revealed the role of BNIP3 in controlling apoptosis, alongside its part in mitochondrial quality management through mitophagy (Wang et al., 2013; Lampert et al., 2019). Thomas et al. (2011) reported that, unlike PINK1 and Parkin, BNIP3 and NIX bind directly to autophagosomes, contributing to the activation of mitophagy. BNIP3 and NIX exhibit a 56% protein sequence similarity, and both contain BCL2 homology domain-3 (BH3). Under normal conditions, the interaction between BCL-xl/BCL2 and the BH3 of Beclin-1 results in the formation of Beclin-1/BCL-xl and Beclin-1/BCL2 complexes, effectively

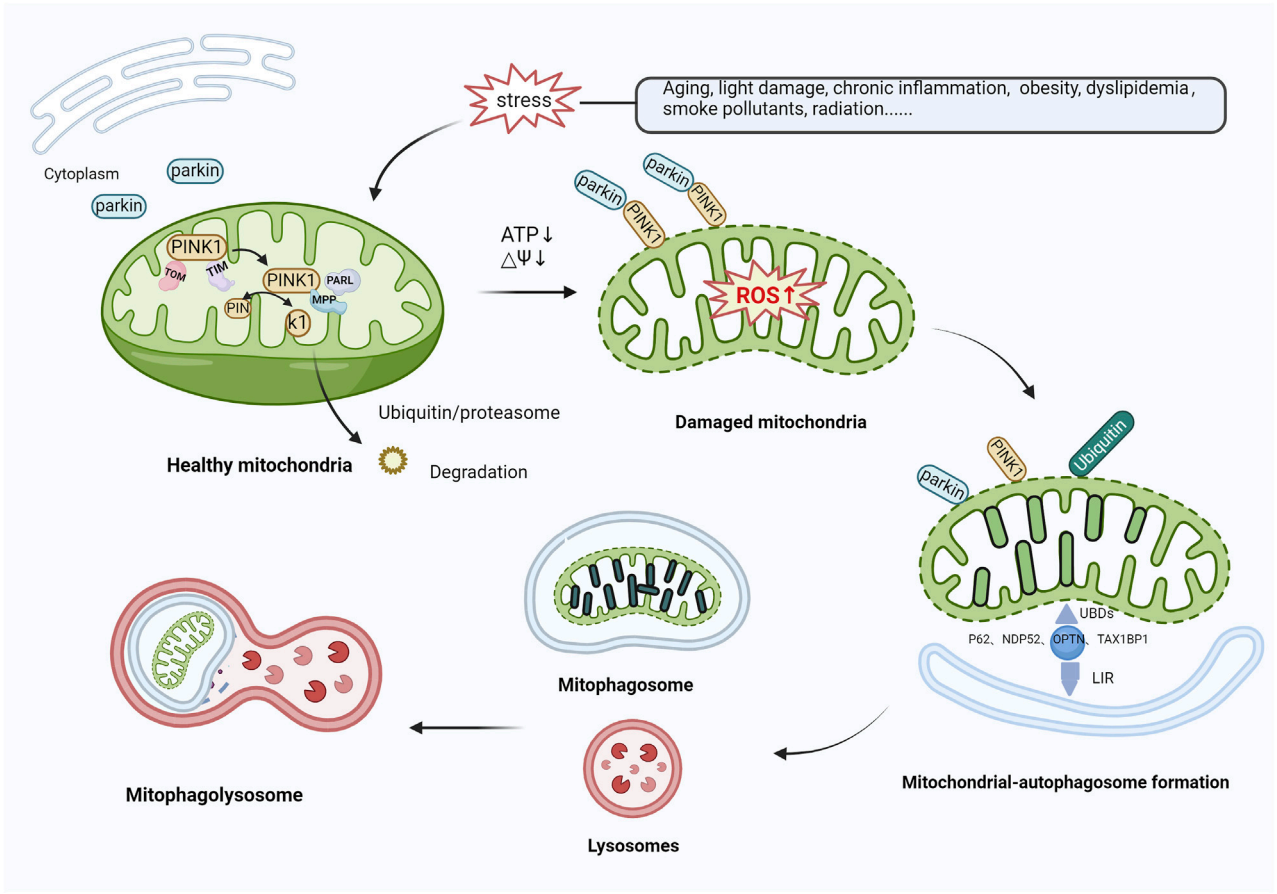

FIGURE 3
Mitophagy activation mediated by PINK1/Parkin. In healthy mitochondria, PINK1 is transported to mitochondria via TOM and TIM, cleaved by PARL and MPP, and then translocated to the cytoplasm, where it is broken down by the ubiquitin/proteasomal pathway. In response to various stressors, the level of ROS production rises, inducing oxidative damage. PINK1 recognizes damaged mitochondria, accumulates in large quantities in the mitochondrial outer membrane, and recruits and phosphorylates Parkin, activating its E3 ubiquitin ligase function. Both proteins translocate from the cytoplasm to the mitochondrial outer membrane and ubiquitinate mitochondrial proteins by forming polyubiquitin chains. Autophagy-related proteins (e.g., P62, NDP52, OPTN, TAX1BP1) then recognize ubiquitinated mitochondria through UBDs on the one hand, and on the other hand, are anchored to the membrane of autophagic vesicles through the LIR to form mitochondrial autophagosomes, which subsequently merge with lysosomes, leading to mitochondrial degradation. Abbreviations: PINK1, phosphatase and tensin homolog (PTEN)-induced kinase 1; TOM, translocase of outer membrane; TIM, Translocase of inner membrane; PARL, presenilin-associated rhomboid-like; MPP, mitochondrial-processing protease; ROS, reactive oxygen species; NDP52, nuclear dot protein 52; OPTN, optineurin; TAX1BP1, TAX1-binding protein-1; UBDs, Ubiquitin-binding domains; LIR, LC3-interacting region.

inhibiting autophagy. In response to stress, BNIP3/NIX are activated by hypoxia-inducible factor-1 (HIF-1) and interact with BCL-xl/BCL2 to release Beclin-1, subsequently activating mitophagy (Choubey et al., 2021). Furthermore, the control of BNIP3/NIX-mediated mitophagy involves phosphorylation—when BNIP3 Ser17 and Ser24 residues are phosphorylated, BNIP3 attaches to Microtubule-associated protein 1 light chain 3 (LC3) or to LC3 homolog GABA type A receptor-associated protein (GABARAP) through the LIR motif. Nevertheless, when NIX Ser34 and Ser35 residues undergo phosphorylation, NIX forms a bond with LC3, leading to the subsequent binding of LC3 to the γ-aminobutyric acid receptor-associated protein (GABAR) complex, ultimately resulting in the formation of the LC3-GABARAP complex. This complex targets LC3 in the damaged mitochondrial outer membrane and ultimately triggers mitophagy (Zhu et al., 2013; Rogov et al., 2017). BNIP3 contributes to ubiquitin-dependent mitophagy by inducing the mitochondrial movement of Parkin and promoting Parkin-mediated mitophagy. Additionally, BNIP3 inhibits the Ras homolog enriched in the brain (Rheb)/mTOR pathway and initiates autophagy (Figure 4) (Lin et al., 2014). Recent studies demonstrated that RPE in AMD has reduced levels of PINK1 and Parkin, and BNIP3/NIX-mediated mitophagy is the key factor in preserving mitochondrial equilibrium (Fisher et al., 2022; Jiménez-Loygorri et al., 2023). Esteban-Martínez and Boya (2018) reported that in response to metabolic stress, the destabilization of HIF1A/HIF-1 led to the upregulation of mitophagy receptor BNIP3/NIX. Furthermore, BNIP3L-dependent mitophagy-induced metabolic shifts in glycolysis are required for retinal ganglion cell (RGC) neurogenesis and the regulation of pro-inflammatory/M1-type macrophage polarization, which is vital for degenerative diseases such as AMD.

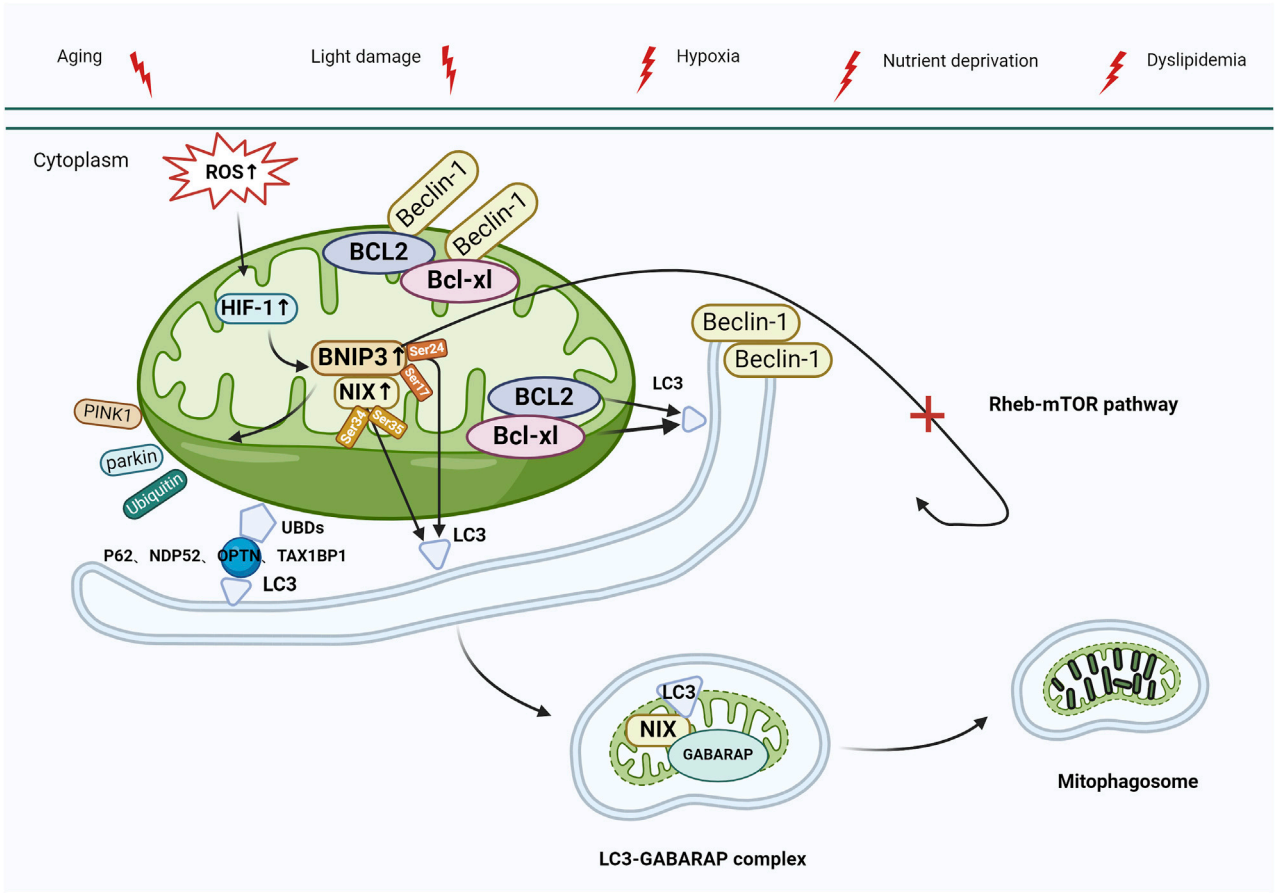

FIGURE 4
Mitophagy activation mediated by BNIP3/NIX. Under stress (e.g., aging, light damage, hypoxia, nutrient deprivation, and dyslipidemia), elevated levels of ROS activate HIF-1, leading to the upregulation of BNIP3/NIX, which interacts with the Beclin-1/BCL-xl and Beclin-1/BCL2 complexes, releasing Beclin-1, which activates autophagy. BNIP3/NIX-mediated mitophagy can also be regulated by phosphorylation. Ser17 and Ser24 phosphorylated on BNIP3 bind to LC3 via LIR, whereas Ser34 and Ser35 phosphorylated on NIX bind to LC3, and then bind to the GABAR complex to form the LC3-GABARAP complex, which targets LC3 to damaged mitochondrial outer membrane, and eventually initiates mitophagy. In addition, BNIP3 plays a role in the control of ubiquitin-dependent mitophagy by initiating mitochondrial movement of Parkin and facilitating Parkin-mediated mitophagy (process as in Figure 3). Finally, BNIP3 inhibits the Rheb-mTOR pathway, which also leads to autophagy activation. Abbreviations: BNIP3, BCL2-interacting protein 3; NIX, NIP3-like protein X; ROS, reactive oxygen species; HIF-1, Hypoxia-inducible factor-1; LC3, Microtubule-associated protein 1 light chain 3; LIR, LC3-interacting region; GABARAP, GABA type A receptor-associated protein; Rheb, Ras homologue enriched in brain; mTOR, target of the rapamycin machinery.

2.3 Mitophagy mediated by FUNDC1

Mitophagy can also be activated via the FUN14 domain-containing 1 (FUNDC1) pathway. FUNDC1 is a protein located in the outer membrane of the mitochondria, and its increased expression has been reported to trigger mitophagy (Kuang et al., 2016; Wu et al., 2016). The identification of FUNDC1 as a mitophagy receptor has been confirmed by recent studies (Mao et al., 2022). Wu et al. (2016) suggested that the site of FUNDC1 action is the mitochondria-associated endoplasmic reticulum membrane (MAM). FUNDC1, as a MAM-associated protein (Li et al., 2014), has an LIR motif at the amino-terminus that engages with LC3, and the deletion or structural changes to the LIR motif impede the connection between FUNDC1 and LC3, resulting in the downregulation of mitosis. The phosphorylation of FUNDC1, similar to BNIP3/NIX and BNIP3, can have either a positive or negative effect. Under physiological conditions, Src and casein kinase 2 prevent FUNDC1 from binding to LC3 by phosphorylating the Tyr18 residue of LIR, reducing mitophagic activity. When hypoxia or mitochondrial depolarization occurs, phosphoglycerate mutase family member 5 (PGAM5) induces the dephosphorylation of Ser13 (Liu et al., 2012; Chen et al., 2014); the dephosphorylated FUNDC1 then interacts with LC3 to activate mitophagy. Simultaneously, the deubiquitinating enzyme ubiquitin specific peptidase 19 (USP19), which is located in the endoplasmic reticulum, accumulates at the MAM and binds to the mitochondrial outer membrane protein FUNDC1, inducing its deubiquitination, promoting the oligomerization of dynamin-related protein 1 (DRP1), and resulting in mitochondrial fission (Zhang et al., 2022). Additionally, the unc-51-like autophagy-activating kinase 1 (ULK1) complex can translocate into the mitochondria, contributing to the phosphorylation of FUNDC1 Ser17 and the activation of mitophagy (Wu et al., 2014; Mercer et al., 2018). Another mitochondria-associated

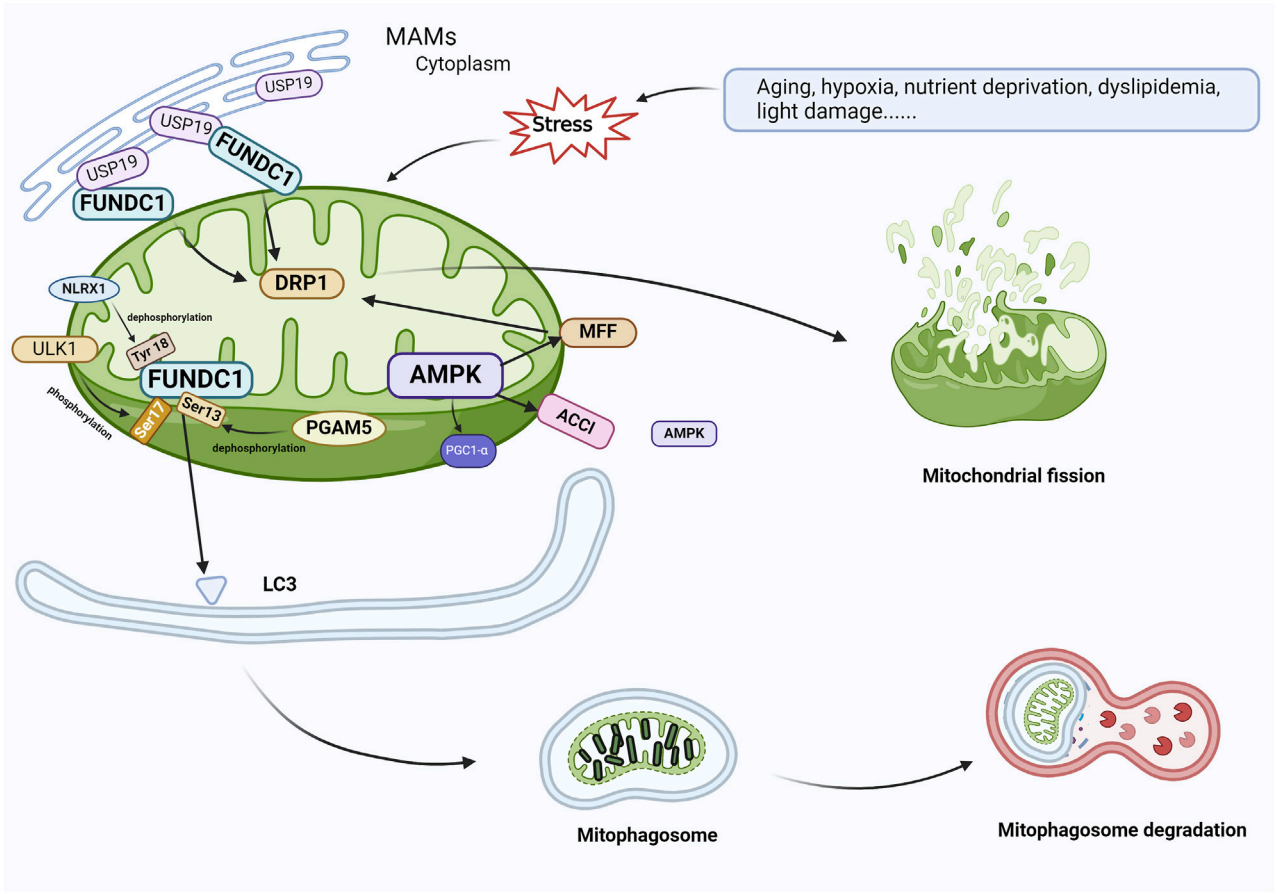

FIGURE 5
FUNDC1 and AMPK are involved in the regulation of mitophagy. In response to cellular stress, PGAM5 induces the dephosphorylation of Ser13, and FUNDC1 and LC3 interact to induce mitophagy. At the same time, USP19, which accumulates on MAM, attaches to FUNDC1 and triggers deubiquitination of FUNDC1, which promotes oligomerization of DRP1 and leads to mitochondrial fission. ULK1 complex translocates to mitochondria and phosphorylates Ser17 of FUNDC1 to initiate mitophagy, and NLRX1 activates FUNDC1 by dephosphorylating FUNDC1 Tyr 18 and induces mitophagy. During AMPK-mediated mitophagy, ACCl and MFF on the mitochondrial outer membrane, which are substrates for the action of AMPK, contact AMPK in the cytoplasm, resulting in the localization of AMPK within or near mitochondria. AMPK phosphorylates MFF and binds to DRP1 in response to oxidative stress, leading to mitochondrial fission, which initiates mitophagy and elimination of damaged mitochondria. Abbreviations: FUNDC1, FUN14 domain-containing 1; AMPK, AMP-activated protein kinase; PGAM5, phosphoglycerate mutase family member 5; USP19, Ubiquitin Specific Peptidase 19; MAM, mitochondria-associated endoplasmic reticulum membrane; DRP1, dynamin-associated protein 1; ULK1, Unc-51-like autophagy-activated kinase 1; NLRX1, nucleotide-binding oligomerization domain (NOD)-like receptor X1; ACC1, acetyl coenzyme A carboxylase 1; MFF, mitochondrial fission factor.

protein, nucleotide-binding oligomerization domain (NOD)-like receptor X1 (NLRX1), an immune system regulator, is known to diminish inflammatory responses, reduce ROS generation, and regulate autophagy. Increased NLRX1 expression reduces the relative levels of FUNDC1 phosphorylation and NLRP3 inflammasome-associated proteins in ARPE-19 cells, thereby preventing the development of AMD (Wang et al., 2023a). The exact mechanism of FUNDC1 action in mitophagy in AMD is not well characterized; however, studies suggest that the FUNDC1 pathway plays an integral role in obesity and metabolic disorders (Tan et al., 2018; Ren et al., 2020), as the excessive intake of saturated fatty acids affects the stability of mitophagy receptor FUNDC1 and mitochondrial mass, leading to mitochondrial dysfunction, obesity, and metabolic disorders (Chen et al., 2023). Wu et al. (2019) also reported that FUNDC1 deficiency in mice inhibits mitophagy, while a high-fat diet impairs mitochondrial function, resulting in high oxidative stress and heightened inflammatory responses. According to these results, the mitophagy receptor FUNDC1 is essential for maintaining mitochondrial quality, controlling inflammatory responses, and regulating metabolic disorders, possibly via mitogen-activated protein kinase (MAPK) signaling.

2.4 Mitophagy mediated by AMPK

AMP-activated protein kinase (AMPK) is also related to the regulation of mitophagy. AMPK, a ubiquitously expressed serine/threonine kinase, is a highly conserved sensor of cellular energy and nutritional status and a major regulator of cellular metabolism (Rey and Tamargo-Gómez, 2023). AMPK activation plays a role in controlling oxidative stress, inflammation, glycolipid metabolism,

mitophagy, and other functions (Cai et al., 2023; Feng et al., 2023; Guo et al., 2023; Zhong et al., 2023; Zhu et al., 2023). AMPK phosphorylates acetyl-CoA carboxylase 1 (ACC1) and mitochondrial fission factor (MFF) on the mitochondrial outer membrane. The interaction among phosphorylated ACC1, MFF, and AMPK in the cytoplasm facilitates the presence of AMPK in or around the mitochondria (Zong et al., 2019). DRP1 is the enzyme that catalyzes mitochondrial fission, while MFF is the primary receptor for DRP1 on the mitochondrial outer membrane. AMPK causes mitochondrial fragmentation by phosphorylating MFF in response to oxidative stress, initiating mitophagy and clearing damaged mitochondrial fragments (Toyama et al., 2016). In another model system, iron overload-induced mesenchymal stem cell (MSC) damage through the AMPK/MFF/DRP1 pathway increased MSC apoptosis, leading to mitochondrial fragmentation and enhanced autophagy (Zheng et al., 2018b). Furthermore, AMPK promotes mitophagy by directly phosphorylating PGC1-α (Figure 5) (Cantó et al., 2009).

Zhuang et al. (2022) reported that inhibition of the AMPK pathway induced RPE cell apoptosis, resulting in mitochondrial injury and the suppression of mitophagy. Xu et al. (2018) showed that increasing AMPK activity diminished DNA injury, reduced oxidative stress, and increased the production of mitochondrial energy, thereby protecting photoreceptors and RPE from acute damage, preventing their degeneration, and hindering the development of AMD. These findings confirm that AMPK-mediated mitophagy removes damaged mitochondria and protects mitochondrial function in response to oxidative stress. Furthermore, Salminen et al. (2012) reported that AMPK induced mitochondria generation by upregulating the transcription of PGC-1α target genes and removed impaired mitochondria via ULK1-dependent mitophagy. AMPK agonists also protect the cells by removing misfolded or damaged proteins from RPE cells through mitophagy.

Su et al. (2020) found that TXNIP was induced significantly in human retinal pigment epithelium, Muller glia, and cone photoreceptor cells under high-glucose conditions for five consecutive days, resulting in oxidative stress, ATP reduction, and decreased mitophagic flux. They proposed that TXNIP regulates Parkin/PINK1-mediated mitophagy in dopaminergic neurons under hyperglycemic conditions (Su et al., 2020). In addition, Devi observed that high glucose increased the expression of TXNIP at the mRNA and protein levels significantly in human RPE cell lines and primary human RPE cells. The upregulation of TXNIP was associated with mitochondrial membrane depolarization, fragmentation, and mitophagy access to lysosomes (Devi et al., 2019). Furthermore, Singh proposed that TXNIP leads to mitochondrial dysfunction, oxidative stress, dysfunctional mitophagy phagocytosis, lysosomal instability and inflammation in DR (Singh et al., 2018). The Nrf2/ARE signaling pathway plays an important role in the coordination of mitochondrial biogenesis and mitophagy, and it is involved in regulation of the expression of protective genes against oxidative stress, and regulation of mitochondrial biology and mitophagy. Consequently, it may be an important target for drugs for the treatment of neurodegeneration (Gureev et al., 2020). All the findings above suggest that TXNIP-mitochondria-lysosome and Nrf2-P62 also mediate mitophagy.

3 Mitophagy: a promising therapeutic target for AMD

As summarized in the previous section, mitophagy is strongly associated with the pathogenesis of AMD. Therefore, drugs that activate mitophagy in RPE cells could provide a novel therapeutic strategy for AMD. Recently, numerous mitophagy modulators that either activate or inhibit mitophagy, including AICAR (5-aminoimidazole-4-carboxamide ribonucleotide), an AMP analogue that maintains the optimal function of mitochondria (Ebeling et al., 2022), PGC-1α (an important regulator of mitochondrial biosynthesis) (Hyttinen et al., 2021), human retinal progenitor cells (hRPCs) (Yu et al., 2021), the mitochondria-targeted antioxidant triphenylphosphine (TPP)-nicotinic acid (Kim et al., 2021), melatonin (Mehrzadi et al., 2020), the mitochondrial activator PU-91 (Nashine et al., 2019), the mitochondria-derived peptide variant Humanin G (HNG) (Nashine et al., 2017), and the mitochondria-targeted antioxidant SkQ1 (Telegina et al., 2020; Fisher et al., 2022), have been discovered. mtDNA repair could be another target for improving mitochondrial function. Poly (ADP-ribose) polymerase (PARP1) active base excision repair (BER) and microhomology-mediated end-joining (MMEJ), among others, restore single- and double-strand breaks in mtDNA. Similar to nuclear DNA, nucleic acid complexes of multiple proteins can protect mtDNA from histone binding-induced ROS damage. However, owing to the complexity of mtDNA, its repair needs to be further investigated (Kaarniranta et al., 2020). Furthermore, these studies are still limited to animal and preclinical experiments, and the potential side effects during AMD treatment must be evaluated. A recent study demonstrated that augmenting mitophagic activity in individuals with AMD can impede the progression of the disease (Amini et al., 2023). The complexity of AMD pathogenesis should not be overlooked, as it encompasses a multitude of signaling crosstalks connecting the various layers of retinal structures to the choroid; as a result, positive outcomes are often difficult to achieve using single-agent therapy.

4 Role of TCM in mitophagy regulation in AMD

TCM treats diseases through various active ingredients, which possess antioxidant, anti-ageing, immunomodulatory, and anti-inflammatory properties as well as the ability to regulate mitophagy, and could thus be considered as an alternative therapeutic strategy for AMD. In China, *Astragalus mongholicus* Bunge, *Poria cocos* (Schw.) Wolf, *Plantago asiatica* L., *Atractylodes macrocephala* Koidz., *Angelica sinensis* (Oliv.) Diels, *Panax ginseng* C.A.Mey., *Salvia miltiorrhiza* Bunge, *Curcuma longa* L. (Pfahler et al., 2022; Vallée, 2022), *Rehmannia glutinosa* (Gaertn.) DC., *Lycium chinense* Mill., and *Glycyrrhiza glabra* L. (Kozlowski et al., 2015; Lee et al., 2015; Peng et al., 2016; Chen et al., 2021; Li et al., 2022a; Cho et al., 2023; Wei and Tong, 2023) are commonly used for the treatment of AMD. Studies have demonstrated that *Fructus lycii* can selectively activate and regulate the AMPK and VEGF pathways to enhance mitophagy, thereby preventing the development of retinopathy (Yang et al., 2022c). Another TCM,

TABLE 1 Main active ingredients, pharmacological actions, and mechanisms of traditional Chinese medicine herbs and botanical drug decoction frequently used to treat AMD.

Single herb	Active ingredients	Pharmacological actions	Mechanisms	References
Poria	Poricoic acid A	Anti-inflammatory, antioxidant, immunomodulatory	Induction of mitochondrial dysfunction, endoplasmic reticulum stress and activation of the AMPK pathway	Yang et al. (2022a)
		Hypoglycemic and antifibrotic	Induction of mitophagy by downregulation of FUNDC1	Wu et al. (2023)
Radix *Angelicae sinensis*	*Angelica sinensis* polysaccharide	Antioxidant and anti-apoptotic	Downregulation of BNIP3 with the activation of mTOR and Notch signaling pathways	Xue et al. (2019)
Radix Panax Ginseng	Ginsenoside Rg1	Attenuates apoptotic and fibrotic responses	Enhancement of SIRT1/PINK1/Parkin-mediated mitophagy	Guan et al. (2023)
		Antioxidant stress	Increased expression of PINK1 and p-AMPK	Xu et al. (2019)
Radix Astragali	Astragaloside IV	Anti-ageing	Activation of Parkin-mediated mitophagy	Li et al. (2022b)
		Antioxidant stress	Enhancement of and restoration of mitochondrial function in an AMPK-dependent manner	Zhu et al. (2022a)
	Astragalus polysaccharide	Anti-apoptotic	Increased expression of BNIP3	Zhang et al. (2021)
Rhizoma *Atractylodis macrocephalae*	Atractylenolide III	Antifibrotic	Activation of AMPK signaling pathway	Huang et al. (2022)
		Antioxidant, antitumor, antiallergic reaction, antimicrobial and cognitive protection	Activation of the AMPK/SIRT1 signaling pathway	Li et al. (2022c)
Salvia miltiorrhiza	Tanshinone IIA	Anti-inflammatory, antioxidant, and anti-apoptotic	Activation of the AMPK/mTOR autophagy pathway by enhancing AMPK phosphorylation and the expression of Bcl-2/Bax, Beclin-1, LC3II/I, and SOD2, while diminishing mTOR phosphorylation, and the expression of NOX2, cleaved caspase-3/caspase-3	Si et al. (2023)
		Antioxidant, anti-apoptotic, regulation of mitophagy	Inhibition of the AMPK/Skp2 pathway by attenuating Parkin-mediated mitophagy	He and Gu (2018)
	Sodium Tanshinone IIA Sulfonate	Antioxidant, anti-inflammatory, anti-apoptotic, regulation of mitochondrial homeostasis	Inhibition of the overproduction of mitochondrial ROS via the AMPK pathway	Zhu et al. (2022b)
Curcuma longa	Curcumin	Anti-inflammatory, antioxidant, maintenance of mitochondrial homeostasis	Promotion of AMPK/PINK1/Parkin-mediated mitophagy	Jin et al. (2022)
		Antioxidant, anti-ageing, autophagy activation	Regulation of the SIRT1/AMPK/mTOR pathway, induction of autophagy	Yang et al. (2022b)
Mingmu Di Huang Pill	Quercetin, lignans and naringenins, strychnosides, paeoniflorin, salvinorin, etc.	Antioxidant, anti-apoptotic	Activation of autophagy articulator-SQSTM1 by AMPK-mediated degradation of autophagic Kelch-like ECH-related protein 1 (Keap1)	Chen et al. (2022)

Mingmu Di Huang Pill, treats AMD by activating the expression of autophagy adaptor-SQSTM1 and AMPK phosphorylation, which can promote the autophagic degradation of Kelch-like ECH-related protein 1 (Keap1), safeguarding RPE cells against oxidative stress damage (Chen et al., 2022) (the main active ingredients, pharmacological actions, and mechanisms of these Chinese medicines are summarized in Table 1). Furthermore, various natural products from TCM, including berberine (BBR), curcumin, artemisinin, paeoniflorin, quercetin, luteolin, naringenin, ivytin (Cao et al., 2022b), urinary apolipid A (UA), ferulic acid, and astaxanthin (Lewis Luján et al., 2022), have been reported to have a mitophagy-regulating effect in numerous diseases

TABLE 2 Natural chemical components and Mitophagy.

Natural chemical components	Pharmacological actions	Mechanisms	References
Urinary apolipid A	Anti-aging, anti-inflammatory, immunomodulatory, anti-tumor	1) Triggering mitophagy by decreasing MMP without interrupting ROS production and the mitochondrial respiratory chain 2) Improvement of immunity through PINK1/Parkin-related pathway	Denk et al. (2022), Lu et al. (2023)
Berberine	Antioxidant, anti-inflammatory, anti-aging, antibacterial, hypotensive, gastric mucosa protection	1) Inhibition of H_2O_2-induced oxidative damage in human retinal pigment epithelial cell line D407 cells by activation of AMPK 2) Restoration of mitophagy via the PINK1-Parkin pathway, thereby attenuating the aging process	Wang et al. (2017), Li et al. (2018), Wang et al. (2023b)
Paeoniflorin	Anti-inflammatory, antioxidant, immunomodulatory	Alleviating Nox1/ROS-associated oxidative stress, mitochondrial dysfunction, and endoplasmic reticulum stress in retinal pigment epithelial cells through activation of AMPK.	Zhu et al. (2018)

by activating several specific pathways, including AMPK, PINK1/Parkin, BNIP3, FUNDC1, as well as LC3 proteins, followed by the induction of autolysosome formation. For example, UA, a novel mitophagy enhancer, initiates mitophagy by decreasing MMP without disrupting the mitochondrial respiratory chain and ROS production. Furthermore, it activates mitophagy by inducing the proper mitochondria function in a dose-dependent manner (Lu et al., 2023) and improves immune function through the PINK1/Parkin-mediated pathway (Denk et al., 2022). A previous study proposed that BBR has anti-inflammatory, antioxidant, antimicrobial, hypotensive, and gastric mucosa protective properties (Wang et al., 2017) as well as the ability to prevent oxidative damage triggered by hydrogen peroxide in human RPE cell line D407 via the activation of AMPK, indicating the potential therapeutic application of BBR for AMD (Li et al., 2018). Wang et al. (2023b) also demonstrated that BBR could act as a potential inducer of mitophagy, inhibiting PINK1 promoter methylation, reversing D-ribose-induced mitochondrial dysfunction, and restoring mitophagy via the PINK1/Parkin pathway, thereby attenuating the ageing process. Lu et al. (2023) reported that curcumin, a diketone extracted from the rhizomes of plants in the Zingiberaceae and Araceae, could potentially enhance Parkin-dependent mitophagy through the AMPK/transcription factor EB (TFEB) signaling pathway to diminish oxidative stress-induced injury to the intestinal barrier and mitochondrial dysfunction (Zhang et al., 2023). By activating AMPK, paeoniflorin, a monoterpene glycoside derived from *Paeonia lactiflora*, mitigates oxidative stress associated with Nox1/ROS in RPE cells, mitochondrial damage, and endoplasmic reticulum stress while protecting ARPE-19 cells and arresting the advancement of retinal degenerative disorders like AMD (Zhu et al., 2018) (Table 2). The latest evidence suggests that TCM or its active components could regulate mitophagy-related proteins, presenting a promising approach to treating AMD.

5 Conclusion and future directions

Mitophagy is an important mitochondrial quality control mechanism that selectively eliminates dysfunctional mitochondria to maintain cellular homeostasis. The regulation of mitophagy is mediated mainly by PINK1/Parkin, BNIP3/NIX, FUNDC1, and AMPK, and its dysfunction is closely related to AMD development. The use of drugs to increase mitophagy in RPE cells provides new ideas for AMD treatment.

Exploration of the relationship between TCM and mitophagy could offer key insights. The findings of such studies could broaden the scope of research on TCM theories by providing insights into how TCM can prevent and control AMD. In recent years, very few reports on TCM treatment of AMD by modulating mitophagy-related pathways have been published. In the present review, the authors focused on the role of mitophagy-related pathways in AMD pathogenesis, drawing on previous studies. In addition, the authors demonstrated potential ways via which TCM could modulate mitophagy to improve AMD based on three aspects: single botanical drugs, botanical drug decoction, and natural chemical components of TCM. However, although multiple pathways can mediate mitophagy, the correlation between the mediating pathways and the exact mechanism of mitophagy-induced AMD has not been elucidated. In future, more animal experiments and clinical experiments should be conducted under various pathological mechanisms to explore the role of mitophagy in AMD. In addition, studies on the regulation of mitophagy by TCM considering specific pharmacodynamics, target pathway, and mechanism of action should be carried out, to clarify the pathways through which the different active ingredients in single botanical drugs regulate mitophagy, and the mitophagy pathways via which different dosages and combinations of TCM could influence the overall AMD regulation, with a view to provide novel clinical insights and methods for the treatment of AMD with TCM.

Author contributions

YY: Conceptualization, Writing–original draft, Writing–review and editing. GW: Project administration, Writing–review and editing. YL: Supervision, Writing–review and editing. ZM: Writing–review and editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This review was funded by the Traditional Chinese Medicine Science and Technology Development Project of Shandong Province (Grant number M-2022156).

Acknowledgments

The figures in this review were created using the BioRender platform. We extend our gratitude to Editage (www.editage.cn) for their expertise in English language editing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 31 May 2024
DOI 10.3389/fphar.2024.1380098

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RECEIVED 01 February 2024
ACCEPTED 10 May 2024
PUBLISHED 31 May 2024

CITATION
Gao C, Song X-D, Chen F-H, Wei G-L and Guo C-Y (2024), The protective effect of natural medicines in rheumatoid arthritis via inhibit angiogenesis.
Front. Pharmacol. 15:1380098.
doi: 10.3389/fphar.2024.1380098

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The protective effect of natural medicines in rheumatoid arthritis via inhibit angiogenesis

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Rheumatoid arthritis is a chronic immunological disease leading to the progressive bone and joint destruction. Angiogenesis, accompanied by synovial hyperplasia and inflammation underlies joint destruction. Delaying or even blocking synovial angiogenesis has emerged as an important target of RA treatment. Natural medicines has a long history of treating RA, and numerous reports have suggested that natural medicines have a strong inhibitory activity on synovial angiogenesis, thereby improving the progression of RA. Natural medicines could regulate the following signaling pathways: HIF/VEGF/ANG, PI3K/Akt pathway, MAPKs pathway, NF-κB pathway, PPARγ pathway, JAK2/STAT3 pathway, etc., thereby inhibiting angiogenesis. Tripterygium wilfordii Hook. f. (TwHF), sinomenine, and total glucoside of Paeonia lactiflora Pall. Are currently the most representative of all natural products worthy of development and utilization. In this paper, the main factors affecting angiogenesis were discussed and different types of natural medicines that inhibit angiogenesis were systematically summarized. Their specific anti-angiogenesis mechanisms are also reviewed which aiming to provide new perspective and options for the management of RA by targeting angiogenesis.

KEYWORDS

rheumatoid arthritis, angiogenesis, mechanisms, natural medicines, ingredients, VEGF

1 Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease whose main pathology is characterized by damage to articular cartilage and bone tissue, synovial hyperplasia, chronic inflammation and pannus formation (Díaz-González and Hernández-Hernández, 2023). Its worldwide prevalence ranges from 0.5% to 2% and the incidence is higher in females than in males (Radu and Bungau, 2021). RA cause progressive joint destruction, lower life expectancy, early unemployment, and considerable disability, all of which have a negative impact on patients' health (Scherer et al., 2020; Conforti et al., 2021; Finckh et al., 2022). The pathogenesis of RA is not yet fully understood and may be related to genes, epigenetics, environmental factors (Giannini et al., 2020). Patients who have suffered irreparable damage to their joints will never regain normal physical functioning, even if clinical remission is achieved at a later stage (Frazzei et al., 2023). Early recognition and treatment can limit radioactive joint damage, preserve joint function, and achieve long-term drug-free remission (Tan and Buch, 2022). Currently, nonsteroidal anti-inflammatory drugs, antirheumatic medicines, glucocorticoids, and immunosuppressants are the most commonly used drugs in the clinical prevention and management of RA (Zhao J. et al.,

2021). The first-line treatment for RA is anti-rheumatic drugs, which interferes with RA symptoms, enhance physical function, and alleviate joint deterioration. However, the progression of damage and severe incapacity is not halted by antirheumatic medicines (Lin et al., 2020). In the last three decades, new treatment alternatives have been developed for both biologics and small-molecule drugs. However, because to the complex pathophysiology of RA, it is difficult for existing drug treatments to achieve satisfactory efficacy (Smolen, 2020).

Angiogenesis, the process by which pre-existing vessels grow into new capillaries, has been implicated in the proliferation of cancers as well as the pathophysiology of inflammatory disorders (Carmeliet, 2003; Folkman, 2006). Some compounds such as nintedanib which targeting angiogenesis have already been used for tumor treatment (Roth et al., 2015). Thus, the blockade of angiogenesis was a viable strategy that could lead to remission of disease progression (Balogh et al., 2019). Angiogenesis is an early indication of RA, and is also one of the hallmarks of RA, which precedes the appearance of other symptoms (Szekanecz et al., 2009). The neovasculature generated in the initial stages of RA transports oxygen and nutrients to the highly dividing synovial tissue, increases synovial cell proliferation, and allows immune cells to invade cartilage and bone tissue (Szekanecz et al., 2005). Once the new vasculature and the proliferative synovial cells form into the vascular cartilage junction and the vascular bone junction, the invasion may injure both bone and cartilage tissue, prompting deformation of the joints and functional impairment (Maeda et al., 2022). Inhibition of neovascularization is a significant aim in the management and recovery of RA because it prevents the occurrence of pannus (Eelen et al., 2020).

Natural medicines has been used for centuries in the management of RA, which has the advantages of abundant resources, minimal toxicity and favourable therapeutic effect (Wang et al., 2021a; Liu X. et al., 2022). Some natural medicines have been offered to patients with RA or have shown encouraging evidence in preclinical studies, indicating potential for future use (Zhou et al., 2018; Liu X. et al., 2022; Luo et al., 2023). Researchers have made major advances in understanding the material basis and mechanism of natural medicines (Li W. et al., 2023). Many researches have demonstrated that natural medicines has significant anti-arthritis potential. The mechanism involved in this effect include immunological regulation, anti-oxidative stress, anti-angiogenesis, inhibition of bone destructionand and decreased expression of inflammatory indicators (Lü et al., 2015; Zhao X. et al., 2021; Liu X. et al., 2022). Firstly, the functions of angiogenesis in RA will be discussed in this review, followed by an increased overview of the angiogenic mediators required for RA. We will then describe and summarize the natural medicines including compounds, extracts, and prescription which inhibit synovial angiogenesis. Natural products primarily regulate the hypoxia-inducing factor (HIF)/vascular endothelial growth factor (VEGF)/angiotensin (Ang) signaling axes, as well as related pathways such as mitogen-activated protein kinase (MAPK), nuclear factor κB (NF-κB), protein kinase B (AKT)/mTOR, peroxisome proliferator-activated receptor-γ (PPARγ), and janus kinase/signal transduction and transcriptional activator 3 (JAK/STAT3). Relevant researches were retrieved from PubMed, Web of Science and China National Knowledge internet databases. The search algorithm was as follows: ("rheumatoid arthritis" OR "anti-rheumatic drugs") AND ("alkaloids" OR "natural medicines" OR "flavonoids" OR "terpenoids" OR.

"polyphenol" OR "natural extracts"). The main information were summarized, as shown in Table 1–3. The major signaling pathways that affect angiogenesis was shown in Figure 1. Studies on natural medicines that inhibit angiogenesis range from 2000 to 2023.

2 Role of angiogenesis in RA

Angiogenesis is the generation of new branches of blood vessels from pre-existing blood vessels, requiring the degeneration of the basement membrane, stimulation, expansion, and migrating of vascular endothelial cells (ECs), and neovascularization (Folkman, 2006). Synovial cells, like usual cells, require an exchange of vital nutrients and oxygen through blood vessels in addition to the elimination of metabolic waste (Taylor and Sivakumar, 2005). Under normal physiological conditions, the vascular system will be in a resting state for a long time, and only when the body is damaged will there be temporary angiogenesis (Maruotti et al., 2006).

However, in the pathology of RA, the oxygen and nutrient requirements of the synovium incrementally increase, and the proliferation and expansion of synovial tissues force the number and density of synovial vessels to increase compensatively (Leblond et al., 2017). The higher the degree of synovial cell growth and the degree of monocyte infiltration, the higher the vascular density and the degree of ECs growth (Taylor and Sivakumar, 2005). Angiogenesis depends on multiple phases, each of which is modulated by particular factors. Angiogenesis is supposed to begin with the stimulation of ECs mainly activated by pro-angiogenic substances especially VEGF. The activated ECs secrete matrix metalloproteinases (MMPs) which fragment the basal membrane and trigger invasion. This causes local blood vessels dilatation, increased vascular permeability, and proteolytic breakdown of the basal membranes of existing capillary endothelial cells. When the basement membrane is disrupted, endothelial tip cells will protrude and migrate approach the site of origin of the angiogenic signaling. Following EC growth, capillary buds form, causing ECs to elongate and organize properly. The expanding buds eventually generate lumens, allowing these tubular structures to connect to nearby blood vessels. In the last stage, pro-angiogenic factors such as Ang1 stabilise blood vessels, and then pericytes are incorporated into the newly constructed basement membrane to facilitate the process of blood flow (Taylor and Sivakumar, 2005; Szekanecz et al., 2010; Rizzi et al., 2017; Eelen et al., 2020). Early neovascularization in RA strengthens tumor-like synovial cell proliferation and allows inflammatory cells to infiltrate cartilage and bone tissue. Inflammation, immunological imbalance, and angiogenesis are all linked together to promote pannus production, which damages joints and causes abnormalities in joints and dysfunction (Costa et al., 2007; Leblond et al., 2017). Numerous investigations have indicated that angiogenesis occurs at the beginning of RA and persists throughout every phase of the disease. There is evidence that the suppressing angiogenesis may improve synovial inflammation and pannus formation. Consequently, angiogenesis is essential to both the initiation of RA and the discovery of effective pharmaceuticals (Semerano et al., 2011; Wang et al., 2021b). The role of angiogenesis in the pathological process of rheumatoid arthritis is shown in Figure 2.

TABLE 1 Effects of compounds on angiogenesis in rheumatoid arthritis.

Categories	Natural product	Plants source	Experiment models	Effective dose	Mechanism	Ref.
Alkaloids	Sinomenine	Sinomenium acutum	CIA rats	30, 100, 300 mg/kg	HIF-1α, VEGF, Ang-1, CD31↓	Feng et al. (2019)
	Berberine	Hydrastis canadensis/Cortex phellodendri/Rhizoma coptidis	CIA rats	200 mg/kg	TNF-α, IL-1β, IL-6, IL-17, VEGF↓; VEGF, CD34↓; p-ERK, p-p38, p-JNK↓	Wang et al. (2014)
	1-Methoxycarbony-β-carboline	Picrasma quassioides	HUVECs	50 μmol/L	Ang, EGF, bFGF, GRO, IGF-1, PLG, MMP-1, TIE-2, uPAR↓	Lin et al. (2018)
			zebrafish	12.5, 25, 50 μmol/L	embryonic angiogenesis in zebrafish↓; angiogenesis in the zebrafish caudal fin regeneration assay↓	
	Matrine	Sophora flavescens Alt	CIA rats	100 mg/kg	IL-1β, IFN-γ, VEGF, PLGF, HIF-α, Ang-1, Ang-2, Tie-2↓; phosphorylation-Akt↓	Ao et al. (2022)
			FLS	0.5, 1.0, 1.5, 2.0 mg/mL	proliferation and migration of RA-FLS↓	
			HUVECs	0.5, 1.0, 1.5, 2.0 mg/mL	proliferation and lumen formation of HUVECs↓	
Flavonoids	Morin	Morus alba L	CIA rats	20, 40, 80 mg/kg	TNF-a, IL-6↓; IL-10↑; CD31, VEGF, bFGF↓	Yue et al. (2018)
			HUVECs	1, 3, 10, 30, 100 mol/L	VEGF induced migration and tube formation↓	
	Morin	Morus alba L./Otostegia persica	CIA rats	80 mg/kg	PTEN↑; PPARγ↓	Zeng et al. (2015)
			HUVECs	10 μM	migration and tube formation↓; PTEN↑, PPARγ↓; PI3K/Akt↓	
	nobiletin	citrus fruits	CIA rats	100, 400 mg/kg	IL-1β, MCP-1, IL-6, TNF-α↓; p38/NF-κB↓	Yang et al. (2017)
	Gambogic acid	Garcinia maingayi gambogic tree	AIA rats	1, 5, 10 mg/kg	IL-1β, IL-6↓; p-Akt, mTOR↑; HIF-1α, VEGF↓	Wu et al. (2017)
	Liquiritin	Glycyrrhiza uralensis	CIA rats	8 mg/kg	IL-1β, TNF-α, IL-6↓; synoviocyte apoptosis↑; VEGF↓	Zhai et al. (2019)
			RA-FLS	0.345, 3.45, 34.5 μmol/L	proliferation↓; nuclear DNA fragmentation↑; cell apoptosis↑; Bcl-2/Bax↓; VEGF, p-JNK, P38↓	
	Genistein	Euchresta japonica Benth. Ex Oliv./Sophora japonica L	CIA mice	5 mg/kg	IL-1β, IL-6, TNF-α↓; VEGF↓	Hu et al. (2016)
	apigenin	parsley/celery/celeriac/chamomile tea	CIA mice	20 mg/kg	VEGF, VEGFR↓	Li et al. (2019b)
Iridoid	Geniposide	Gardenia jasminoides Ellis	AIA mice	60 mg/kg	CD31, VEGF, p-VEGFR2, SphK1, p-SphK1, S1PR1↓	Wang et al. (2022a)
			HUVECs	25, 50, 100 μM	VEGFR2, p-VEGFR2, p-PKC, ERK1/2, p-ERK1/2, SphK1, p-SphK1, S1PR1↓	
	Geniposide	Gardenia jasminoides Ellis	AIA rats	60 mg/kg	vimentin, CD31, VEGF, VEGFR2, p-VEGFR2, Erk1/2, p-Erk1/2, SphK1, p-SphK1, S1PR1↓	Wang et al. (2021c)
			FLS/VEC	25, 50, and 100 μM	proliferation, migration, tube formation, S1P secretion↓	
	Geniposide	Gardenia jasminoides Ellis	AIA rats	30, 60 and 120 mg/kg	Dnmt1-mediated PTEN gene hypermethylation↓	Bu et al. (2022a)
			HUVECs	25, 50, 100 μM	DNA methylation, Dnmt1↓	

(Continued on following page)

TABLE 1 (*Continued*) Effects of compounds on angiogenesis in rheumatoid arthritis.

Categories	Natural product	Plants source	Experiment models	Effective dose	Mechanism	Ref.
	Geniposide	Gardenia jasminoides Ellis	AIA rats	30, 60 and 120 mg/kg	PTEN↑; p-PI3K, p-Akt↓	Bu et al. (2022b)
			HUVECs	25, 50, 100 μM	proliferation, migration, and tubule formation↓	
	geniposide	Gardenia jasminoides Ellis	AIA rats	30, 60, 120 mg/kg	CD31, VEGF, Ang-1↓; ES↑	Sun et al. (2020)
			FLSs	5, 10, 20 μM	SphK1, S1PR1, VEGF↓	
	Geniposide	Gardenia jasminoides Ellis	AIA	30, 60, 120 mg/kg	CD31, SphK1, p-Erk1/2↓	Deng et al. (2021)
			FLSs/VECs	25,50,100 μM	SphK1, p-Erk1/2, S1P↓	
Monoterpene	Paeoniflorin-6′-O-benzene sulfonate	Paeonia lactiflora Pall	AIA rats	50 mg/kg	CXCL12, CXCR4↓	Zhang et al. (2019)
			HUVECs	1×10^{-9}, 1×10^{-8}, 1×10^{-7}, 1×10^{-6}, 1×10^{-5} mol/L	GRK2↓	
Sesquiterpene	artesunate	Artemisia annua L	RA FLS	0.5, 1.0, 5.0, 10, 20 μM	VEGF, IL-8↓; HIF-1α↓	He et al. (2011)
Diterpene	Triptolide	Tripterygium Wilfordii Hook f	CIA rats	11, 22, 45 mg/kg	TNF-α, IL-17, VEGF, VEGFR, Ang-1, Ang-2, Tie2↓, ERK, p38, JNK↓	Kong et al. (2013)
			HFLS–RA cells	1, 10 and 50 ng/mL	tube formation↓, chemotactic migration↓	
			HUVEC	1, 10 and 50 ng/mL	chemotactic migration↓	
Triterpene	Pristimerin	Celastrus aculeatus Merr	AIA rats	0.40,0.8 mg/kg	vessel density↓; TNF-α, Ang-1, MMP-9↓; VEGF, p-VEGFR2↓	Deng et al. (2015)
			Rat aortic ring assay	0.5, 1.0, 2.0 μM	sprouting vessels of the aortic ring↓	
			HFLS-RA	0.50, 0.75, 1.0 μM	migration↓	
			HUVECs	0.125, 0.25, 0.50 μM	VEGF-induced proliferation, migration and tube formation↓, VEGF-induced VEGFR2↓; PI3K, AKT, mTOR, ERK1/2, JNK, and p38↓	
	β-Sitosterol	many kinds of plants	CIA mice	100 mg/kg	VEGFR2, p-VEGFR2↓	Qian et al. (2021)
			HUVECs	10 and 20 μM	proliferation and migration of HUVECs↓	
Polyphenol	Chebulinic acid	Fructus Chebulae	CIA mice/DBA/1J	50 mg/kg	CD31, VEGF↓	Lu et al. (2020)
			HSMECs	1.7 μM	Erk1/2, p38 MAPK, AKT↓	
	Resveratrol	Polygonum cuspidatum	CIA rats	200, 400 mg/kg	IL-1β, MCP-1, IL-6, TNF-α, ROS↓	Yang et al. (2018)
			RSC-364	25, 50 μmol/L	HIF-1α↓; p38↓; c-Jun↓	
Anthraquinones	Emodin	Rheum palmatum L	synoviocytes	0.01, 0.1, 1, 10, 100 mM	TNF-a, IL-6, IL-8, PGE2, MMP-1, MMP-13, VEGF, COX-2, HIF-1a, HDAC1↓	Ha et al. (2011)
Coumarin	Scopoletin	Erycibe obtusifolia Benth	AIA rats	50, 100 mg/kg	blood vessel formation↓; VEGF, bFGF, IL-6↓	Pan et al. (2010)
	Scopolin	Erycibe obtusifolia Benth	AIA rats	25, 50, 100 mg/kg	IL-6, VEGF, FGF-2↓	Pan et al. (2009)
Mineral drug	arsenic trioxide	—	CIA mice	1.0, 2.0, 5.0 mg/kg	TSP-1, TGF-β1, CTGF, VEGF↓	Zhang et al. (2017)
			RA-FLS	0.5, 1, 2 μM		

↑, increase, upregulate, promote or improve; ↓, suppress, downregulate, reduce, or inhibit.

3 The major mediator and signaling pathways that regulates angiogenesis

Angiogenesis is mainly regulated by an appropriate ratio of promoting and inhibitory forces (Eelen et al., 2020). Once this balance is broken, it will activate the angiogenesis into new vessels or suppress the vasculature angiosclerosis. Synovial neovascularization can be aided by a variety of mediators, such as growth factors, cytokines, chemokines and so on. Conversely, synovial angiogenesis is hampered by endogenous vascular

TABLE 2 Effects of natural medicine extracts in rheumatiod arthritis.

Phytochemicals	Animal/ Strains	Dose	Effects and molecular mechanisms	Ref.
Anemone ffaccida Fr. Schmidt	CIA rats	200, 400 mg/kg	CD31, VEGF↓	Rao et al. (2023)
	HUVECs	5, 7.5, 10, 15, 20, 30 μg/mL	VEGFR, p-PI3K, p-AKT, p-mTOR↓	
	HFLS-RA	2.5, 5, 10, 20, 40, 80 μg/mL	Proliferation, migration↓	
Davallia bilabiata	chicken embryos	0.1, 0.5 mg/mL	MMP-2, MMP-14↓	Liu et al. (2017)
	HUVECs	0.25, 0.5 mg/mL/	VEGF-A, VEGF-B, VEGF-C, VEGF-D↓; VEGFR-1, VEGFR-2, VEGFR-3↓	
Cissus quadrangularis	AIA rats	50, 100, 200 mg/kg	TNF-α, IL-1β, IL-6, TNF-R1, VEGF, MMP-3, MMP-9↓	Kumar et al. (2015)
total Saponins of *Panax japonicus*	CIA mice	50, 150 mg/kg	HIF-1α, VEGF↓; SRC, STAT 3↓	Guo et al. (2020)
Dendrobium huoshanense stem polysaccharide	CIA mice	0.1095, 0.4380 g/kg	CD90↓; CD31↓; RANKL↓; OPG↑; VEGF, IL-17, IL-1β, IL-6, TNF-α, MMPs, GM-CSF, M-CSF, CCL5, CXCL2↓; IL-10, TGF-β1↑; NF-κB, MAPKs, PI3K/AKT, JAK1/STAT3↓; Treg cell↑; Th17 cell↓	Shang et al. (2021)
evening primrose oil	AIA rats	5 g/kg	Ang-1, TNF-a↓; SOD↓; lipid peroxidation↑	El-Sayed et al. (2014)
Rhus verniciflua Stokes	CIA mice	50 mg/kg	synovial inflammatory cells↓	Lee et al. (2009)
	FLS	0–1,000 μg/mL	TNF-α, IL-6, IL-8, MCP-1, VEGF↓; ERK 1/2, p-JNK, p38 MAPK↓	
saponins from *Nigella glandulifera* seeds	CIA rats	10, 50, 250 mg/kg	IFN-γ, TNF-α, IL-1β, IL-6, IL-17A↓; IL-4, IL-10↑; $CD4^+CD25^+$ Tregs↑; Foxp3↑; OPG/RANKL ratio↑; p-p65↑	Jiang et al. (2022)
	HFLS-RAs	10, 30, 100 μg/mL	proliferation, migration, adhesion↓; f TNF-α, IL-17A, Ang-2, Tie-2↓	
	HUVECs	10, 30, 100 μg/mL	proliferation, migration, adhesion↓; Ang-2, Tie-2↓	
total saponins of Rhizoma *Dioscorea nipponica*	CIA rats	25 mg/kg	VEGF, Ang-2, Tie-2↓; MVD, VEGF, STAT3, NF-κB↓	Liang et al. (2016)

↑, increase, upregulate, promote or improve; ↓, suppress, downregulate, reduce, or inhibit.

inhibitors, including angiostatin, interleukin-4 (IL-4), interleukin-13 (IL-13) and interferon. Among them, VEGF, HIF-1, Ang-1, Ang-2 are recognized as most important angiogenic factors that affect synovial angiogenesis (Szekanecz et al., 2005; Maruotti et al., 2006).

3.1 VEGF

VEGF is a homologous dimeric glycoprotein which involved in regulating neovascularization. The VEGF family is a group of angiogenic cytokines that comprises placental growth factor (PLGF), VEGF-A→VEGF-F (Costache et al., 2015; Hamilton et al., 2016). The importance of VEGF-A (commonly referred to as VEGF) in the regulation of angiogenesis will be the primary topic of this review (Apte et al., 2019). VEGF experience alternative exon splicing, resulting in various isoforms. Among them, VEGF165 is the most widely expressed isoform in tissues (Woolard et al., 2009). VEGF has two high-affinity receptors, VEGFR-1 and VEGFR-2, which are predominantly distributed on ECs, and a small amount of other cells such as hematopoietic stem cells and monocytes are also expressed, but it is currently believed that only ECs have a response to VEGF (Melincovici et al., 2018). Studies have shown that VEGF specifically acts on vascular ECs and can affect their proliferation, differentiation and other functions. The VEGF-dependent signaling pathway plays a central part in angiogenesis during RA pathophysiology (Liang et al., 2014). Under normal conditions, vascular ECs renewal is slow and VEGF expression is low. During tissue injury and inflammation, hypoxia, platelet-derived growth factor (PDGF), fibroblast growth factors (FGFs), etc., Can increase VEGF by 3–20 times (Melincovici et al., 2018). VEGF signaling modulates the activation of various kinases during vasculogenesis and angiogenesis, which in turn controls cell division, movement, longevity, and permeability of the vessel (Ahmad and Nawaz, 2022).

3.2 Ang

Ang serves as one of the growth factors requested for the initial angiogenesis. At the present time, the Ang family is the sole known pro-angiogenic factor having both promoting and inhibitory actions, and it consists four members, Ang-1→Ang-4, which all share the EC tyrosine kinase receptor Tie-2 (Saharinen et al., 2017). Ang-1 and Ang-2 are two key angiogenin regulators involved in vascular development, endothelial eruption, vascular wall remodeling and parietal cell recruitment. According to research,

TABLE 3 Effects of natural medicine prescriptions in rheumatoid arthritis.

Prescription	Composition	Models	Dose	Effects and molecular mechanisms	Toxicity	Clinical dosage	Ref.
YuXueBi tablet	*Boswellia carteri* Birdw., *Commiphora myrrha* (Nees) Engl., *Clematis chinensis* Osbeck, *Cyathula officinalis* Kuan, *Curcuma longa* L., *Carthamus tinctorius* L., *Salvia miltiorrhiza* Bunge, *Cyperus rotundus* L., *Ligusticum chuanxiong* Hort, *Astragalus membranaceus* (Fisch.) Bunge, and *Angelica sinensis* (Oliv.) Diels	CIA rats	0.1, 0.2, 0.4 g/kg	CD31, VEGF↓; LOX, Ras, Raf-1, p-MEK p-ERK↓	eye edema, Sore throat	2.5 g p.o tid	Su et al. (2022)
		HUVECs	12.5, 25, 50, 100 μg/mL	migration, invasion, tube formation↓; LOX, Ras, Raf-1, p-MEK, MEK, p-ERK, MEK↓			
Huatan Tongluo decoction	Dannanxing (*Rhizoma Arisaematis Cum Bile*), Taoren (*Semen Persicae*), Jiangcan (*Bombyx Batryticatus*), Baijiezi (*Semen Sinapis*) and Shancigu (Pseudobulbus Cremastrae)	CIA rats	7.5 g/kg	CD34, VEGF, VEGFR2↓	gastrointestinal discomfort, dizziness	Potion p.o bid	Chen et al. (2019a)
Shexiang Zhuifeng analgesic plaster	Artificial Musk, *Aconitum kusnezoffii* Reichb., *Aconitum carmichaeli* Debeaux, *Boswellia carterii* Birdw., *Commiphora myrrha* (T.Nees) Engl., *Strychnos nux*-vomica L., *Eugenia caryophyllata* Thunb., *Cinnamomum cassia* (L.) J.Presl, *Schizonepeta tenuifolia* (Benth.) Briq., *Saposhnikovia divaricate* (Trucz.) Schischk., *Geranium wilfordii* Maxim., *Periploca sepium* Bunge, *Centella asiatica* (L.) Urban, *Drynaria fortunei* (Kunze ex Mett.) J.Sm., *Angelica dahurica* (Hoffm.) Benth. and Hook.f. Ex Franch. and Sav., *Kaempferia galanga* L., *Zingiber officinale* Roscoe, Camphor, Borneol, Menthol, Methyl salicylate, *Liquidambar formosana* Hance, *Atropa belladonna* L	CIA rats	0.63, 2.52 cm^2	IL6, VEGF, TNF-α↓; HIF-1, p-AKT, p-mTOR↓	rash, pruritus, anaphylactic shock	External use 1 patch qd	Wang et al. (2023)
		RAW 264.7 cells	12.5, 25, 50 μM	NO↓			
Kunxian Capsule	*Tripterygium wilfordii* Hook. F. (*Tripterygium hypoglaucum* (H. Lév.) Hutch), *Epimedium brevicornu* Maxim, *Lycium barbarum* L. and *Cuscuta chinensis* Lam	Zebra fish	3.5, 7, 14, 21 μg/mL	PI3K, AKT, MAPK, ERK1, ERK2, VEGF2, VEGFR, FGF-2↓	gastrointestinal discomfort, abnormal liver function, leukopenia, paramenia, oligospermia	0.6 g p.o tid	Ma et al. (2023)
Sidaxue	*Spatholobus suberectus* Dunn, *Sargentodoxa cuneata* (Oliv.) Rehd. Et Wils., *Toddalia asiatica* L. Lam., *Periploca forrestii* Schltr	CIA rats	10, 20, 40 g/kg	IL-2, IL-6, TNF-α, VEGF, PI3K, AKT, p-AKT, NF-κBp65, p-NF-κBp65, STAT1, PTGS2↓	-	-	Wu et al. (2022)
Qianghuo Shengshi Decoction	*Ramulus Cinnamomi*, *Epimedium brevicornum* Maxim, Rhizoma Wenyujin Concisum, *Poria cocos*, *Scutellaria baicalensi*	AIA rats		IL-6, MMP-9, TNF-α, VEGFA↓	gastrointestinal discomfort, abnormal liver function, rash, infect, subcutaneous hemorrhage	Potion p.o bid	Zeng et al. (2021b)
Fengshi Gutong capsule	*Aconiti Radix Cocta*, Aconiti Kusnezoffii Radix Cocta, *Carthami Flos*, *Chaenomelis fructus*, *Mume Fructus*, Ephedrae Herba, *Glycyrrhizae Radix* et Rhizoma	CIA rats	300, 900 mg/kg	RF, VEGF, TNF-α, IL-6↓; ICAM-1, IL-1β, p-Akt↓	gastrointestinal discomfort, renal damage	0.6–1.2 g p.o bid	Lin et al. (2021)
		RAW264.7	Different concentration	IL-1β, iNOS↓			

(Continued on following page)

TABLE 3 (*Continued*) Effects of natural medicine prescriptions in rheumatoid arthritis.

Prescription	Composition	Models	Dose	Effects and molecular mechanisms	Toxicity	Clinical dosage	Ref.
		HUVEC	Different concentration	migration↓			
Qing-Luo-Yin Extract	*Sophora flavescens* Ait., *Phellodendron amurense* Rupr., *Sinomenium acutum* Rehd. Et Wils. And *Dioscorea hypoglauca* Palib	CIA rats	0.3 g/kg	MMP-3↓, TIMP-1↑	-	Potion p.o bid	Li et al. (2003)
Wen Luo Yin	Radix Aconiti Lateralis Preparata, Cinnamomi Ramulus, Atractylodis Macrocephalae Rhizoma, Selaginellae Herba	CIA rats	3.45, 6.9, 13.8 g/kg	TNF-α, IL-1β, VEGF↓	-	Potion p.o tid	Liu et al. (2013)
		HFLS-RA	8, 16, 32 mg/mL	migration, adhesion↓; TNF-α, IL-17, VEGF, Ang-1, Ang-2↓			
		HUVEC	8, 16, 32 mg/mL	migration, adhesion↓; VEGFR, Ang-2↓			
Wutou Decoction	*Aconitum carmichaeli* Debeaux., *Ephedra sinica* Stapf, *Paeonia lactiflora* Pall., *Astragalus mongholicus* Bunge, *Glycyrrhiza uralensis* Fisch. ex DC.	CIA rats	3.75, 7.5 g/kg/day	CD31, VEGF, Ang-1,VEGFR2, TEK, PI3K, AKT, mTOR, HIF-1α↓	gastrointestinal discomfort	Potion p.o tid	Ba et al. (2021)
		FLS	1, 10 mg/mL	proliferation, migration, invasion↓; apoptosis↑; VEGF, Ang-1↓			
		HUVEC	1, 10 mg/mL	proliferation, migration, invasion, tube formation↓			
Wutou decoction	*Aconitum carmichaeli* Debeaux., *Ephedra sinica* Stapf, *Paeonia lactiflora* Pall., *Astragalus mongholicus* Bunge, *Glycyrrhiza uralensis* Fisch. ex DC.	CIA rats	0.95, 1.9, 3.8 g/kg	CD31↓; VEGF, VEGFR2, IL-1β, IL-17, TGF-β, PDGF, PIGF, Ang I, Ang II↓	gastrointestinal discomfort	Potion p.o tid	He et al. (2018)
		FLS	0.2, 1, 5 μg/mL	IL-1β, IL-17, VEGF, TGF-β, PDGF, PIGF, Ang I, Ang II↓			
		HUVECs	0.2, 1, 5 μg/mL	migration, invasion, tube formation↓; VEGFR2↓; AKT, ERK1/2, JNK, p38↓			

↑, increase, upregulate, promote or improve; ↓, suppress, downregulate, reduce, or inhibit.

the Ang/Tie2 signaling pathway is directly associated to the development of blood vessels in pathological diseases such as RA and tumor (Fagiani and Christofori, 2013). There is 60% similarity between Ang1 and Ang2, in addition, they all have high binding affinity with Tie2. Ang-1 adheres to and phosphates the Tie2 receptor, which stimulates it and facilitates the survival of cells and vascular integrity. Ang-1/Tie2 signalling also inhibits NF-κB pathway thus decreasing the inflammatory factor cascade and maintaining vascular stability (Mitola et al., 2008). Overall, the Ang1/Tie2 signaling pathway builds vascular stability and quiescence under physical environments (Fukuhara et al., 2010). In pathological conditions, Ang-2 expression is increased, which pushes the emergence of RA's clinical hallmarks. As natural antagonist of Ang-1, Ang-2 is employed to cause EC instability and prevent Ang-1-induced Tie2 phosphorylation. In fact, the Ang/Tie signal pathway and the VEGF/VEGFR signaling pathway are related to each other and coordinate with each other to regulate the processes of vascular growth, maturation and degeneration (Akwii et al., 2019).

3.3 HIF-1

Hypoxia predominantly modulates VEGF expression by means of HIF, a heterodimeric transcription factor with α (HIF-1α) and β (HIF-1β) subunits (Hamilton et al., 2016; Yang et al., 2021). Under proper condition of oxygen, HIF-1α expression is limited. Hypoxia causes HIF-1α to aggregate and bond to HIF-1β, generating a stable active dimer HIF-1 (Balamurugan, 2016). HIF-1 encourages synovial angiogenesis by prompting synoviocytes to release VEGF, which influences the progression of RA. Additionally, synovial angiogenesis boosted HIF-1α and VEGF amounts of expression in synovial tissues (Westra et al., 2010; Konisti et al., 2012).

3.4 Growth factors

Growth factors including FGF, PDGF, transforming growth factor-β (TGF-β), insulin-like growth factor 1 (IGF-I), hepatocyte growth factor (HGF) and epidermal growth factor (EGF) also

TABLE 4 Animal model for rheumatoid arthritis commonly used.

	name	Molding method	Suitable species	Molding time	Position
induced model	collagen-induced arthritis (CIA)	CII + CFA	rat, mice, monkey	21–25 days	base of the tail, dorsal skin
	collagen-antibody-induced arthritis (CAIA)	CII + LPS	mice	7 days	base of the tail
	adjuvant-induced arthritis (AIA)	CFA + tubercle *bacillus*	rat	10–14 days	base of the tail, hind paw region
	streptococcal cell wall induced arthritis (SCWIA)	PG-PS	rat, mice	3 days	intra-articular
	COMP induced arthritis	IFA + COMP	rat, mice	45 days	base of the tail
	pristane induced arthritis (PIA)	2,6,10,14-tetramethylpentodecane	rat, mice	2 months	intradermal injection
	antigen induced arthritis	mBSA + CFA	mice, rats, guinea pigs, rabbits	6 days	subcutaneous or intradermal injections, intra-articular
	proteoglycan induced arthritis (PGIA)	Proteoglycan + DDA	rabbit, dogs, mice	35 days	intra-articular
	G6PI induced arthritis	G6PI + CFA	mice	15 days	base of the tail
genetic models	K/BxN mice	-	mice	10–14 days	-
	TNF-α mice	-	mice	3-4 w	-
	SKG mice	-	mice	14 days	-
	IL-1ra−/− transgenic mice	-	mice	5 w	-

promote angiogenesis (Szekanecz and Koch, 2009). FGF is secreted by ECs in the pannus and includes alkaline FGF and acidic FGF. The former (bFGF) can stimulate microvascular ECs to secrete metalloproteinases and destroy vascular endothelial matrix. aFGF not only produces collagenase and plasminogen, which degrades ECs' basement membranes, but it also has a potent mitogenic impact and directly induces the proliferation of vascular ECs. (Malemud, 2007). PDGF and TGF-β both helps blood vessels function properly (Carmeliet and Jain, 2011). PDGF is needed for the recruitment of pericytes to newly established vessels. During angiogenesis, sprouting ECs secrete PDGF which stimulates proliferation and migration of mural cells during vessel maturation (Armulik et al., 2005). The reinduction of vascular smooth muscle cells around neovasculature is dependent on TGF-β, which is required for the growth and function of the vasculature (Armulik et al., 2005).

3.5 Pro-inflammatory cytokines

Cytokines leave their mark throughout the whole course of RA' biological development (Kondo et al., 2021). Pro-inflammatory cytokines including TNF-α, IL-1, IL-6, IL-15, IL-17, IL-18 promote synovial vessel formation (Szekanecz and Koch, 2009). Studies on the effects of TNF-α and IL-6 on angiogenesis are the most in-depth and extensive (Kondo et al., 2021). Tumor necrosis factor (TNF-α) may stimulate a multitude of immune cells in the RA cartilage tissues to produce pro-angiogenic factors, which in consequently results in persistent inflammation of the synovial tissue during the progression of the disease (Chen Z. et al., 2019; Kondo et al., 2021). IL-6 is produced by monocytes and macrophages of RA synovium, which can activate vascular ECs(Zegeye et al., 2020). It not only boosts pannus formation but also aggravates bone resorption (Pandolfi et al., 2020). Anti-inflammatory cytokines, such as interferon-α (IFN-α), IFN-γ, IL-4, IL-12, IL-13 and leukemia inhibitory factor (LIF) are mediators that inhibit angiogenesis (Szekanecz and Koch, 2009).

3.6 Others

Angiogenesis regulation is a complex process. Except the above-mentioned factors, there are also chemokines/receptors, extracellular adhesion molecules and proteolytic enzyme possess a contribution to make in the controlling of synovial angiogenesis (Maruotti et al., 2006). Matrix metalloproteinases (MMPs) and plasminogen activators participate in the dissolution of synovial cartilage during the maintenance of angiogenesis. Other factors that both encourage and inhibit angiogenesis that are mentioned have been reviewed in the literature (Paleolog, 2002; Balogh et al., 2019). Plenty of studies on RA have proved that extracellular adhesion molecules play a critical part in leukocyte migration into synovial tissue (Volin, 2005).

3.7 Signaling pathways

In addition, various additional signaling pathways are also associated in angiogenesis. MAPK, NF-κB, JAK-STAT, PI3K, and PPARγ signaling pathways have been extensively studied for their role in inducing angiogenesis. The PPARγ signaling pathway is

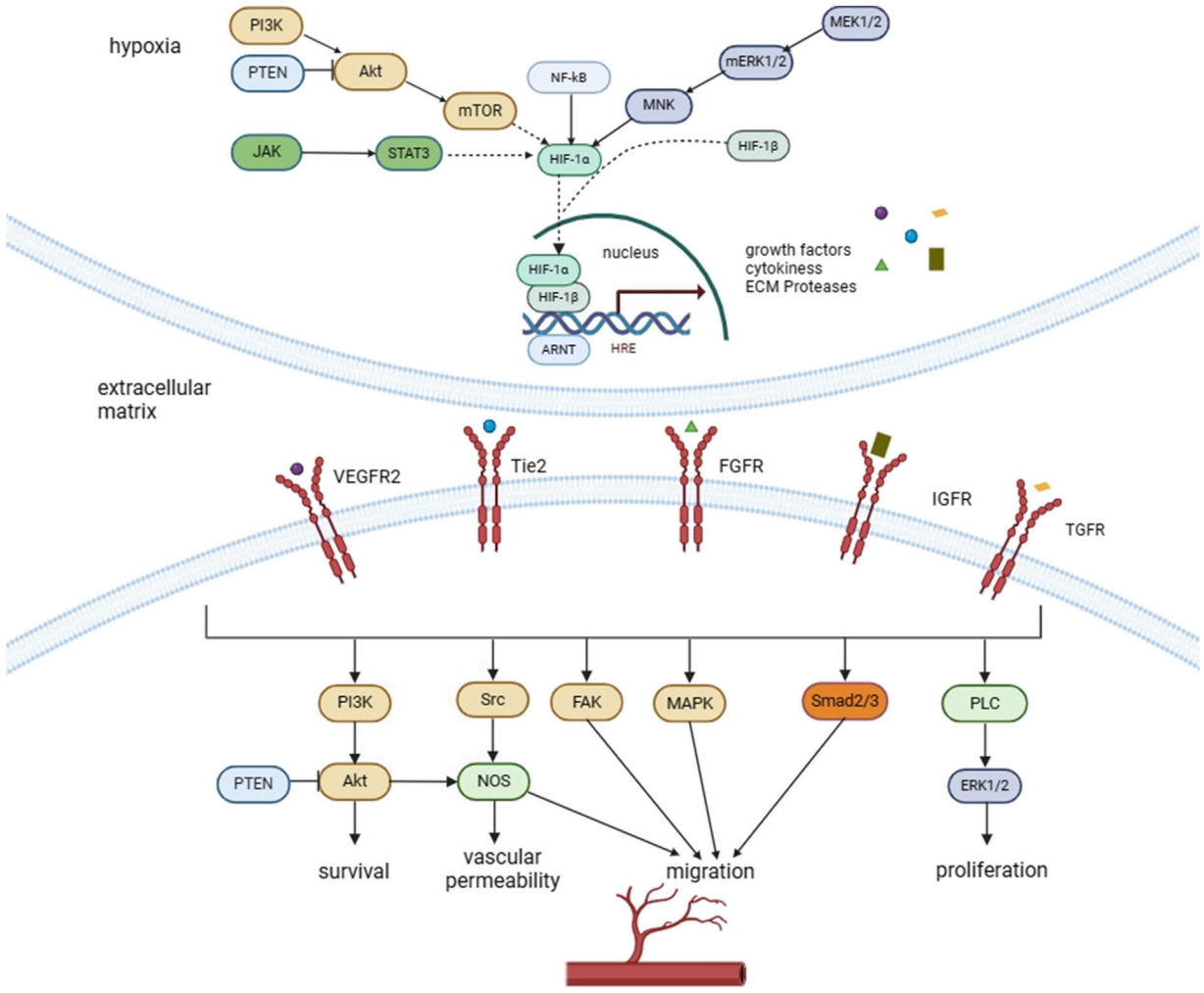

FIGURE 1
Major signaling pathways that affect angiogenesis.

capable of promoting angiogenesis by enhancing VEGF expression. Signaling pathways such as MAPK, NF-κB, and JAK/STAT3 may indirectly boost angiogenesis. MAPK mediates various cellular physiological processes and abnormal activation of MAPK signaling pathway can lead to RA. MAPK signaling pathway is a tertiary enzyme linked system with four activation pathways: ERK1/2, JNK/SAPK, p38 and ERK5. In the pathological process of RA, MAPKs not only regulate the production of pro-inflammatory cytokines and matrix protein degrading enzyme, but also play an important role in the downstream signaling cascade of cytokine receptors (Liu et al., 2021). MAPK is the up-stream signal of the NF-κB pathway and is regarded as an intersection of various signalling pathways (Yeung et al., 2018). NF-κB is an important transcription factor in the cytoplasm. The expression of a large number of genes in immune and inflammatory responses is regulated by NF-κB. The role of NF-κB in the pathogenesis of RA has attracted the attention of researches. Activation of NF-κB signaling pathway can lead to T cell activation to induce the production of various pro-inflammatory cytokines. It can also induce abnormal proliferation of RA-FLS to stimulate osteoclast proliferation and activation, which results in joint deformity and bone erosion (Liu et al., 2021). After activation of JAK-STAT pathway, it can activate the activity of immune cells, especially T cells, and inhibit FLS autophagy, resulting in continuous proliferation of RA synovium and cartilage erosion (Di Benedetto et al., 2021). PI3K pathways have a key role in angiogenesis which involved in stimulating HIF(Zeng et al., 2016). These multiple pathways interact and collaborate to control diverse angiogenic activities in vascular cells. Animal model for rheumatoid arthritis commonly used are shown in Table 4.

4 Natural medicine targeting angiogenesis to prevent and treat RA

4.1 Natural active ingredients

Natural medicines contain abundant anti-angiogenic active ingredients, including alkaloids, flavonoids, terpenoids, polyphenol and so on. Figure 3 illustrates the chemical structures

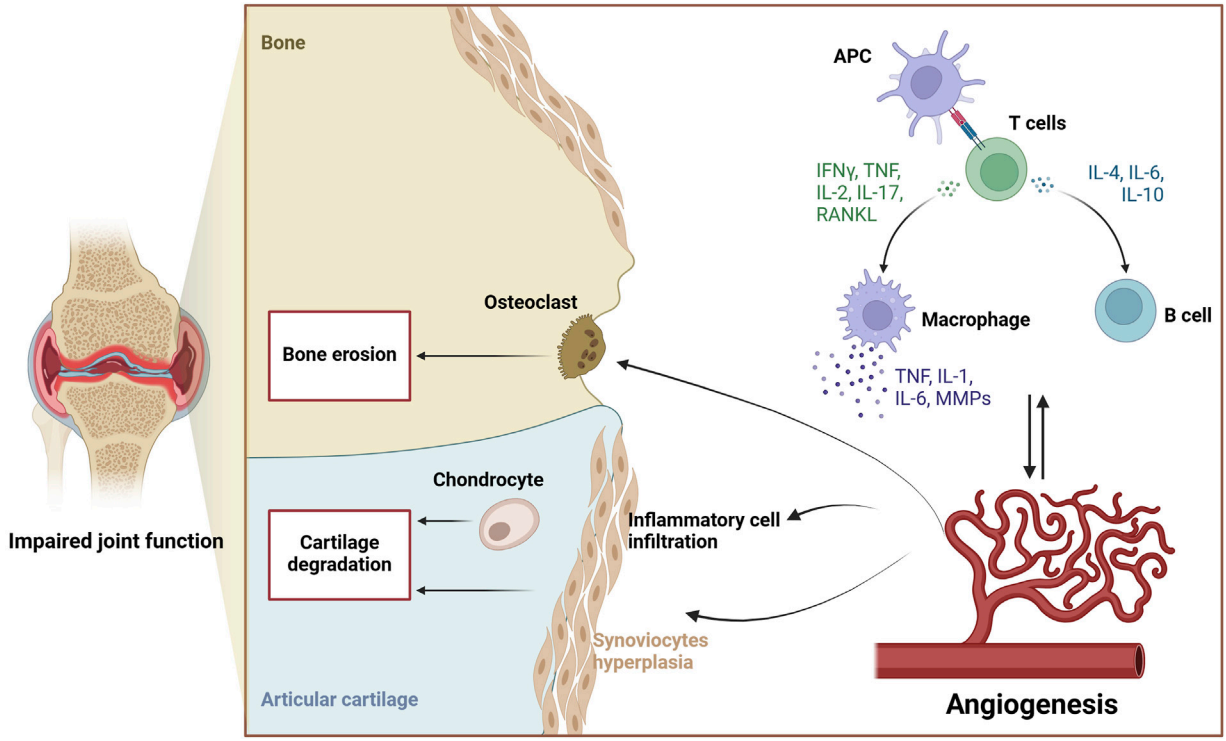

FIGURE 2
The role of angiogenesis in the pathological process of rheumatoid arthritis.

of the active components obtained from natural medicines. Table 1 summarizes the models and mechanisms of all these components against rheumatoid arthritis.

4.1.1 Alkaloids

Alkaloids occur naturally as organic molecules with no less than one basic nitrogen atom embedded in their ring structure. Alkaloids can be divided into organic amines, pyrrolidines, pyridines, isoquinolines, indoles, scopolanes, imidazoles, quinazolines, purines, steroids, terpenes and others according to their structure (Bhambhani et al., 2021). Alkaloids are employed in the clinical treatment of a number of illnesses. For example, berberine is used to treat enteritis that achieve significant benefits (Cao et al., 2023). Alkaloids are found in numerous natural medicines, including *tricuspidus*, *aconitum*, *ephedra*, *corydalis*, and *tetrandrhexis*. They are also extensively distributed in nature (Bhambhani et al., 2021). Many alkaloids have therapeutic effects on experimental arthritis (Lu et al., 2022), among which sinomenine has been used in clinic.

Sinomenine, the main component of *Sinomenium acutum*, has been traditionally utilized as a natural medicine for RA. Sinomenine has been proven in modern pharmacological research to be capable of remarkable anti-inflammatory, pain reliever, and immunosuppressive effects (Li J. M. et al., 2023). In collagen-induced arthritis (CIA) mice, sinomenine can significantly reduce the swelling, erythema expansion, arthritis index, cartilage deterioration, bone erosion and the quantity of CD31-positive cells in the synovium. In addition, sinomenine can drastically lower the levels of HIF-1α, VEGF and Ang-1 in the peripheral blood. The HIF-1α-VEGF-Ang-1 axis is considered to be essential for the inhibitory effect of sinomenine on angiogenesis (Feng et al., 2019). Besides sinomenine, other alkaloids also provide inhibitory effects on RA angiogenesis as well.

Berberine, a quaternary ammonium alkaloid, as an active ingredient in various therapeutic materials (such as *Hydrastis canadensis*, *Cortex phellodendri*), has a number of pharmacological actions which include antimicrobial and anti-diarrhea (Song et al., 2020). Wang et al. found that berberine considerably reduced the hyperplasia of synovial tissue and inflammatory responses in CIA rats, while also suppressing cytokines and VEGF in the plasma. Subsequent investigations into beberine's mechanism showed that it inhibited the activation of phospho-extracellular signal-regulated kinase (p-ERK), phosphorylated p38 mitogen-activated protein kinase (p-P38), and phosphorylated c-Jun N-terminal kinase (p-JNK). Additionally, it markedly decreased the generation of VEGF and CD34 ($p < 0.05$). These discoveries indicate that berberine has both anti-inflammatory and anti-angiogenic potential, meaning that it may have an important therapeutically value in RA (Wang et al., 2014). Matrine is a prominent bioactivated alkaloid gained from *Sophora flavescens* Alt, and extensive research have corroborated its wide-ranging biological activities, including anticancer, anti-inflammatory and antiviral (Sun et al., 2022). In cellular experiments, matrine suppressed the migration and proliferation of fibroblast-like synoviocytes (FLS). Additionally, it decreased the growth and lumen formation of human umbilical vein endothelial cells (HUVECs). *In vivo*, matrine has the ability to suppress the expression of several proteins, including IL-1β, interferon gamma (IFN-γ), VEGF, PLGF, HIF-α, Ang-1, Ang-2, Tie-2, and

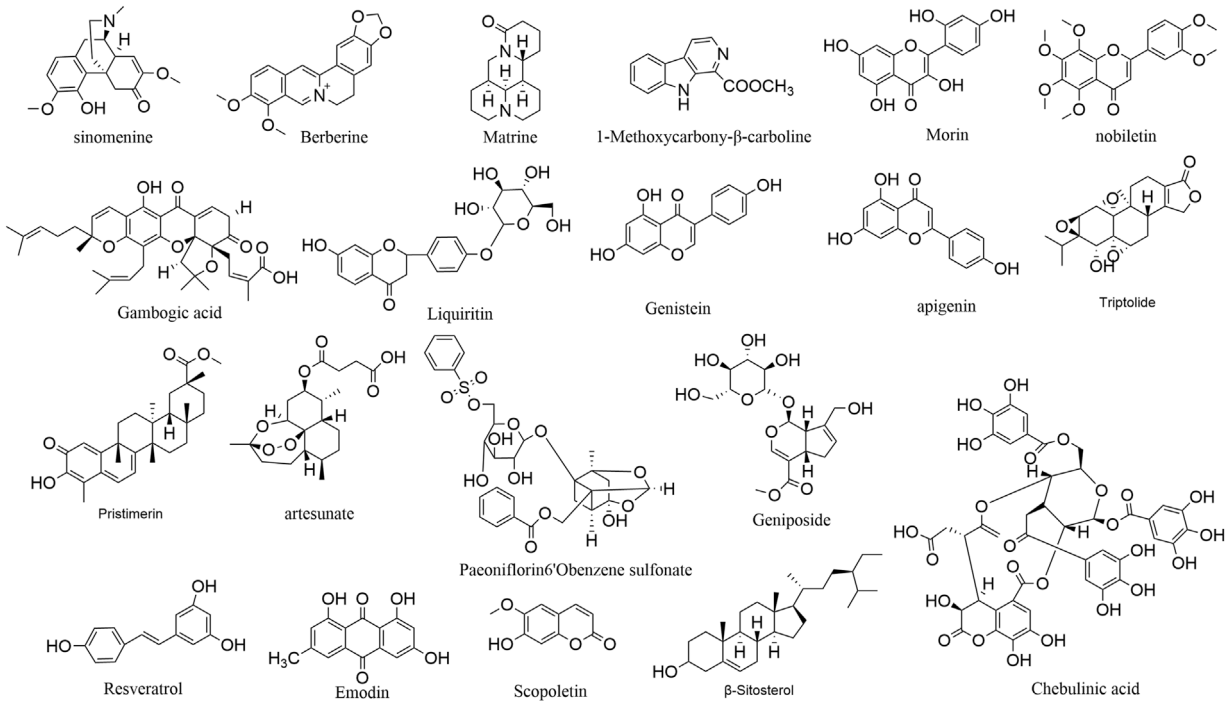

FIGURE 3
The chemical structures of compounds in natural medicine with antiangiogenesis effects.

phosphorylated -akt in ankle of CIA rats. Further investigation of the mechanism revealed that matrine inhibits angiogenesis by suppressing the PI3K/Akt signalling pathway and the HIF-VEGF-Ang axis, thereby alleviating RA symptoms (Ao et al., 2022). *Picrasma quassioides* is a natural medicine, using dried branches and leaves, rich in alkaloids, triterpenoids. *Picrasma quassioides* has anti-tumor, anti-inflammatory, antiviral and antihypertensive activities, according to modern pharmacological investigations (Lee et al., 2019). Several alkaloids with anti-angiogenic activity were discovered by zebrafish bioassay-guided screening. Following screening, 1-Methoxycarbony-β-carboline (MCC) had the highest anti-angiogenic index. Further studies confirmed that MCC inhibits HUVECs activity, migration, invasion and lumen formation, as well as downregulates several angiogenesis-ralated proteins, like Ang, epidermal growth factor (EGF), bFGF, growth-related oncogene (GRO), insulin like growth factor 1 (IGF-1), plasminogen (PLG), and matrix metallopeptidase-1 (MMP-1). In zebrafish tail fin regeneration experiments, it was observed that MCC prevented the formation of blood vessels. These findings point to MCC's potential therapeutic utility as a powerful natural angiogenesis inhibitor for RA via numerous biological targets (Lin et al., 2018).

Collectively, most alkaloids perform as antiangiogenic drugs by inhibiting angiogenic factors, lumen formation, and proliferation of RA-FLS. The two biggest obstacles to alkaloid study are their complicated structures and insufficient bioavailability. The use of modern preparation strategies and biochemical transformation techniques can address the problem of low bioavailability (Cheng et al., 2018; Valentová, 2023). Chemical synthesis provides a way to prepare the active components of natural medicines. With the application of green chemistry, biocatalysis, and computer-aided drug design, more efficiency has been gained in the synthesis of highly complex natural compound. Many difficulties, such as many chiral centers, complex synthesis steps, easily formed isomerism, and chemical agent pollution, will be solved or considerably improved by the use of the above technology (Wang et al., 2019; Chakrabarty et al., 2021; Peña et al., 2023).

4.1.2 Flavonoids

Flavonoids are a group of compounds with a C6-C3-C6 skeleton, consisting of three carbon atoms connected to two benzene rings (Dias et al., 2021). These compounds are abundant in natural medicines and are mostly bonded to sugars to the formation of glycosides. They have favorable therapeutic effects such as immune enhancement, anti-aging and antioxidant which some of them including quercetin have been applied to dietary supplements (Wen et al., 2021; Shen et al., 2022).

Morin is a kind of flavonoid that is found in a diverse range of plant sources. A series of investigations have revealed that morin has potent antioxidant and anticancer effects (Rajput et al., 2021). At the same time, multiple research have confirmed that morin has potent anti-inflammatory effects (Caselli et al., 2016). *In vitro*, Yue et al. investigated the impact of morin on HUVECs migration through wound healing and transwell experiments. Morin blocks VEGF-induced HUVEC movement and tubular formation through initiating PPARγ. Morin raised expression levels of phosphatase and tensin homolog deleted on chromosome ten (PTEN), whereas suppressing PI3K/Akt signaling. Animal experimentation have demonstrated that morin can relieve symptom of CIA rats, reduce synovial angiogenesis and upregulate synovial PTEN

expression in synovial membrane. In brief, morin, a putative PPARγ agonist, attenuates angiogenesis and joint inflammation by acting on the PPARγ-PTEN-PI3K/Akt pathway (Yue et al., 2018). Zeng et al. also used the CIA rat model and observed that morin can strongly downregulate the serum pro-inflammatory factors TNF-α and IL-6, upregulate the anti-inflammatory factor interleukin-10 (IL-10) and ameliorate the pathologic damage of the joint tissue. Morin was able to drastically reduced the production of CD31, VEGF and bFGF in the synovial tissue of CIA rats, while also decreasing the serum VEGF levels. These findings indicate that morin has anti-rheumatoid potential via inhibiting synovial angiogenesis (Zeng et al., 2015).

Nobiletin is a natural constituent widely occurring in the peel of *citrus* fruits (such as Pericarpium *Citri Reticulatae*) belonging to the rutaceaceae family (Chen Y. Y. et al., 2023). It can inhibit inflammation, regulates blood lipids and act as an antioxidant (Nakajima and Ohizumi, 2019). Yang et al. employed bovine type II collagen to establish a CIA model in SD rats. Nobiletin was able to drastically lower the levels of nuclear factor kappa-B kinase α (IκBα), phosphorylated p38, p-p65, and TNF-α in CIA rats. These results point to the fact that nobiline inhibited the p38/NF-κB signaling pathway, which in turn prevented the development of RA through decreased angiogenesis and inflammation (Yang et al., 2017). Garcinia, a resin, which has a number of different pharmacological activities such as anti-tumor, detoxification and hemostasis, and its main active ingredient is garcinic acid (Jia et al., 2015; He et al., 2023). Gambogic acid has potently inhibited right paw swelling in adjuvant-induced arthritis (AIA) rats, increased the pain threshold, decreased the clinical arthritis score, and downregulated pro-inflammatory markers. *Garcinia cambogia* also relieves RA by regulating the PI3K/Akt/mTOR signaling pathway, resulting in decreased inflammation and angiogenesis. Therefore, Gambogic acid might be used as a prospective treatment in the future medical management of RA (Wu et al., 2017). Liquiritin is a natural flavonoid isolated from licorice root, which has antioxidant, antibacterial and anti-tumor effects (Qin et al., 2022). Zhai et al. reported that liquiritin greatly suppressed the proliferation of IL-1β-induced RA-FLS, enhanced nuclear DNA fragmentation, altered mitochondrial membrane potential and accelerated cell apoptosis. Liquiritin can also downtregulate the proportion of B-cell lymphoma 2/Bcl-2-associated X protein (Bcl-2/Bax), inhibit VEGF expression and c-Jun N-terminal kinase (JNK) and P38 phosphorylation. In addition, liquiritin was able to improve the rheumatism score, inflammatory infiltrations and angiogenesis in CIA rats and induce synovial tissue apoptosis. Therefore, liquiritin may improve RA by controlling inflammation, blocking MAPK signalling and inhibiting angiogenesis (Zhai et al., 2019). Genistein, which is present in many medical herbs such as *Euchresta japonica* Benth. Ex Oliv. And *Sophora japonica* L., is an isoflavone compound that possesses a series of biological effects including anti-angiogenesis and joint protection properties (Sharifi-Rad et al., 2021). Genistein is able to reduce the expression of the pro-inflammatory factors in the serum of CIA mice, inhibit bone degradation, reduce synovial inflammation, inhibit VEGF expression, and block the angiogenesis of synovial tissue (Hu et al., 2016). Apigenin widespread in warm tropical vegetables, fruits and medicinal plants (Salehi et al., 2019). The study by Li et al. has indicated that apigenin is able to inhibit the proliferation of synovial cells *in vitro*. *In vivo*, apigenin has a very protective effect on CIA mice and can prevent angiogenesis in CIA mice by blocking VEGF and VEGFR. Furthermore, apigenin may inhibit the nuclear factor-κ b ligand receptor activator/receptor activator of nuclear factor-κ b/osteoprotegerin (RANKL/RANK/OPG) signaling pathway, which may help protect bones and joints from damage. Therefore, apigenin may influence the pathogenesis of RA by inhibiting synovial hypertrophy, angiogenesis, and osteoclastogenesis, thereby ameliorating arthritis manifestations (Li Y. et al., 2019).

In conclusion, flavonoids have significant antiangiogenic effects. At the same time, it has the advantages of low toxicity and abundant resources which make it has a strong potential for RA treatment (Hughes et al., 2017).

4.1.3 Terpenoids

Terpenoids, which are widely distributed in higher plants, fungi, microorganisms, insects, and marine creatures, are characterized as hydrocarbons and their derivatives based on the carbon structure of 2-methylbutadiene and 3-diene (also known as isoprene) (Bergman et al., 2019; Avila, 2020; Amirzakariya and Shakeri, 2022). Terpenoids are classified as monoterpenes, sesquiterpenes, diterpenes, disesquiterpenes, triterpenes, tetriterpenes, and polyterpenes based on the amount of isoprene units they possess. Terpenoids are the most abundant class of natural substances, and more than 40,000 compounds have been identified, accounting for 60% of all natural compounds. Terpenoids are rich in diversity and have multiple biological functions (Bergman et al., 2019). In the past few decades, specialists and scholars all over the world have paid increasing attention to the anti-arthritis properties of terpenoids and their mechanisms (Lü et al., 2015). The bioactive compounds of terpenoids with anti-angiogenic effects include triptolide (TP), geniposide, Paeoniflorin-6′-O-benzene sulfonate, artesunate, Pristimerin, etc.

TP is an epoxidized diterpene lactone and a major active ingredient of *Tripterygium wilfordii* Hook. f. (Gao et al., 2021). Numerous investigations have ascribed the anti-arthritis potential of TP to its immunosuppressive, anti-inflammatory, activation of apoptosis, cartilage protection and gene regulation (Yuan et al., 2019). According to Kong et al.'s research, TP was also reported to significantly inhibit the phosphorylation of extracellular signal-regulated kinase (ERK), p38 and JNK at the protein level and to downregulate the expression of angiogenic activators. In cellular assays, TP inhibited HUVEC luminal formation and chemotactic migration of RA-FLS and ECs. The above experiments provided evidence that TP may be able to inhibit angiogenesis and is an excellent candidate as a novel therapeutic drug for RA (Kong et al., 2013). Pristimerin is a naturally occurring quinonemethide triterpenoidis which contain in *Celastrus aculeatus* Merr. (Wang et al., 2022a). In past years, more and more research has been undertaken on the anti-arthritis pharmacological effect of Pristimerin (Tong et al., 2014; Lv et al., 2022). In AIA rats, Pristimerin has been shown to significantly decrease the density of synovial vessels in inflamed joints and serum pro-angiogenic factors such as matrix metallopeptidase-9 (MMP-9). Pristimerin has also been observed to decrease the expression of VEGF and its receptor in the synovium. *In vitro*, pristimerin inhibited rat aortic

ring vessel sprouting and HFLS-RA and HUVEC migrations. Pristimerin also inhibited VEGF-induced HUVEC proliferation and luminal formation and downregulated the levels of activated PI3K, AKT, mTOR, ERK1/2, JNK and p38 (Deng et al., 2015). A sesquiterpene lactone isolated from *Artemisia annua* L., artemisinin is a useful medication for the treatment of malaria (Ma et al., 2020). Artesunate is derived from artemisinin, which is used mainly as an antimalarial drug (Zhang J. et al., 2022). Artesunate may not only modulate T and B cell immunological function and limit the generation of autoantibodies, but it could also directly alter FLS and osteoclasts, inhibit synovitis and osteoclastogenesis, and therefore suppress joint inflammation and bone degradation associated with RA. As a result, artesunate may be a viable treatment option for RA in the clinic. Artesunate suppresses the production of VEGF and IL-8 as well as the nuclear expression and translocation of HIF-1α in RA-FLS, which indicates that it could inhibit angiogenesis through those pathways (He et al., 2011). Paeoniflorin, which has both an antiinflammatory and immunomodulatory function, is one of the principle active constituents of genus *Paeonia*. But its oral bioavailability is low, about 3%–4% (Zhang X. X. et al., 2022). Paeoniflorin six-o'-benzenesulphonate (CP-25) is a lipophilic molecule obtained by structural modification of paeoniflorin (Tu et al., 2019). CP-25 is a remarkable inhibitor of pannus formation in AIA rats. The specific mechanism could be associated to the lowered the plasma membrane distribution of G protein-coupled receptor kinase 2 (GRK2) in ECs, the more marked suppression of ERK1/2 in the cytoplasm by GRK2, the decreased phosphorylation of ERK1/2 (Zhang et al., 2019). Sterol is an abundant phytosterol and Qian et al. recently showed that β-sitosterol inhibits angiogenesis both *in vitro* and *in vivo*. In the cellular experiments, β-sitosterol suppressed the HUVECs' growth. Notably, in CIA mice, treatment with β-sitosterol was more effective than axitinib in inhibiting VEGFR2/p-VEGFR2 (Qian et al., 2021).

Geniposide is iridoid glucoside, which easily soluble in water, and is the main pharmacodynamic component of *Gardenia jasminoides* Ellis. Geniposide has a considerable impact on illnesses of digestive, circulatory, and neurological systems. Additionally, Geniposide has anti-inflammatory and soft tissue injury healing properties (Liu L. et al., 2022). Geniposide treats RA by inhibiting angiogenesis through multiple pathways. Geniposide can block angiogenesis, rebalance pro/anti-angiogenic factors and suppress activation of VEGF and S1P signalling pathways in the synovium in the AIA rat model, according to the study by Wang et al. Geniposide decreases VEGFR2/PKC/ERK1/2-mediated sphingosine kinase 1 (SphK1) translocation, prevents activation of sphingosine 1-phosphate/sphingosine 1-phosphate receptor 1 (S1P/S1PR1) signalling, thereby limiting VEGF-induced angiogenesis (Wang et al., 2022b). Wang et al. also investigated the anti-angiogenic potential of geniposide, the main therapeutic targets of which are to lower the expression of VEGF, to reestablish the dynamic balance between pro- and anti-angiogenic factors, to block the SphK1/S1P signalling pathway and to decrease the secretion of S1P(Wang et al., 2021c). Bu et al. figured out that geniposide improved the severity of inflammation and angiogenesis in AIA rats. Geniposide has an anti-angiogenesis effect *in vitro* via decreased HUVEC migration, proliferation, and tubule formation. According to the mechanistic study, geniposide blocked the activation of the PI3K-Akt signalling pathway, upregulated the expression of PTEN and indirectly inhibited angiogenesis (Bu et al., 2022b; a). In experimental arthritis, geniposide confirmed anti-angiogenesis actions by preventing Dnmt1-mediated hypermethylation of the PTEN gene (Bu et al., 2022b). Sun et al. discovered that geniposide dose-dependently reduced inflammatory manifestation and synovial microvessel density (MVD) in AIA rats. Geniposide can lower VEGF and Ang-1 production, increase ES secretion, and inhibit abnormal FLS proliferation (Sun et al., 2020). Deng et al. discovered that geniposide decreased S1P secretion and the interaction between FLSs and ECs via decreased expression of p-Erk1/2 and SphK1. These findings provided insight into the process of angiogenesis in the inhibition of geniposide. These data confirm that Geniposide is able to inhibit angiogenesis through several pathways and is expected to be developed as an angiogenesis inhibitor (Deng et al., 2021).

Although terpenoids have a relatively significant effect in anti-arthritis, some of them may cause serious liver and kidney damage when used, which is not to be ignored. For instance, TP is a pharmacologically active ingredient of Tripterygium wilfordii, but its clinical application is limited due to a restricted therapy window and multi-organ toxicity. Triptolide can disrupt a number of cellular structures and functions, including membrane injury, mitochondrial disruption, metabolic malfunction (Xi et al., 2017; Cui et al., 2023). Therefore, reducing the toxicity of terpenoids and increasing their bioavailability through structural modification are very important for further application of terpenoids.

4.1.4 Polyphenol

Polyphenols, as natural metabolites widely found in natural medicines, have a high variety of biological capacities such as anti-oxidation, anti-inflammation, anti-fibrosis and anti-tumor (Luca et al., 2020). Additionally, they have low toxicity and will not accumulation in body, which are potential active ingredients of great development value for dietary supplement and drugs (Gamage et al., 2023).

Chebulinic acid, obtained from *Fructus Chebulae*, is a polyphenol which has also been described to reduce inflammatory manifestations in CIA mice by inhibiting CD31 expression and VEGF. Degenerative changes and inflammatory damage in osteoarthritis were also notably attenuated by chebullinic acid administration. Further studies were performed showing that chebullinic acid greatly inhibited the activation of Erk1/2, p38 MAPK and AKT phosphorylation in human synovial microvascular endothelial cells (HSMECs). This result of this study point to the possible benefit of using chebulinic acid to treat RA targeting angiogenesis (Lu et al., 2020). Resveratrol is a widely existing polyphenol substance, which has pharmacologic benefits such as anti-oxidation, anti-inflammatory and cardiovascular protection, and is widely used in healthcare products and cosmetics (Tian and Liu, 2020; Zhang L. X. et al., 2021). According to Yang et al. research, resveratrol decreased the levels of HIF-1α, MAPK and JNK in IL-1β treated RSC-364 cells. In CIA rats, resveratrol decreased production of diverse pro-inflammatory cytokines, monocyte chemoattractant protein-1

(MCP-1), and reactive oxygen species (ROS). As a result, resveratrol looks to have great potential for clinical validation as an angiogenesis inhibitor (Yang et al., 2018).

4.1.5 Others

Ingredients such as emodin, scopoletin, scopolin and arsenic trioxide can also alleviate RA by inhibiting angiogenesis. Emodin is a natural anthraquinone compound found in a variety of natural medicines such as *Rheum palmatum* L. (Dong et al., 2016). It can dramatically suppress the expression of cytokines, prostaglandin E2 (PGE2), MMP-1, matrix metallopeptidase-13 (MMP-13), VEGF, cyclooxygenase 2 (COX-2), HIF-1a, histone deacetylase 1 (HDAC1) in synovial cells (Ha et al., 2011). In a rat model of AIA, scopoletin inhibits neoangiogenesis and reduces overexpression of VEGF, bFGF and IL-6 in synovial tissue (Pan et al., 2010). Arsenic trioxide (As_2O_3) is a highly toxic medicine. It has been clinically used in China as early as 1996 for the treatment of leukemia with remarkable efficacy (Hoonjan et al., 2018). At present, it has been confirmed that the anti-tumor mechanism of As_2O_3 is predominantly by inhibiting the proliferation of tumor vascular ECs and inducing their apoptosis, and inhibiting the formation of tumor neovascularization and new lymphatic vessels (Chen J. et al., 2023). Therefore, several studies have used As_2O_3 to treat RA (Li C. et al., 2019; Niu et al., 2022). In CIA mice and FLS, As_2O_3 dramatically reduced the expression of thrombospondin-1 (TSP-1), transforming growth factor-1 (TGF-1), connective tissue growth factor (CTGF), and VEGF (Zhang et al., 2017). Unfortunately, it has been discovered that As_2O_3 has a nephrotoxic effect during leukemia treatment, causing swelling, denaturation, and necrosis of renal tubular ECs. As a result, the use of As_2O_3 in the therapy of RA should be approached with caution (Hoonjan et al., 2018).

The content of active components in natural medicines is very low, which greatly limits their development and application. For example, the first-line anti-cancer drug paclitaxel exists in the bark of Taxus brevifolia, which accounts for about 0.004% (Alqahtani et al., 2019). Most of the active substances in natural medicines are directly extracted from plants, but the efficiency of extraction is very low, and a large number of uncontrolled production will lead to the destruction of plant resources (Stanifer et al., 2017; Liu G. Q. et al., 2022).

4.2 Natural extracts

Natural medicines treatment of RA has been occupied a very important position since ancient times, including plant extracts and animal extracts. At present, the commercial preparations of natural medicines include tripterygium glycoside tablets, sinomenine hydrochloride enteric-coated tablets, etc., (Zhang Y. et al., 2021; Liu X. et al., 2022). Modern pharmacology has demonstrated that natural medicines extracts have anti-arthritis effects by targeting angiogenesis. Both the total extract and the effective parts had antiangiogenic effect. The natural extracts with anti-arthritis activity were summarized in Table 2.

Anemone flaccida F. Schmidt is a perennial plant of the genus Anemone L. in the Ranunculacae family whose roots can be traditionally used as natural medicine. It is mainly rich in triterpenoid saponins, and it has been reported that *A. flaccida* F. Schmidt is effective in the prevention of RA by inhibiting bone differentiation and reconstruction (Kong et al., 2015; Liu et al., 2015). Research by Rao et al. has indicated that *A. flaccida* F. Schmidt effectively suppresses synovial proliferation and angiogenesis in arthritic joints and inhibited VEGFR/PI3K/AKT signaling pathway (Rao et al., 2023).

Davallia bilabiata is an essential medicine for the healing of bone injury, which can be used to treat osteoporosis, osteoarthritis (Yang et al., 2014). Liu et al. evaluated the potential anti-angiogenic benefits of *D. bilabiata* through neovascularisation experiments with chicken chorioallantoic membrane (CAM) and migration and tube formation experiments with HUVECs. According to the experimental findings, *D. bilabiata* exhibited anti-angiogenic properties. The anti-angiogenic efficacy of *D. bilabiata* has probably been due to its modulation on matrix metallopeptidase-2 (MMP-2)/tissue inhibitor of metalloproteinase-2 (TIMP-2) balance, suppression of MMP-2 activity and blocking of VEGF ligand/receptors. *Davallia bilabiata* may therefore be a very promising antiangiogenic drug for the management of RA (Liu et al., 2017). *Cissus quadrangularis*, a natural medicine, that has been previously observed to have significant osteoprotective effects (Bafna et al., 2021; Kaur et al., 2021). Administration of *C. quadrangularis* resulted in reduction of serum cytokines, oxidative stress damage and markers of angiogenesis. In addition, the oral LD_{50} of *C. quadrangularis* was over 2000 mg/kg body weight, indicating a high safety profile (Kumar et al., 2015). Saponins from *Nigella glandulifera* seeds, the primary active components, may relieve pain and swelling (Zhao et al., 2013; Zeng L. et al., 2021). In CIA model, rats received saponins10 mg, 50 mg or 250 mg per day for 24 days. The authors found that saponins from *N. glandulifera* seeds modified the immuno-inflammatory response by recovering cytokines, as well as increasing the proportions of Tregs in the peripheral blood vessels and forkhead box protein P3 (Foxp3) levels in knee joints. Saponins from *N. glandulifera* seeds alleviate synovitis, bone degradation, and angiogenesis through the OPG/RANKL/NF-κB and Ang/Tie-2 pathways (Jiang et al., 2022). Total saponins of Rhizoma *Dioscorea nipponica* also have anti-angiogenic effects, the main target of which is to reduce VEGF, Ang-2, and Tie-2 in synovial membrane. In addition to inhibiting angiogenesis by reducing VEGF, total saponins of Rhizoma *D. nipponica* can also inhibit MVD, STAT3 expression, and DNA-binding activity of NF-κB (Liang et al., 2016). The Flavonol-rich Rhus verniciflua Stokes exhibited anti-arthritic potential by decreasing the levels of pro-inflammatory factors, MCP-1 and VEGF in FLS, but downregulated the anti-inflammatory cytokine in CIA mice, resulting in reduced arthritic angiogenesis (Lee et al., 2009). *Dendrobium huoshanense* stem polysaccharide (cDHPS) was able to comprehensively and effectively alleviate arthritis symptoms in CIA mice by inhibiting NF-κB, MAPKs, PI3K/AKT and JAK1/STAT3 signalling pathways. These discoveries indicated that cDHPS has the potential to be utilized in generating of functional foods or medications for the administration of RA (Shang et al., 2021). Moreover, *Spirulina platensis* (Ali et al., 2015), evening primrose oil (El-Sayed et al., 2014) and total Saponins of *Panax japonicus* (Guo et al., 2020) rely on their antiangiogenic effect to prevent the occurrence of arthritis.

Natural extracts can directly or indirectly affect synovial angiogenesis through multiple components and multiple targets. However, there are many components in natural extracts, and the

content of effective substances in different batches of plants is different. How to ensure the stable and reliable efficacy of different batches of medicinal materials is difficult. There are still great difficulties in the consistency of natural extracts quality, which seriously hinders the clinical transformation process (Lu et al., 2021).

4.3 Prescriptions

There are about 5,000 kinds of natural medicines, and the prescriptions formed by the combination of different natural medicines are countless. Natural medicines has the characteristics of multi-component comprehensive action on multiple targets, which can effectively treat difficult diseases. At the same time, it is praised by more and more people because of its low price and safety (Ziemska et al., 2019; Tang et al., 2020). Following the holism concept of natural medicines, natural medicines prescription offer the benefits of individualized treatment, lower cost and fewer side effects and are proving their worth in managing RA (Liu X. et al., 2022). According to the different types of syndrome of RA in clinical practice, TCM prescription has achieved remarkable clinical effect on RA relief. Classic prescriptions such as Wutou decoction have a history of more than 1,800 years, which is a summary of clinical experience and is still used in RA therapy (Ba et al., 2021; Wu et al., 2021; Xie et al., 2021). With the development of preparation technology, modern preparations have been developed according to the classic prescriptions.

Wutou decoction has been proven in earlier research to have anti-inflammatory and antinociceptive properties. It also seems to prevent the progressive development of chronic arthritic joints and to reverse the symptoms of CIA or AIA rats. The research of He et al. have confirmed that Wutou decoction has remarkable anti-angiogenesis capacities via mediating VEGFR2 and PI3K/AKT/mTOR/HIF-1α signaling pathway (He et al., 2018). Wutou Decoction significantly reduced the generation of angiogenic activators such as VEGFR2, TGF-β, platelet-derived growth factor receptor (PDGF-R) in CIA rats synovium (Ba et al., 2021). Wenluo Yin, a classic prescription composed of a variety of cold-dispersing and pain-relieving herbal medicines. It has been recommended to accelerate blood circulation and so alleviate pain (Li et al., 2002; Guan et al., 2019). Liu et al. evaluated the antiangiogenic efficacy of the Wenluo drink by means of animal tests (CIA rats) and cellular tests (HFLS-RA, HUVEC). The findings indicate that Wenluo drink might significantly lower the density of macrovascular and capillary vessels in synovium. Wenluo Yin inhibits angiogenesis by downregulating various angiogenesis activators (Liu et al., 2013). Qingluo Yin is an empirical prescription for treating dampness-heat arthralgia. It is composed of four kinds of medicinal plant (Li et al., 2002). In CIA rats, Qingluo Yin can suppress angiogenesis, it might achieve so by reestablishing the balance between MMP3 and TIMP-1 in the synovium (Li et al., 2003).

Modern preparations for RA treatment by inhibit angiogenesis include tablets, capsules and patches. Most natural medicines preparations such as Yuxuebi tablet, Kunxian capsule, Shexiang zhuifeng analgesic plaster are used for RA joint swelling, pain and stiffness. YuXueBi tablet decreased disease activity index, bone degradation and angiogenesis in CIA rats. Its anti-angiogenesis mechanism is believed to be the inhibition of CD31 and VEGF production as well as the inhibition of lysyl oxidase (LOX)/Ras/Raf-1 pathway (Su et al., 2022). Shexiang Zhuifeng analgesic plaster significantly decreased the concentrations of cytokines and VEGF in CIA rats, while also lowering the protein overexpression of the AKT/mTOR/HIF-pathway. Shexiang Zhuifeng analgesic plaster decreased nitric oxide (NO) production in RAW 264.7 cells (Wang et al., 2023). In zebrafish embryos, Kunxian Capsule displayed anti-angiogenic actions through the controlling of the PI3K/AKT-MAPK-VEGF pathway (Ma et al., 2023). Sidaxue is a Miao prescriptions known in the research work of Miao medicine. It has been reported that Sidaxue can decrease chronic synovitis and formation of pannus in CIA rats. Network pharmacology investigations suggest that Sidax may primarily impact the PI3K-AKT, TNF-α and NF-κB pathways in RA. Animal studies have confirmed that SX ameliorates inflammatory symptoms, inhibits angiogenesis, downregulates inflammatory factors and regulates the expression of signal transducer and activator of transcription 1 (STAT1), and prostaglandin endoperoxide synthase 2 (PTGS2), resulting in bone protection in CIA rats (Wu et al., 2022). Fengshi Gutong capsule can inhibit the angiogenesis and disease progression of joint synovial tissue in CIA rats, perhaps via inhibiting rheumatoid factor (RF), VEGF, cytokines, intercellular cell adhesion molecule-1 (ICAM1), and Akt (Lin et al., 2021). In addition, Qianghuo Shengshi Decoction (Zeng Z. et al., 2021) and Huatan Tongluo decoction (Chen J. et al., 2019) can also improve RA by inhibiting angiogenesis.

Even though the constitution of the prescriptions of the natural medicines is extremely complex, and the active pharmacological compounds are hard to identify, they are still frequently applied as medicinal products in clinical practice due to their effectivity and the individualization of the dosage. The anti-angiogenic prescriptions are listed in Table 3.

5 Conclusions and future directions

Angiogenesis is directly related to a series of inflammatory cell mediators which are part of the overall pathogenic process of RA (Bagli et al., 2004). It has been well established that many kinds of regulating factors are of great relevance in the process of synovial angiogenesis, it is clear that when treating synovial angiogenesis, the inflammatory reaction closely related to it should also be treated. Hence, inhibiting angiogenesis is a very attractive approach for the management of RA by preventing inflammatory infiltration and bone destruction (Maruotti et al., 2006). This paper is a review of the natural medicines components, herbs and prescription that have been found to inhibit angiogenesis in a recent period.

Natural medicines, including bioactives compounds, extracts, and prescriptions have emerged as promising therapeutic alternatives for RA treatment in the modern society. There are many similarities between natural medicines theory of “collaterals” and modern theory of angiogenesis. Most natural medicines with the effect of “clearing collaterals” have better anti-angiogenesis effect (Li and Xu, 2011; Lee et al., 2019; Huang et al., 2020; Jin et al., 2023). It suggests that natural medicines have its own advantages in the intervention of angiogenesis. Tripterygium wilfordii Hook. f. (TwHF), sinomenine, and total glucoside of Paeonia lactiflora Pall. Are probably the most valuable

of all the natural products. They are the prescription drug approved by the China Food and Drug Administration for RA treatment. A randomised controlled clinical trial comprising 269 patients compared TwHF with methotrexate (MTX) in the treatment of active RA. TwHF monotherapy was not found to be inferior to, and MTX + TwHF was better than, MTX monotherapy in the control of disease activity in patients with active RA (Lv et al., 2015). In recent years, there have been many investigations of triptolide, which is one of the main chemical components of TwHF. (5R)-5-hydroxytriptolide (LLDT-8) is a new derivative of triptolide with potentially potent immunosuppressive and anti-inflammatory activities which was developed at the Shanghai Institute of Materia Medica. In fact, a phase II clinical trial of this compound has been completed in RA patients (Fan et al., 2018). A open-label, 24-week, parallel randomized controlled trial has shown that the therapeutic effect of methotrexate + sinomenine (MTX + SIN) is similar to methotrexate + leflunomide (MTX + LEF). Notably, gastrointestinal adverse reactions and hepatotoxicity were significantly reduced in patients treated with MTX + SIN compared to patients treated with MTX + LEF ($p < 0.05$) (Huang et al., 2019). Another clinical trial in RA patients showed that SIN treatment markedly improved disease activity and reduced RA-specific clinical indicators such as RF. SIN was found to be effective in remissioning RA, even though it produced a slightly lower remission rate than MTX (Liu W. et al., 2018). Chen et al. demonstrate the hepatoprotective and additive role of total glucoside of Paeonia lactiflora Pall. In combination with MTX and LEF in the therapy of active RA (Chen et al., 2013).

In recent years, the scientific research on natural medicines has made great progress, especially in explaining the material basis, the mechanism, the rationality of the formulation, the quality standard and so on. But, there are still many outstanding problems in the research and development of natural medicines. For example, compared with the pathological process such as synovial inflammation and joint destruction, the study of natural medicines on synovial angiogenesis is relatively insufficient (Cutolo et al., 2022; Komatsu and Takayanagi, 2022; Maeda et al., 2022). The basic research on the efficacy and mechanism of natural medicines is still very weak. Although there have been new developments in the study of angiogenesis models, the models adopted by natural medicines are still relatively simple, and the study on the complex model that can dynamically observe the process of synovial angiogenesis has not been reported (Liu M. et al., 2018). Additionally, most of these studies are currently at the preclinical phase, more specific and adequate clinical research to explore and harness the full potential of natural medicines is sorely lacking.

Since the 1960s, the research on natural medicines has developed rapidly, especially Tu Youyou, who was awarded the Nobel Prize in 2015 for her findings on artemisinin, making the world realize the huge development potential of natural medicines (Tu, 2016). Natural medicines has been applied to the management of tens of thousands of confirmed cases of COVID-19, achieving higher cure rates and lower mortality rates (Low et al., 2023). Natural medicines are an invaluable source for the discovery and development of new drugs. With the ongoing improvement of chemical and biological research techniques, more and more new natural products with novel skeletal structures have been discovered, providing more information on the structure of new drugs and forming a larger library of candidate natural products (Zhang et al., 2020; Chopra and Dhingra, 2021). The comprehensive use of genomics, transcriptomics, proteomics, metabolomics, phenomics and synthetic biology techniques enables the analysis of biosynthetic pathways and key enzymes of complex natural products. Furthermore, the utilization of natural medicines as safe and efficacious drugs could also be promoted by multi-omics technologies and network pharmacology which elucidating the complex mechanism of natural medicines (Chen and Song, 2016; González-López et al., 2021; Pandita et al., 2021). It is also applied in bionic cultivation and synthetic biology of natural products to achieve scientific, standardized and sustainable development of natural products (Clarke and Kitney, 2020; Jamieson et al., 2021; Zhu et al., 2021). Natural medicines are becoming more widely-used due to the rapid advancement of modern preparation technique. The new preparation plays an increasingly crucial role in improving the utilization rate, increasing the solubility and reducing the toxicity of natural medicines (Patra et al., 2018). The defects of natural medicines, such as short half-life in human body and easy to produce accumulated toxicity, can be significantly improved by structural modification using computer aided drug design (Thomford et al., 2018; de Sousa et al., 2022; Mullowney et al., 2023). For example, liu et al. designed and synthesized a series of triptolide-glucose conjugates. These conjugates are well dissolved in water and selectively inhibit the growth of tumour cells, which is worth further exploration (Liu Y. et al., 2022). Therefore, exploring natural products with novel structure and elucidating the mechanism of their anti-angiogenesis will provide more valuable anti-RA lead compounds for the research of innovative drugs. The recognition of lead compounds targeting angiogenesis is an essential contribution to accelerating the early treatment of RA, which may enable the generation of novel therapeutic targets and approaches for the management and prevention of irreversible joint damage.

In conclusion, the efficacy of natural medicines and its natural products in the prevention of RA through anti-angiogenic activity is undoubted. This review elucidates the anti-angiogenic action and mechanism of various prescriptions, herbs and compounds, and provides reference for the application and understanding of natural medicines. It will help to treat RA in its earlier phases, avoiding disability and potentially improving patients' quality of life.

Author contributions

CG: Funding acquisition, Writing–original draft, Data curation. X-DS: Writing–original draft. F-HC: Investigation, Supervision, Writing–review and editing. G-LW: Conceptualization, Methodology, Supervision, Writing–review and editing. C-YG: Conceptualization, Methodology, Supervision, Writing–review and editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This work is supported by Natural Science Foundation of Jiangxi Province (NO. 20212BAB216068), Jiangxi Provincial Administration of Traditional Chinese Medicine (NO. 2020A0350), Doctoral Foundation of the First Affiliated Hospital of Gannan Medical university (NO. QD074), Project of Health Commission of Jiangxi Province

(202130626), The Science and Technology Project of Ganzhou (2022DSYS9969).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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frontiers | Frontiers in Pharmacology

TYPE Systematic Review
PUBLISHED 18 June 2024
DOI 10.3389/fphar.2024.1376637

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RECEIVED 25 January 2024
ACCEPTED 29 April 2024
PUBLISHED 18 June 2024

CITATION
Zhao F-Y, Xu P, Kennedy GA, Zheng Z, Zhang W-J, Zhu J-Y, Ho Y-S, Yue L-P, Fu Q-Q and Conduit R (2024), Commercial Chinese polyherbal preparation Zao Ren An Shen prescription for primary insomnia: a systematic review with meta-analysis and trial sequential analysis.
Front. Pharmacol. 15:1376637.
doi: 10.3389/fphar.2024.1376637

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Commercial Chinese polyherbal preparation Zao Ren An Shen prescription for primary insomnia: a systematic review with meta-analysis and trial sequential analysis

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Background: Natural products are widely used for primary insomnia (PI). This systematic review with trial sequential analysis (TSA) aimed to summarize evidence pertaining to the effectiveness and safety of Zao Ren An Shen (ZRAS) prescription, a commercial Chinese polyherbal preparation, for treating PI.

Methods: Controlled clinical trials appraising ZRAS compared to controls or as an add-on treatment were systematically searched across seven databases until January 2024. Cochrane ROB 2.0 and ROBINS-I tools were adopted to determine risk of bias. Quality of evidence was assessed using the GRADE framework.

Results: We analyzed 22 studies, involving 2,142 participants. The effect of ZRAS in reducing Pittsburgh Sleep Quality Index scores was found to be comparable to benzodiazepines [*MD* = 0.39, 95%*CI* (−0.12, 0.91), *p* = 0.13] and superior to Z-drugs [*MD* = −1.31, 95%*CI* (−2.37, −0.24), *p* = 0.02]. The addition of ZRAS to hypnotics more significantly reduced polysomnographically-recorded sleep

Abbreviations: AE(s), adverse event(s); AIS, Athens Insomnia Scale; CAM, Complementary and alternative medicine; CBT-i, Cognitive behavioral therapy for insomnia; CCPP, Commercial Chinese polyherbal preparation; CENTRAL, Cochrane Central Register of Controlled Trials; CFDA, China Food and Drug Administration; CQVIP, Chongqing VIP database; GRADE, Grades of Recommendation, Assessment, Development, and Evaluation; ISI, Insomnia Severity Index; NRCT(s), Non-randomized controlled clinical trial(s); PI, Primary insomnia; PPRC, Pharmacopoeia of the People's Republic of China; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; PSQI, Pittsburgh Sleep Quality Index; RCT(s), Randomized control trial(s); ROB, Risk of Bias; ROB 2.0, Revised Cochrane Risk of Bias tool for randomised trials; ROBINS-I, Cochrane Risk of Bias in Non-randomized Studies-of Interventions; SCMM, Shennong's Classic of Materia Medica; SinoMed, China biomedical literature service system; TCM, Traditional Chinese medicine; TSA, Trial sequential analysis; WHO, World Health Organization; ZRAS, Zao Ren An Shen.

onset latency [*MD* = −4.44 min, 95%*CI* (−7.98, −0.91), *p* = 0.01] and number of awakenings [*MD* = −0.89 times, 95%*CI* (−1.67, −0.10), *p* = 0.03], and increased total sleep time [*MD* = 40.72 min, 95%*CI* (25.14, 56.30), *p* < 0.01], with fewer adverse events than hypnotics alone. TSA validated the robustness of these quantitative synthesis results. However, the quality of evidence ranged from very low to low. The limited data available for follow-up did not support meta-synthesis.

Conclusion: While ZRAS prescription shows promising effectiveness in treating PI, the overall quality of evidence is limited. Rigorously-designed randomized control trials are warranted to confirm the short-term efficacy of ZRAS and explore its medium-to-long-term efficacy.

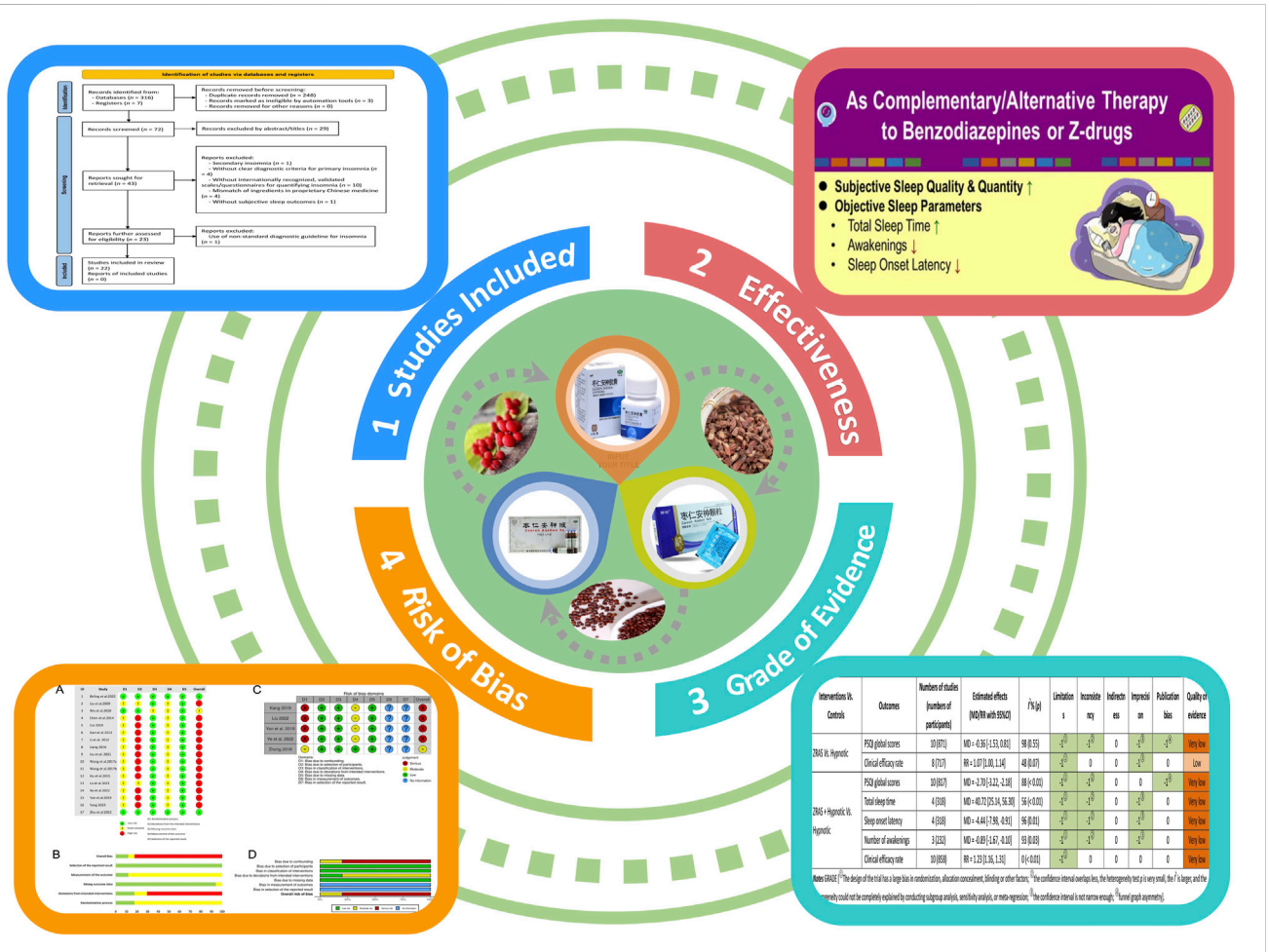
Systematic Review Registration: (https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=471497), identifier (CRD42023471497).

KEYWORDS

Zao Ren An Shen, insomnia, sleep quality, botanical drugs, Chinese medicine, systematic review, meta-analysis, clinical trials

1 Background

Approximately 30% of the general population worldwide suffers from chronic insomnia (Roth, 2007), with primary insomnia (PI) accounting for an estimated 25% of these cases (Roth and Roehrs, 2003). PI manifests as frequent sleep disturbances, where the subjective complaint of trouble falling asleep, maintaining sleep or non-restorative sleep, or the experience of sleep that is non-



GRAPHICAL ABSTRACT

TABLE 1 The information about the botanical drugs contained in ZRAS.

Botanical drug	Plant	Family
Ziziphi spinosae semen	*Ziziphus jujuba* Mill. var. *Spinosa* (Bunge) Hu ex H. F. Chou	Rhamnaceae
Schisandrae chinensis fructus	*Schisandra chinensis* (Turcz.) Baill	Schisandraceae
Salviae miltiorrhizae radix et rhizoma	*Salvia miltiorrhiza* Bunge	Lamiaceae

refreshing, cannot be directly attributed to a comorbid psychiatric and/or medical disorder (Shekleton et al., 2010). Insufficient sleep duration has been linked to a variety of deleterious health outcomes, cardiovascular disease, diabetes, weight gain and obesity (Grandner et al., 2016), heightened susceptibility to anxiety and depressive disorders (Neckelmann et al., 2007), and increased mortality (Grandner et al., 2016). Insomnia also imparts tremendous societal and economic ramifications, resulting from workplace absenteeism, reduced productivity, and increased accident rates (Zhou et al., 2017). The combined direct and indirect healthcare costs associated with insomnia have been estimated at up to $100 billion annually (Taddei-Allen, 2020).

A proportion of patients worldwide living with insomnia resort to various types of complementary and alternative medicine (CAM) treatments (Xie et al., 2013; Zhao et al., 2021), including Chinese medicines (Birling et al., 2019; Birling et al., 2022). Commercial Chinese polyherbal preparation (CCPP) refers to Chinese medicinal products, used under the guidance of the traditional Chinese medicine (TCM) theory, that have been manufactured into solution, pill, powder, ointment, capsule, tablet, concentrated extract granules, etc. (Chao et al., 2021).

Zao Ren An Shen (ZRAS; Chinese character: 棗仁安神) is a CCPP composed of three botanical drugs: Ziziphi spinosae semen, Schisandrae chinensis fructus and Salviae miltiorrhizae radix et rhizoma (Birling et al., 2020; Birling et al., 2022) (Table 1). Ziziphi spinosae semen refers to the dried seed of *Ziziphus jujuba* Mill. var. *Spinosa* (Bunge) Hu ex H. F. Chou (Zhou et al., 2018). Schisandrae chinensis fructus is the dry ripe fruits of *Schisandra chinensis* (Turcz.) Baill. (Yang et al., 2022). Salviae miltiorrhizae radix et rhizoma refers to the dried root of *Salvia miltiorrhiza* Bunge (Wang et al., 2017). Despite being a modern Chinese medicinal preparation, the three botanical drugs in ZRAS have been used in TCM for mental illness for thousands of years. All these three were first documented in the ancient TCM monograph *Shennong's Classic of Materia Medica* (SCMM) over 2500 years ago (Wang et al., 2017; Zhou et al., 2018; Yang et al., 2022). Ziziphi spinosae semen is annotated in the SCMM for its efficacy in calming the *Heart* and tranquilizing the *Mind* and is recommended for treating restlessness, palpitations, insomnia and excessive dreaming (Guan et al., 2022). It is also the most commonly used single botanical drug for treating insomnia in TCM (Zhou et al., 2018). The SCMM records the efficacy of Schisandrae chinensis fructus in nourishing the *Kidney* and calming the *Mind*, and it is therefore often used to treat insomnia and depression (Yang et al., 2022). Salviae miltiorrhizae radix et rhizoma is used to treat insomnia due to its documented efficacy in clearing the *Heart* and eliminating restlessness, as noted in the SCMM (Wang et al., 2017).

ZRAS is manufactured as CCPP in the form of granules, capsules, or solution, with the former two included in the *Pharmacopoeia of the People's Republic of China* (PPRC), 2020 version (https://ydz.chp.org.cn/#/main) for insomnia treatment. The prescription, preparation methods, characteristics, therapeutic functions, dosage and administration, specifications, precautions, and storage method of ZRAS capsules [Sinopharm Group Tongjitang (Guizhou) Pharmaceuticals Co., Ltd.; SFDA Approval No. Z20010033)] and ZRAS granules (Heilongjiang province Jiren Pharmaceuticals Co., Ltd.; SFDA Approval No. Z20053837) are comprehensively elucidated in the PPRC, as presented in Supplementary Appendix S1.

Chemical analysis suggests that jujubosides, schisandrin, triterpene saponin glycosides, flavonoids, and alkaloids are present in Ziziphi spinosae semen, Schisandrae chinensis fructus and/or Salviae miltiorrhizae radix et rhizoma, which may account for the biological effects of ZRAS (Birling et al., 2020). These metabolites regulate sleep-wake cycles by either enhancing the metabolism of gamma-aminobutyric acid or modulating the serotonergic systems (Birling et al., 2020).

ZRAS is the most extensively used and researched CCPP for insomnia (Birling et al., 2020; Birling et al., 2022; Yu et al., 2022). However, a double-blind randomized control trial (RCT) from Australia found that ZRAS capsule to be no more effective than placebo for insomnia (Birling et al., 2022), contrasting with findings from two similar RCTs conducted in China (Liu and Nan, 2009; Wu and Jiang, 2020). These inconsistent findings promoted us to conduct a systematic review. Two systematic reviews (Birling et al., 2020; Chen et al., 2020) published in peer-reviewed journals earlier covered similar topics but did not specifically focus on PI. Instead, they combined evidence on both PI and secondary insomnia. This approach introduces additional variability in quantitative synthesis, potentially skewing the actual therapeutic efficacy evaluation of ZRAS and complicating the identification of its specific indications. Moreover, these reviews were conducted in 2018 (Birling et al., 2020) and 2019 (Chen et al., 2020) respectively. New evidence may be available. Our study exclusively focused on PI, incorporating recent clinical evidence to minimize bias and obtain more objective conclusions. Additionally, we utilized trial sequential analysis (TSA) to appraise the robustness of quantitative synthesis results (Sanfilippo et al., 2021), a component lacking in previous reviews.

By combining all available data, we aimed to objectively assess the role and safety of ZARS in PI treatment and thus, enable clinicians to make critically-evaluated evidence-based treatment decisions about its use.

2 Materials and methods

2.1 Registration

The current review followed the *Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 Statement* guidelines (Page et al., 2021). A prospective protocol was registered in PROSPERO (Identifier: CRD42023471497).

2.2 Eligibility criteria

2.2.1 Inclusion criteria based on the PICOS framework

In line with the PICOS framework, formally published RCTs and non-randomized controlled clinical trials (NRCTs) were included, irrespective of language and date restrictions. Patients must be diagnosed with insomnia as per standard diagnostic criteria (Suppelmentary Appendix S2.1). Interventions were restricted to ZRAS, either as a standalone treatment or in combination with standard care for PI, i.e., hypnotics/sedatives, or cognitive behavioral therapy for insomnia (CBT-i). Controls included waitlist-control, placebo-ZRAS, or standard care. Placebo-hypnotic combined with ZRAS was only allowed as an eligible intervention when the same hypnotics combined with placebo-ZRAS was applied to controls. Primary outcomes were restricted to internationally recognized, validated scales/questionnaires for quantifying insomnia, i.e., Pittsburgh Sleep Quality Index (PSQI), Insomnia Severity Index (ISI) and Athens Insomnia Scale (AIS).

2.2.2 Exclusion criteria for the study

To ensure the reliability of the results, studies adopting only self-developed sleep questionnaires were not considered. Secondary outcomes included sleep diary, objective sleep parameters measured with actigraphy or polysomnography, clinical efficacy rate, and adverse events (AEs). The studies were also excluded if they: 1) only included objective sleep outcomes without subjective measures; 2) employed ZRAS as a decoction rather than a CCPP; and/or 3) used compounded ZRAS. This review does not discuss compounded ZARS products due to the disparity in drug ingredients. For instance, an in-hospital medication preparation called "Compounded ZRAS Capsule" (SFDA Approval No. Z20080002), manufactured by the First Affiliated Hospital of Anhui University of Chinese Medicine, consists of six ingredients: Ziziphi spinosae semen, Platycladi semen, Aucklandiae radix, Polygalae radix, Angelicae sinensis radix and Coptidis rhizoma (Xu et al., 2015). Another compounded product, "Quick-Acting ZRAS Capsule," manufactured by Chongqing Pharmaceutical Factory of Chinese Medicine (SFDA Approval No. 008351), mainly comprises Ziziphi spinosae semen and *L*-Tetrahydropalmatine (Luo et al., 1999).

2.3 Search strategy and data extraction

A systematic search was conducted across seven electronic databases [Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE (via PubMed), EMBASE, China biomedical literature service system (SinoMed), China National Knowledge Infrastructure (CNKI), Chongqing VIP database (CQVIP) and Wanfang database and two trial registries [WHO International clinical trials registry platform search portal and US ClinicalTrials.gov] from their inceptions through January 2024. The search strategies with search terms were detailed in Supplementary Appendix S2.2.

Search outcomes were exported to *Rayyan* for duplicate removal. Initial screening of titles and abstracts facilitated the exclusion of obviously irrelevant studies, with full texts reviewed when necessary. Two independent reviewers selected eligible trials, achieving consensus on inclusion. A spreadsheet was utilized for data extraction, encompassing trial identification details, participant grouping specifics (number of patients, gender ratio per group, duration of PI, patients' TCM syndrome patterns), diagnostic criteria, intervention protocols (e.g., dosage form, dose, frequency, etc.), prescription in controls, outcome measures, results, follow-up details, and AEs.

2.4 Evaluation of risk of bias in individual studies

Two investigators (W-JZ and JY-Z) independently appraised the included trials. A substantial level of agreement (*Kappa* = 0.86) was achieved, and all discrepancies were solved by discussion followed by consensus and arbitrated by a third assessor (FY-Z). The methodological quality of the RCTs and NRCTs were appraised using Revised Cochrane Risk of Bias tool for randomised trials (ROB 2.0) (Sterne et al., 2019) and Cochrane Risk of Bias in Non-randomized Studies-of Interventions (ROBINS-I) (Sterne et al., 2016), respectively.

3 Data analysis

We analyzed all studies qualitatively. Outcomes measured in at least three trials were merged for quantitative meta-analysis using Review Manager 5.4.1 software. Continuous variables (i.e., sleep scales/questionnaires scores and objective sleep parameters) were pooled using the inverse variance method, while dichotomous variables (i.e., clinical efficacy rate) were pooled using the Mantel-Haenszel method. Statistical heterogeneity was appraised using the Chi^2 test and was quantified by I^2 statistic. Following the recommendations of Tufanaru *et al.* (Tufanaru et al., 2015), the random-effects model was employed as the default model, as we aimed to generalize the conclusions beyond the actual studies included in the meta-analysis. The fixed-effects model was only considered in cases where there was no statistical heterogeneity among the effect sizes ($I^2 = 0$), or when the number of pooled studies was less than five and the heterogeneity was acceptable, i.e., $p > 0.10$ and $I^2 \leq 50\%$. We adopted TSA for primary outcomes, assessing whether the sample sizes were adequate enough to generate a statistically significant result. TSA was performed with a two-tailed type I error rate of 5% and 80% power using TSA 0.9.5.10 Beta software.

In case where there was significant clinical heterogeneity and the data permitted, subgroup analyses were performed based on different study types (RCT or NRCT), dosage forms of ZRAS, therapeutic dosage (high dosage with treatment ≥4 weeks; low dosage with treatment <4 weeks), comparator interventions (pharmacotherapy or psychotherapy), and medications in the controls (benzodiazepines, Z-drugs, melatonin receptor agonist, dual orexin receptor antagonist, or sedative antidepressants). We also conducted meta-regression analysis and sensitivity analysis if sufficient studies were available ($n \geq 10$). Univariate or multifactor meta-regressions were carried out by taking publication year, study sample size, study types, dosage forms of ZRAS, therapeutic dosage and/or medications in the controls as covariates if data permitted. In addition to identifying the sources of heterogeneity, sensitivity

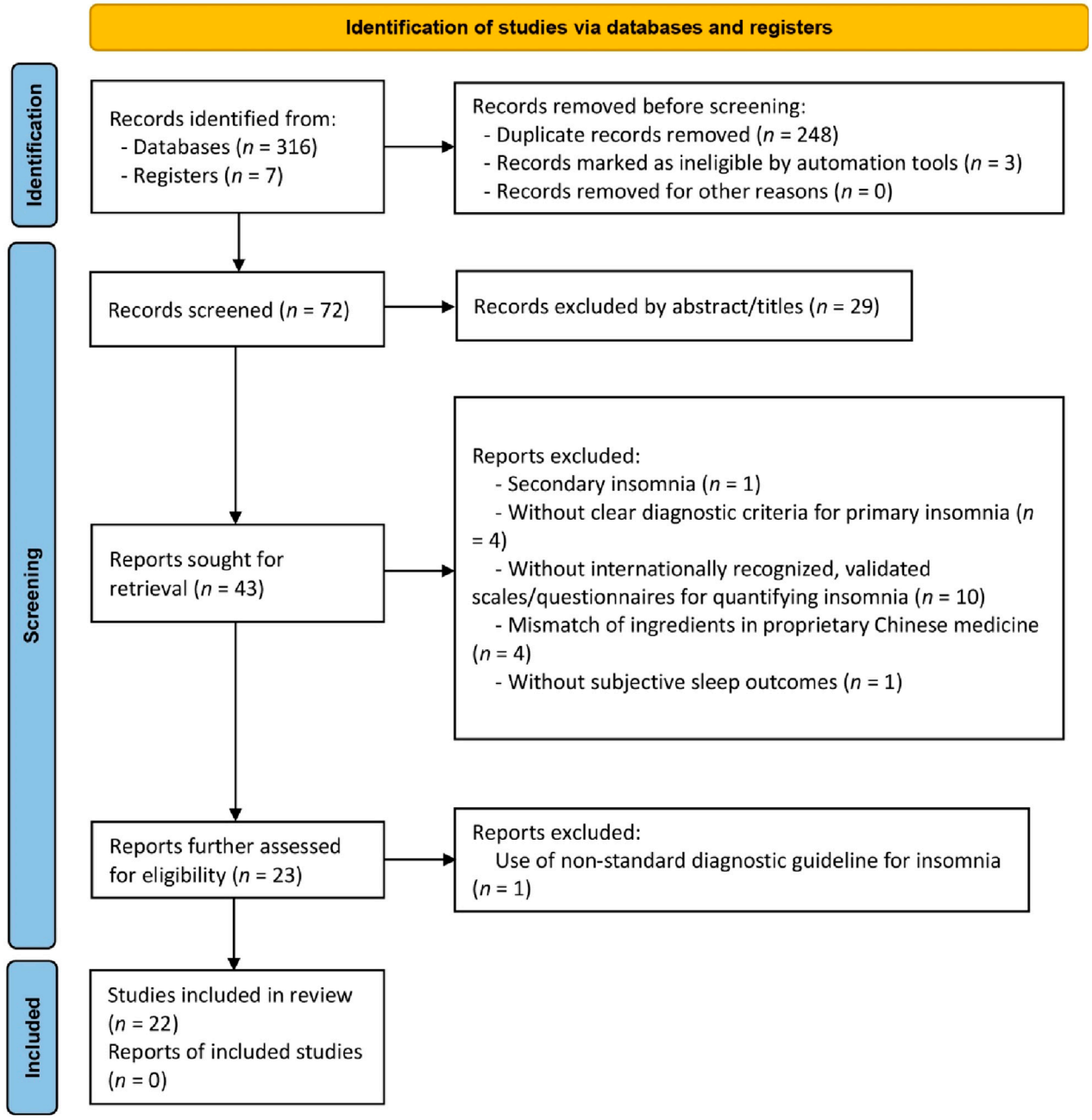


FIGURE 1
Flow diagram of the study selection process.

analysis with influence analysis method was also used to check robustness of the conclusions derived from meta-synthesis.

With STATA 18.0 software, we employed linear regression analysis (*Egger's* test) to detect the potential publication bias for outcomes measured in at least ten trials.

4 Assessment on the certainty of evidence

The overall quality of evidence obtained from the meta-synthesis was appraised using the Grades of Recommendation, Assessment, Development, and Evaluation (GRADE) framework (Atkins et al., 2004). The certainty of evidence was categorized into four levels, ranging from "High" to "Very low." The GRADE approach classifies bodies of RCTs as initially starting at high certainty and bodies of non-randomized studies at initially starting at low certainty (Schwingshackl et al., 2021). Accordingly, evidence bodies studied exclusively in RCTs were initially assigned *a priori* ranking of "High," whereas other evidence bodies were initially assigned the ranking of "Low." Subsequently, evidence bodies might undergo further upgrading when multiple high-quality studies yield consistent results or downgrading due to identifiable bias (Goldet and Howick, 2013).

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TABLE 2 Characteristics of the included studies.

Author, year	Study type	Group/size (M = male, F = female)	Age (year)	Disease duration (d = day; m = month; y = year)	Diagnostic system	TCM syndrome Pattern	ZRAS interventions	Dosage form of ZRAS	Prescription in control group (placebo /Western medication)	Outcome measure tool	ZRAS/ZRAS + Hypnotics/Sedatives compared with controls (waitlist, placebo-ZRAS, Hypnotics/Sedatives, or CBT-i)	Follow-up	Adverse events
Birling *et al.* 2022 (Birling et al., 2022)	RCT	- ZRAS/n = 38 (9M, 29F) - placebo-ZRAS/n = 47 (17M, 30F)	- ZRAS/52.0 ± 13.0 - placebo-ZRAS/50.0 ± 16.0	- ZRAS/NR - placebo-ZRAS/NR	DSM-5	NR	0.76g*3′ qn for 4 weeks	capsule	placebo-ZRAS 0.72g*3′ qn for 4 weeks	(i) ISI (ii) DASS (iii) FSS (iv) AQoL (v-i) sleep diary [TST (min)] (v-ii) sleep diary [SOL (min)] (v-iii) sleep diary [WASO (min)] (vi-i) actigraphy [TST (min)] (vi-ii) actigraphy [SOL (min)] (vi-iii) actigraphy [WASO (min)]	(i) compared with placebo-ZRAS $p > 0.05$ (ii) compared with placebo-ZRAS $p > 0.05$ (iii) compared with placebo-ZRAS $p > 0.05$ (iv) compared with placebo-ZRAS $p > 0.05$ (v-i) compared with placebo-ZRAS $p > 0.05$ (v-ii) compared with placebo-ZRAS $p > 0.05$ (v-iii) compared with placebo-ZRAS $p > 0.05$ (vi-i) compared with placebo-ZRAS $p > 0.05$ (vi-ii) compared with placebo-ZRAS $p > 0.05$ (vi-iii) compared with placebo-ZRAS $p > 0.05$	4-week follow-up (no significant differences in efficacy outcomes between two groups)	- ZRAS/n = 10 [dry mouth (2); frequent night waking (1); facial skin rash (1); urinary urgency (n = 1); NR (5)] - placebo-ZRAS/n = 16 [NR]
Liu *et al.* 2009 (Liu and Nan, 2009)	RCT	- ZRAS/n = 30 (12M, 18F) - placebo-ZRAS/n = 30 (13M, 17F)	- ZRAS/36.9 ± 11.5 - placebo-ZRAS/37.8 ± 11.4	- ZRAS/8.9 ± 2.8m - placebo-ZRAS/9.8 ± 2.3m	ICSD-3	NR	0.45g*5′ qn for 3 weeks	capsule	placebo-ZRAS 0.45g*5′ qn for 3 weeks	(i) PSQI (ii) clinical efficacy rate	(i) compared with placebo-ZRAS $p < 0.05$ (ii) compared with placebo-ZRAS $p < 0.05$	no follow-up	NR
Liu *et al.* 2009 (Liu and Nan, 2009)	RCT	- ZRAS/n = 30 (12M, 18F) - Estazolam/n = 30 (13M, 17F)	- ZRAS/36.9 ± 11.5 - Estazolam/37.2 ± 11.4	- ZRAS/8.9 ± 2.8m - Estazolam/9.3 ± 2.2m	ICSD-3	NR	0.45g*5′ qn for 3 weeks	capsule	Estazolam 1mg qn for 3 weeks	(i) PSQI (ii) clinical efficacy rate	(i) compared with Estazolam $p > 0.05$ (ii) compared with Estazolam $p > 0.05$	no follow-up	NR
Wu *et al.* 2020 (Wu and Jiang, 2020)	RCT	- ZRAS/n = 120 (48M, 72F) - placebo-ZRAS/n = 120 (45M, 75F)	- ZRAS/51.1 ± 8.3 - placebo-ZRAS/50.9 ± 9.0	- ZRAS/12.1 ± 3.5m - placebo-ZRAS/12.1±4.1m	DSM-5	NR	0.45g*5′ qn for 4 weeks	capsule	placebo-ZRAS 0.45g*5′ qn for 4 weeks	(i) PSQI (ii) clinical efficacy rate	(i) compared with placebo-ZRAS $p < 0.05$ (ii) compared with placebo-ZRAS $p < 0.01$	no follow-up	- ZRAS/n = 14 [fatigue (2); dizziness (4); drowsiness (1); gastrointestinal discomfort (2); muscle soreness (1); dry mouth (2); constipation (2)] - placebo-ZRAS/n = 12 [fatigue (4); dizziness (2); drowsiness (1); gastrointestinal discomfort (2); muscle soreness (1); dry mouth (1); constipation (1)]
Zhu *et al.* 2022 (Zhu et al., 2022)	RCT	- ZRAS + placebo-Zolpidem/n = 32 (11M, 21F) - placebo-ZRAS + Zolpidem/n = 35 (10M, 25F)	- ZRAS + placebo-Zolpidem/51.0 ± 13.0 - placebo-ZRAS + Zolpidem/44.0 ± 13.0	NR	DSM-5	NR	0.45g*5′ for 4 weeks	capsule	Zolpidem 5mg + placebo-ZRAS qn for 4 weeks	(i) ISI (ii) clinical efficacy rate	(i) compared with placebo-ZRAS + Zolpidem $p > 0.05$ (ii) compared with placebo-ZRAS + Zolpidem $p > 0.05$	2-week follow-up (no significant differences in efficacy outcomes between two groups)	- ZRAS + placebo-Zolpidem/n = 2 [constipation (1); abdominal pain (1)] - placebo-ZRAS + Zolpidem/n = 6 [drowsiness (2); dry mouth/bitter taste (1); constipation and urinary urgency (1); gastrointestinal discomfort (1); head painful distension (1)]
Zhu *et al.* 2022 (Zhu et al., 2022)	RCT	- ZRAS + Zolpidem/n = 32 (10M, 22F) - placebo-ZRAS + Zolpidem/n = 35 (10M, 25F)	- ZRAS + Zolpidem/48.0 ± 12.0 - placebo-ZRAS + Zolpidem/44.0 ± 13.0	NR	DSM-5	NR	0.45g*5′ for 4 weeks	capsule	Zolpidem 5mg + placebo-ZRAS qn for 4 weeks	(i) ISI (ii) clinical efficacy rate	(i) compared with placebo-ZRAS + Zolpidem $p < 0.05$ (ii) compared with placebo-ZRAS + Zolpidem $p < 0.05$	2-week follow-up (lower ISI in ZRAS + Zolpidem group)	- ZRAS + Zolpidem/n = 3 [drowsiness (1); dry mouth (1); dizziness (1)] - placebo-ZRAS + Zolpidem/n = 6 [drowsiness (2); dry mouth/bitter taste (1); constipation and urinary urgency (1); gastrointestinal discomfort (1); head painful distension (1)]
Kang 2019 (Kang, 2019)	NRCT	- ZRAS + Zolpidem/n = 43 (22M, 21F)	- ZRAS + Zolpidem/43.9 ± 1.8	- ZRAS + Zolpidem/1.9 ± 0.7y - Zolpidem/1.7 ± 0.5y	GDTICA	NR	0.45g*5′ for 4 weeks	capsule	Zolpidem 10mg qn for 4 weeks	(i) PSQI (ii-i) PSG [TST (min)] (ii-ii) PSG [SE (%)]	(i) compared with Zolpidem $p < 0.05$ (ii-i) compared with Zolpidem $p < 0.05$ (ii-ii) compared with Zolpidem $p < 0.05$	no follow-up	- ZRAS + Zolpidem/n = 0 - Zolpidem/n = 0

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TABLE 2 (*Continued*) Characteristics of the included studies.

Author, year	Study type	Group/size (M = male, F = female)	Age (year)	Disease duration (d = day; m = month; y = year)	Diagnostic system	TCM syndrome Pattern	ZRAS interventions	Dosage form of ZRAS	Prescription in control group (placebo /Western medication)	Outcome measure tool	ZRAS/ZRAS + Hypnotics/Sedatives compared with controls (waitlist, placebo-ZRAS, Hypnotics/Sedatives, or CBT-i)	Follow-up	Adverse events
		- Zolpidem/n = 43 (24M, 19F)	- Zolpidem/43.6 ± 1.5							(ii-iii) PSG [REM (min)] (ii-iv) PSG [SOL (min)] (ii-v) PSG [WASO (min)] (iii) HADS (iv) PHQ-9 (v) WEMWBS (vi) clinical efficacy rate (vii) serum 5-HT (viii) serum orexin-A (ix) serum cortisol (x) serum IL-2 (xi) serum IL-6	(ii-iii) compared with Zolpidem $p < 0.05$ (ii-iv) compared with Zolpidem $p < 0.05$ (ii-v) compared with Zolpidem $p < 0.05$ (iii) compared with Zolpidem $p < 0.05$ (iv) compared with Zolpidem $p < 0.05$ (v) compared with Zolpidem $p < 0.05$ (vi) compared with Zolpidem $p < 0.05$ (vii) compared with Zolpidem $p < 0.05$ (viii) compared with Zolpidem $p < 0.05$ (ix) compared with Zolpidem $p < 0.05$ (x) compared with Zolpidem $p < 0.05$ (xi) compared with Zolpidem $p < 0.05$		
Xu *et al.* 2022 (Xu and Zhou, 2022)	RCT	ZRAS + Eszopiclone /n = 48 (34M, 14F) Eszopiclone /n = 48 (31M, 17F)	ZRAS + Eszopiclone /71.0 ± 6.7 Eszopiclone/71.4 ± 7.0	ZRAS + Eszopiclone /3.5 ± 0.4m Eszopiclone/3.7 ± 0.4m	GDTICA	NR	5g qn for 4 weeks	granule	Eszopiclone 2mg qn for 4 weeks	(i) PSQI (ii-i) PSG [SWS (min)] (ii-ii) PSG [SE (%)] (ii-iii) PSG [WASO (min)] (iii) serum BDNF (iv) serum NPY (v) serum TNF-α (vi) clinical efficacy rate	(i) compared with Eszopiclone $p < 0.05$ (ii-i) compared with Eszopiclone $p < 0.05$ (ii-ii) compared with Eszopiclone $p < 0.05$ (ii-iii) compared with Eszopiclone $p < 0.05$ (iii) compared with Eszopiclone $p < 0.05$ (iv) compared with Eszopiclone $p < 0.05$ (v) compared with Eszopiclone $p < 0.05$ (vi) compared with Eszopiclone $p < 0.05$	no follow-up	- ZRAS + Eszopiclone/n =6 [nausea (2); headache (1); fatigue (1); dizziness (2)] - Eszopiclone/n = 5 [nausea (1); headache (1); fatigue (2); dizziness (1)]
Yan *et al.* 2018 (Yan et al., 2018)	NRCT	- ZRAS + Estazolam/ n = 35 (16M, 19F) - Estazolam/n = 35 (17M, 18F)	- ZRAS + Estazolam/46.2 ± 5.0 - Estazolam/48.1 ± 5.1	- ZRAS + Estazolam/ 57.0 ± 5.4d - Estazolam/58.0 ± 4.0d	ICD-10	NR	0.45g*5″ for 4 weeks	capsule	Estazolam 1-2mg qn for 4 weeks	(i) PSQI (ii-i) PSG [TST (min)] (ii-ii) PSG [SOL (min)] (ii-iii) PSG [ATs (times)] (iii) clinical efficacy rate	(i) compared with Estazolam $p < 0.01$ (ii-i) compared with Estazolam $p < 0.01$ (ii-ii) compared with Estazolam $p < 0.01$ (ii-iii) compared with Estazolam $p < 0.05$ (iii) compared with Estazolam $p < 0.05$	no follow-up	- ZRAS + Estazolam/n = 2 [fatigue (1); dizziness (1)] - Estazolam/n = 9 [drowsiness (4); dizziness (3); dry mouth (2)]
Yang 2023 (Yang, 2023)	RCT	- ZRAS + Estazolam/ n = 30 (19M, 11F) - Estazolam/n = 30 (20M, 10F)	- ZRAS + Estazolam/39.6 ± 2.1 - Estazolam/39.4 ± 2.2	- ZRAS + Estazolam/ 11.3 ± 2.1m - Estazolam/11.2 ± 2.2m	GDTICA	deficiency of *Heart*-blood	5g qn for 4 weeks	granule	Estazolam 1-2mg qn for 4 weeks	(i) PSQI (ii-i) PSG [TST (min)] (ii-ii) PSG [SOL (min)] (ii-iii) PSG [ATs (times)] (iii) clinical efficacy rate	(i) compared with Estazolam $p < 0.01$ (ii-i) compared with Estazolam $p < 0.05$ (ii-ii) compared with Estazolam $p < 0.05$ (ii-iii) compared with Estazolam $p < 0.05$ (iii) compared with Estazolam $p < 0.05$	no follow-up	- ZRAS + Estazolam/n = 2 [dry mouth (1); dizziness (1)] - Estazolam/n = 2 [nausea (1); dry mouth (1)]
Ye *et al.* 2022 (Ye and Lin, 2022)	NRCT	ZRAS + Eszopiclone/ n = 51 (24M, 27F) Eszopiclone/n = 51 (23M, 28F)	ZRAS + Eszopiclone /54.5 ± 10.7 Eszopiclone/54.1 ± 10.3	ZRAS + Eszopiclone /11.4 ± 0.9m Eszopiclone/11.3 ± 0.8m	CCMD-3	hyperactivity of *Fire* due to *Yin* deficiency	5g qn for 4 weeks	granule	Eszopiclone 2-3mg qn for 4 weeks	(i) PSQI (ii-i) PSG [TST (min)] (ii-ii) PSG [SOL (min)] (ii-iii) PSG [ATs (times)] (iii) clinical efficacy rate	(i) compared with Eszopiclone $p < 0.05$ (ii-i) compared with Eszopiclone $p < 0.05$ (ii-ii) compared with Eszopiclone $p < 0.05$ (ii-iii) compared with Eszopiclone $p < 0.05$ (iii) compared with Eszopiclone $p < 0.05$	no follow-up	- ZRAS + Eszopiclone/n = 2 [acid reflux (1); dry mouth (1)] - Eszopiclone/n = 10 [drowsiness (1); dry mouth (5); dizziness (4)]
Lu *et al.* 2023 (Lu et al., 2023)	RCT	- ZRAS + placebo-Zolpidem/n = 10 (5M, 5F) - placebo-ZRAS + Zolpidem/n = 17 (7M, 10F)	- ZRAS + placebo-Zolpidem/55.0 ± 13.4 - placebo-ZRAS + Zolpidem/49.5 ± 14.5	- ZRAS + placebo-Zolpidem/NR - placebo-ZRAS + Zolpidem/NR	ICSD-3	NR	0.45g*5″ for 4 weeks	capsule	Zolpidem 10mg qn + placebo-ZRAS 0.45g*5″ for 4 weeks	(i) PSQI (ii) ISI (iii) HAMA (iv) HAMD	(i) compared with placebo-ZRAS + Zolpidem $p < 0.05$ (ii-i) compared with placebo-ZRAS + Zolpidem $p < 0.05$ (iii) compared with placebo-ZRAS + Zolpidem $p > 0.05$	no follow-up	NR

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TABLE 2 (*Continued*) Characteristics of the included studies.

Author, year	Study type	Group/size (M = male, F = female)	Age (year)	Disease duration (d = day; m = month; y = year)	Diagnostic system	TCM syndrome Pattern	ZRAS interventions	Dosage form of ZRAS	Prescription in control group (placebo /Western medication)	Outcome measure tool	ZRAS/ZRAS + Hypnotics/Sedatives compared with controls (waitlist, placebo-ZRAS, Hypnotics/Sedatives, or CBT-i)	Follow-up	Adverse events
											(iv) compared with placebo-ZRAS + Zolpidem $p > 0.05$		
Lu *et al.* 2023 (Lu et al., 2023)	RCT	- ZRAS + Zolpidem/n = 14 (6M, 8F) - placebo-ZRAS + Zolpidem/n = 12 (2M, 10F)	- ZRAS + Zolpidem/48.5 ± 11.7 - placebo-ZRAS + Zolpidem/46.0 ± 12.7	- ZRAS + Zolpidem/NR - placebo-ZRAS + Zolpidem/NR	ICSD-3	NR	0.45g*5″ for 4 weeks	capsule	Zolpidem 5mg qn for 4 weeks	(i) PSQI (ii) ISI (iii) HAMA (iv) HAMD	(i) compared with placebo-ZRAS + Zolpidem $p < 0.05$ (ii-i) compared with placebo-ZRAS + Zolpidem $p < 0.05$ (iii) compared with placebo-ZRAS + Zolpidem $p > 0.05$ (iv) compared with placebo-ZRAS + Zolpidem $p > 0.05$	no follow-up	NR
Zhong 2018 (Zhong, 2018)	NRCT	ZRAS + Oxazepam/n = 48 (NR) Oxazepam/n = 48 (NR)	42.4 ± 3.6	10.2 ± 1.5m	CCMD-3	NR	0.45g*5″ for 4 weeks	capsule	Oxazepam 15-30mg tid for 4 weeks	(i) PSQI (ii) clinical efficacy rate	(i) compared with placebo-ZRAS $p < 0.05$ (ii) compared with placebo-ZRAS $p < 0.05$	no follow-up	NR
Chen *et al.* 2014 (Chen et al., 2014)	RCT	- ZRAS/n = 60 (21M, 39F) - Zopiclone/n = 60 (20M, 40F)	- ZRAS/45.6 ± 12.1 - Zopiclone/45.2 ± 11.5	- ZRAS/13.9 ± 11.5m - Zopiclone/13.1 ± 11.9m	CCMD-3	deficiency of *Heart*-blood	5g qn for 4 weeks	granule	Zopiclone 7.5mg qn for 4 weeks	(i) PSQI (ii) clinical efficacy rate	(i) compared with Zopiclone $p > 0.05$ (ii) compared with Zopiclone $p < 0.05$	no follow-up	- ZRAS/n = 2 [acid reflux (1); mouth-numbing (1)] - Zopiclone/n = 16 [drowsiness (1); dry mouth (1); dizziness (4); headache (1); bitter taste (9)]
Cui 2019 (Cui, 2019)	RCT	- ZRAS/n = 43 (21M, 22F) - Zopiclone/n = 47 (25M, 22F)	- ZRAS/70.0 ± 6.0 - Zopiclone/70.0 ± 6.0	- ZRAS/1.2 ± 0.5y - Zopiclone/1.1 ± 0.5y	GDTICA	NR	0.45g*5 qn for 4 weeks	capsule	Zopiclone 3mg qn for 4 weeks	(i) PSQI	(i) compared with Zopiclone $p > 0.05$	no follow-up	- ZRAS/n = 2 [dry mouth (1); sweat (1)] - Zopiclone/n = 9 [constipation (1); dry mouth (3); headache (2); sweat (3)]
Gan *et al.* 2013 (Gan et al., 2013)	RCT	- ZRAS/n = 60 (23M, 37F) - Alprazolam/n = 60 (23M, 37F)	- ZRAS/67.2 ± 5.0 - Alprazolam/66.5 ± 9.2	- ZRAS/19.4 ± 6.1m - Alprazolam/20.6 ± 8.7m	CCMD-3	NR	0.45g*5″ qn for 4 weeks	capsule	Alprazolam 0.8mg qn for 4 weeks	(i) PSQI (ii) clinical efficacy rate	(i) compared with Alprazolam $p > 0.05$ (ii) compared with Alprazolam $p > 0.05$	no follow-up	- ZRAS/n = 8 [fatigue (6); diarrhea (2)] - Alprazolam/n = 32 [fatigue (12); dizziness (11); drowsiness (7); hangover effects (2)]
Li *et al.* 2012 (Li and Gong, 2012)	RCT	- ZRAS/n = 30 (13M, 17F) - Estazolam/n = 30 (15M, 15F)	- ZRAS/37.8 - Estazolam/37.4	- ZRAS/NR - Estazolam/NR	CCMD-2-R	NR	0.45g*5″ qn for 2 weeks	capsule	Estazolam 1 mg qn for 2 weeks	(i) PSQI (ii) clinical efficacy rate	(i) compared with Estazolam $p > 0.05$ (ii) compared with Estazolam $p > 0.05$	no follow-up	- ZRAS/n = 3 [fatigue] - Estazolam/n = 26 [fatigue (8); dizziness (10); drowsiness (5); hangover effects (3)]
Liang 2016 (Liang, 2016)	RCT	- ZRAS/n = 40 (NR) - Estazolam/n = 40 (NR)	68.1 ± 13.6	9.6 ± 2.2m	CCMD-3	NR	0.45g*5″ qn for 4 weeks	capsule	Estazolam 1 mg qn for 4 weeks	(i) PSQI (ii) clinical efficacy rate	(i) compared with Estazolam $p > 0.05$ (ii) compared with Estazolam $p > 0.05$	no follow-up	- ZRAS/n = 6 [fatigue (2); dizziness (1); drowsiness (3)] - Estazolam/n = 21 [fatigue (7); dizziness (6); drowsiness (8)]
Liu *et al.* 2021 (Liu and Gong, 2021)	RCT	- ZRAS/n = 52 (30M, 22F) - Eszopiclone/n = 52 (29M, 23F)	- ZRAS/48.3 ± 6.6 - Eszopiclone/48.6 ± 7.4	- ZRAS/9.5 ± 3.3m - Eszopiclone/9.4 ± 3.4m	ICSD-3	NR	0.45g*5″ for 4 weeks	capsule	Eszopiclone 3mg qn for 4 weeks	(i) PSQI (ii) GAD-7 (iii) FSS	(i) compared with Eszopiclone $p < 0.05$ (ii) compared with Eszopiclone $p < 0.05$ (iii) compared with Eszopiclone $p < 0.05$	no follow-up	- ZRAS /n =3 [muscle soreness (1); constipation (1); dizziness (1)] - Eszopiclone/n = 7 [muscle soreness (1); constipation (3); dizziness (1); nausea (1); dry mouth (1)]
Wang J. *et al.* 2017 (Wang J. et al., 2017)	RCT	- ZRAS/n = 52 (39M, 25F) - Eszopiclone/n = 64 (38M, 26F)	- ZRAS/42.5 ± 11.3 - Eszopiclone/42.6 ± 10.1	- ZRAS/10.1 ± 3.8m - Eszopiclone/10.3 ± 2.4m	GDTICA	NR	0.45g*5″ for 4 weeks	capsule	Eszopiclone 3mg qn for 4 weeks	(i) PSQI (ii) clinical efficacy rate	(i) compared with placebo-ZRAS $p < 0.01$ (ii) compared with placebo-ZRAS $p < 0.01$	no follow-up	- ZRAS /n = 2 [mouth-numbing (1); acid reflux (1)] - Eszopiclone/n = 9 [mouth-numbing (1); dizziness/headache (3); dry mouth/bitter taste (5)]
Wang X. *et al.* 2017 (Wang X. et al., 2017)	RCT	- ZRAS/n = 41 (20M, 21F) - Estazolam/n = 41 (19M, 22F)	- ZRAS/58.5 ± 5.2 - Estazolam/55.2 ± 5.3	- ZRAS/3.8 ± 0.9m - Estazolam/3.3 ± 0.5m	CCMD-3	NR	0.45g*5″ for 3 weeks	capsule	Estazolam 1mg qn for 3 weeks	(i) PSQI (ii) clinical efficacy rate	(i) compared with Estazolam $p < 0.05$ (ii) compared with Estazolam $p < 0.05$	no follow-up	- ZRAS/n = 5 [fatigue] - Estazolam/n = 23 [fatigue (11); dizziness (5); drowsiness (5); dry mouth (2)]
Hu *et al.* 2015 (Hu and Sheng, 2015)	RCT	- ZRAS + Estazolam/n = 50 (27M, 23F)	- ZRAS + Estazolam/49.8 ±	- ZRAS + Estazolam/16.4 ± 4.1m	ICD-10	NR	5g qn for 12 weeks	granule	Estazolam 1-2mg qn for 12 weeks		(i) compared with Estazolam $p < 0.05$ (ii) compared with Estazolam $p < 0.05$	no follow-up	- ZRAS + Estazolam/n = 7 [fatigue (6); diarrhea (1)]

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TABLE 2 (*Continued*) Characteristics of the included studies.

Author, year	Study type	Group/size (M = male, F = female)	Age (year)	Disease duration (d = day; m = month; y = year)	Diagnostic system	TCM syndrome Pattern	ZRAS interventions	Dosage form of ZRAS	Prescription in control group (placebo /Western medication)	Outcome measure tool	ZRAS/ZRAS + Hypnotics/Sedatives compared with controls (waitlist, placebo-ZRAS, Hypnotics/Sedatives, or CBT-i)	Follow-up	Adverse events
		- Estazolam/n = 43 (25M, 18F)	13.3 - Estazolam/45.3 ± 16.5	- Estazolam/15.1 ± 3.2m						(i) PSQI (ii) clinical efficacy rate			- Estazolam/n = 20 [drowsiness (9); dizziness (7); drowsiness (4)]
Yan *et al.* 2019 (Yan et al., 2019)	RCT	- ZRAS + Estazolam/ n = 39 (27M, 12F) - Estazolam/n = 39 (28M, 11F)	- ZRAS + Estazolam/39.6 ± 10.5 - Estazolam/39.4 ± 11.0	- ZRAS + Estazolam/ 12.3 ± 1.1m - Estazolam/11.9 ± 1.3m	GDTICA	NR	0.45g*5″ for 3 weeks	capsule	Estazolam 1mg qn for 3 weeks	(i) PSQI (ii) SCL-90 (iii) clinical efficacy rate	(i) compared with Estazolam $p < 0.05$ (ii) compared with Estazolam $p < 0.05$ (iii) compared with Estazolam $p < 0.05$	no follow-up	- ZRAS + Estazolam/n = 2 [drowsiness (1); fatigue (1)] - Estazolam/n = 8 [drowsiness (2); fatigue (1); dry mouth (1); dizziness (4)]
Liu 2022 (Liu, 2022)	NRCT	- ZRAS + Estazolam/ n = 60 (29M, 31F) - Estazolam/n = 50 (28M, 22F)	- ZRAS + Estazolam/45.5 ± 10.2 - Estazolam/45.5 ± 10.2	- ZRAS + Estazolam/ 2.6 ± 0.7y - Estazolam/2.6 ± 0.7y	GDTICA	deficiency of *Heart*-blood	0.45g*5″ for 4 weeks	capsule	Estazolam 1mg qn for 4 weeks	(i) PSQI (ii) serum 5-HT (iii) serum GABA (iv) clinical efficacy rate	(i) compared with Estazolam $p < 0.05$ (ii) compared with Estazolam $p < 0.05$ (iii) compared with Estazolam $p > 0.05$ (iv) compared with Estazolam $p < 0.05$	no follow-up	- ZRAS + Estazolam/n =3 [fatigue (2); dizziness (1)] - Estazolam/n = 6 [drowsiness (3); fatigue (2); dry mouth (1)]

***Abbreviations*:** NR, no report; 5-HT, 5-hydroxytryptamine; AQoL, Assessment of Quality of Life; ATs, awakening times; BDNF, brain-derived neurotrophic factor; CCMD-2-R, Chinese Classification of Mental Disorders (Second Edition, Revised); CCMD-3, Chinese Classification of Mental Disorders (Third Edition); GDTICA, Guideline for the diagnosis and treatment of insomnia in Chinese adults; DASS, Depression Anxiety Stress Scales 21-item; DSM-V, Diagnostic and Statistical Manual of Mental Disorders (Fifth Edition); FSS, Fatigue Severity Scale; GABA, Gamma-amino butyric acid; GAD-7, 7-item Generalized Anxiety Disorder; HADS, Hospital Anxiety and Depression Scale; HAMA, Hamilton Anxiety Scale; HAMD, Hamilton Depression Scale; ICD-10, International Classification of Diseases (Tenth Revision); ICSD-3, International classification of sleep disorders (Third Edition); ISI, Insomnia Severity Index; LI-2, interleukin-2; LI-6, interleukin-6; NPY, neuropeptide Y; NRCT, non-randomized controlled clinical trial; PHQ-9, Patient Health Questionnaire-9; PSG, polysomnography; PSQI, Pittsburgh Sleep Quality Index; REM, rapid eye movement sleep; RCT, randomized control trial; SCL-90, Symptom Check List-90; SE, sleep efficiency; SOL, sleep onset latency; SWS, slow-wave sleep; TNF-α, tumor necrosis factor-α; TST, total sleep time; WASO, wake after sleep onset; WEMWBS, Warwick-Edinburgh Mental Wellbeing Scale; ZRAS, Zao Ren An Shen.

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5 Results analysis

The initial search yielded 323 articles. Following duplicate removal and thorough full-text screening, 17 RCTs and five NRCTs, composing a total of 2,142 participants from these 22 studies, met the predefined criteria (Figure 1). A summary of the discarded studies with specific reasons for irrelevance is provided in Supplementary Appendix S3.

5.1 Description of studies

Among the 22 included trials (Table 2), three compared ZRAS against placebo-ZRAS, eight against hypnotics alone, and 11 evaluated ZRAS combined with hypnotics versus hypnotics alone. None of these trials included CBT-i or waitlist-control. The PPRC-recommended dose for ZRAS capsule or granule was consistently administered across all trials. A variety of hypnotics were used as active controls, with Estazolam (9/20), Eszopiclone (4/20), Zolpidem (3/20), Zopiclone (2/20), Alprazolam (1/20) and Oxazepam (1/20), in descending order of frequency of use.

Two trials (Birling et al., 2022; Zhu et al., 2022) employed the ISI as the primary outcome measure in evaluating sleep quality and quantity, while others relied on PSQI. Six trials further recorded changes in objective sleep parameters using actigraphy (Birling et al., 2022) or polysomnography (Yan et al., 2018; Kang, 2019; Xu and Zhou, 2022; Ye and Lin, 2022; Yang, 2023). Sleep diary was adopted in only one trial (Birling et al., 2022). Additionally, 18 studies compared the clinical efficacy rate across interventions, albeit with varying grading criteria (Supplementary Appendix S4).

In all 22 included trials, the ZRAS capsules/granules used in 21 trials were sourced from the same pharmaceutical company, with a consistent batch number (See ***Background*** section). Furthermore, the content of each botanical drug in the ZRAS manufactured by this pharmaceutical company is consistent with the description of ZRAS in the Chinese Pharmacopoeia (https://ydz.chp.org.cn/#/main). The remaining trial was conducted in Australia, where the ZRAS used was manufactured by a local pharmaceutical company. However, the author of that trial highlighted that this company adhered to the standards of ZRAS described in the Chinese Pharmacopoeia during prescription production. Based on this consistency of sourcing and content, it can be considered that the prescriptions used in all trials are consistent and comparable.

All studies, except three (Liu and Nan, 2009; Zhong, 2018; Lu et al., 2023), reported AEs. Fatigue was the only AE associated with ZRAS with an incidence >3%, while eight types of AEs provoked by hypnotics had an incidence exceeding 3%. Although fatigue and dizziness were prevalent across all groups, the reporting rates were significantly lower in either ZRAS or ZRAS combined with hypnotic groups compared to the hypnotic group (6.2% in ZRAS *Vs*. 4.7% in combined group *vs*. 14.0% in hypnotic for fatigue; 2.8% in ZRAS *vs*. 2.9% in combined group *vs*. 11.2% in hypnotic for dizziness). The incidence of other symptoms such as dry mouth/bitter taste, excessive daytime drowsiness/sleepiness, gastrointestinal symptoms and headache/head painful distension was also lower in the combined group compared to the hypnotic group (Supplementary Appendix S5).

5.2 Study quality evaluation

5.2.1 Assessing RCTs quality: ROB 2.0 indicates moderate to high ROB

Among the included RCTs, only three adequately described randomization methods, and employed valid allocation concealment using sealed blinding codes (Wu and Jiang, 2020; Birling et al., 2022; Zhu et al., 2022). They were also judged as having low RoB on the "deviations from intended interventions" domain due to participant-personnel double-blinding achieved via placebo-ZRAS capsules and/or placebo-hypnotics provided by pharmaceutical companies. Two studies were of some concern in this domain as only participants were blinded (Liu and Nan, 2009; Lu et al., 2023). Only two RCTs reported blinding of outcome evaluators (Birling et al., 2022; Zhu et al., 2022). One trial raised concerns in the "missing outcome data" domain due to an 8.3% dropout rate and exclusion of these cases from results analysis (Wu and Jiang, 2020). The remaining RCTs were appraised as having low RoB in this domain, either due to no participant withdrawal (Liu and Nan, 2009; Li and Gong, 2012; Gan et al., 2013; Chen et al., 2014; Hu and Sheng, 2015; Liang, 2016; Wang J. et al., 2017; Wang X. et al., 2017; Cui, 2019; Yan et al., 2019; Liu and Gong, 2021; Xu and Zhou, 2022; Lu et al., 2023; Yang, 2023) or by addressing missing data through both per protocol and intention-to-treat analyses (Birling et al., 2022; Zhu et al., 2022). All RCTs were rated as having low RoB in the "selection of reported outcomes" domain. Overall, two RCTs were rated as having low RoB, one had some concerns, and the rest were considered as having high RoB (Figures 2A, B).

5.2.2 Assessing NRCTs quality: ROBINS-I indicates moderate to serious ROB

Regarding NRCTs, four (Yan et al., 2018; Kang, 2019; Liu, 2022; Ye and Lin, 2022) were assessed as having serious RoB in the "confounding" and moderate RoB in "deviations from intended interventions" domains because of potential impacts of confounding factors and intentional interventions on results. No bias existed in the participant selection. Given all participants completed the entire intervention without classification bias in this process, all included NRCTs were judged as low ROB in both "classification of interventions" and "missing data" domains. None of the NRCTs provided trial pre-registration information or described blinding of outcome assessors. Overall, one NRCT had moderate ROB, and the remaining were rated as having serious ROB (Figures 2C, D).

5.3 Analyses of outcome measures

5.3.1 ZRAS *vs* placebo-ZRAS: inconsistent findings detected

Three RCTs (*participants* = 385) addressed this comparison. Since none of the outcomes were utilized across all three studies, the results were only qualitatively described. Among these trials, two reported a significant reduction in PSQI scores with ZRAS ($p < 0.05$), highlighting its superiority over placebo-ZRAS ($p < 0.05$). However, another trial indicated a non-significant effect of ZRAS on ISI scores ($p > 0.05$), and found no statistical difference in ISI scores between ZRAS and placebo-ZRAS ($p > 0.05$). All three trials

acknowledged the safety and tolerability of ZRAS without any significant adverse events.

5.3.2 ZRAS *vs* hypnotic

Eleven trials (*participants* = 938) fell into this comparison and they were all RCTs. Meta-analyses were conducted for PSQI scores and clinical efficacy rate.

5.3.2.1 PSQI global scores: no significant differences detected

Ten trials (*participants* = 871) compared PSQI scores between ZRAS and hypnotic, revealing no significant differences [*MD* = −0.36, 95%*CI* (−1.53, 0.81), p = 0.55] (Figure 3). The *Z*-curve for PSQI in TSA exceeded the required information size (650), affirming the sufficiency of sample size in generating the current results (Supplementary Appendix S6).

Subgroup analyses were performed due to high heterogeneity. A significant interaction effect was identified between different hypnotics used in the controls (Chi^2 statistic 7.98, df = 1, p < 0.01). There was no significant difference between ZRAS and benzodiazepines in reducing PSQI scores [*MD* = 0.39, 95%*CI* (−0.12, 0.91), p = 0.13]. Whereas, ZRAS induced a more significant reduction in PSQI scores compared to Z-drugs [*MD* = −1.31, 95%*CI* (−2.37, −0.24), p = 0.02]. No interaction was noted in other subgroups (Supplementary Appendix S7).

Meta-regression indicated a potential and weak association of heterogeneity with study sample size (I^2 = 93.95%, Tau^2 = 1.29, p = 0.04), while publication year (I^2 = 96.15%, Tau^2 = 1.82, p = 0.26), therapeutic dosage (I^2 = 96.29%, Tau^2 = 1.80, p = 0.20), dosage forms of ZRAS (I^2 = 98.07%, Tau^2 = 2.30, p = 1.00), and hypnotics in the controls (I^2 = 86.08%, Tau^2 = 1.29, p = 0.07) could not explain heterogeneity (Supplementary Appendix S8).

Sensitivity analysis revealed minimal impact of individual trials on the pooled estimate effects of PSQI scores, suggesting overall robustness of the results (Supplementary Appendix S9).

5.3.2.2 Clinical efficacy rate: no significant differences detected

Eight trials (*participants* = 717) employed clinical efficacy rate as an outcome (Liu and Nan, 2009; Li and Gong, 2012; Gan et al., 2013; Chen et al., 2014; Liang, 2016; Wang J. et al., 2017; Wang X. et al., 2017; Zhu et al., 2022). However, no significant differences were found between the groups [*RR* = 1.05, 95%*CI* (0.96, 1.15), p = 0.31] (Figure 3).

5.3.3 ZRAS combined with hypnotic *vs* hypnotic

This category comprised 11 trials (*participants* = 884), involving RCTs and NRCTs. Meta-analysis was carried out for PSQI, clinical efficacy rate, and polysomnographically-recorded total sleep time, sleep onset latency and number of awakenings.

5.3.3.1 PSQI global scores: ZRAS combined with hypnotic significantly reduces PSQI scores compared to hypnotic alone

All trials, except one (Zhu et al., 2022), used PSQI scores as an outcome (*participants* = 817). Pooled analysis favored ZRAS combined with hypnotic in reducing PSQI scores [*MD* = −2.70, 95%*CI* (−3.22, −2.18), p < 0.01] (Figure 4). The adequacy of sample size in this comparison was validated by TSA (Supplementary Appendix S10).

Subgroup analyses identified a significant interaction effect between different study types (Chi^2 statistic 8.30, df = 1, p < 0.01). In both RCTs and NRCTs, combined therapies showed more significant effects than hypnotic alone in reducing PSQI scores, with no heterogeneity in RCT design (I^2 = 0) (Supplementary Appendix S11). No interaction was identified in other subgroups.

Meta-regression indicated a potential and weak link of heterogeneity to study type (I^2 = 72.27%, Tau^2 = 0.19, p = 0.03), but not to publication year (I^2 = 84.06%, Tau^2 = 0.27, p = 0.09), study sample size (I^2 = 88.71%, Tau^2 = 0.40, p = 0.60), therapeutic dosage (I^2 = 87.14%, Tau^2 = 0.38, p = 0.42), dosage forms of ZRAS (I^2 = 81.21%, Tau^2 = 0.30, p = 0.19), and hypnotics in the controls (I^2 = 81.62%, Tau^2 = 0.41, p = 0.76) (Supplementary Appendix S12).

Sensitivity analysis confirmed the overall robustness of the results (Supplementary Appendix S13).

5.3.3.2 Objective sleep parameters: ZRAS combined with hypnotic significantly improve sleep parameters compared to hypnotic alone

Total sleep time and sleep onset latency were assessed in four RCTs (*participants* = 318) (Yan et al., 2018; Kang, 2019; Ye and Lin, 2022; Yang, 2023); number of awakenings was assessed in three RCTs (*participants* = 232) (Yan et al., 2018; Ye and Lin, 2022; Yang, 2023). These parameters, collected via polysomnography, favored ZRAS combined with hypnotic in increasing total sleep time [*MD* = 40.72 *min*, 95%*CI* (25.14, 56.30), p < 0.01], shortening sleep onset latency [*MD* = −4.44 *min*, 95%*CI* (−7.98, −0.91), p = 0.01], and reducing number of awakenings [*MD* = −0.89 *times*, 95%*CI* (−1.67, −0.10), p = 0.03] (Figure 4). Subgroup analyses for total sleep time and sleep onset latency found no interactions.

5.3.3.3 Clinical efficacy rate: ZRAS combined with hypnotic significantly improve clinical efficacy rate compared to hypnotic alone

Ten RCTs (*participants* = 858) used clinical efficacy rate as an outcome. The results favored ZRAS combined with hypnotic, showing a higher clinical efficacy rate than administering hypnotic alone [*RR* = 1.23, 95%*CI* (1.16, 1.31), p < 0.01] (Figure 4).

5.3.4 ZRAS *vs* CBT-i, or ZRAS combined with CBTi *vs* CBTi: no trials addressed these comparisons

No trials were under these two comparisons.

5.4 Publication bias test

We performed publication bias test based on PSQI in both comparisons, revealing statistically significant effect (p < 0.01 for ZRAS *Vs.* hypnotic; and p = 0.02 for ZRAS combined with hypnotic *Vs.* hypnotic) (Supplementary Appendix S14).

5.5 Certainty and quality of evidence

The certainty and quality of evidence from meta-analyses of seven outcomes are outlined in Supplementary Appendix S15. In

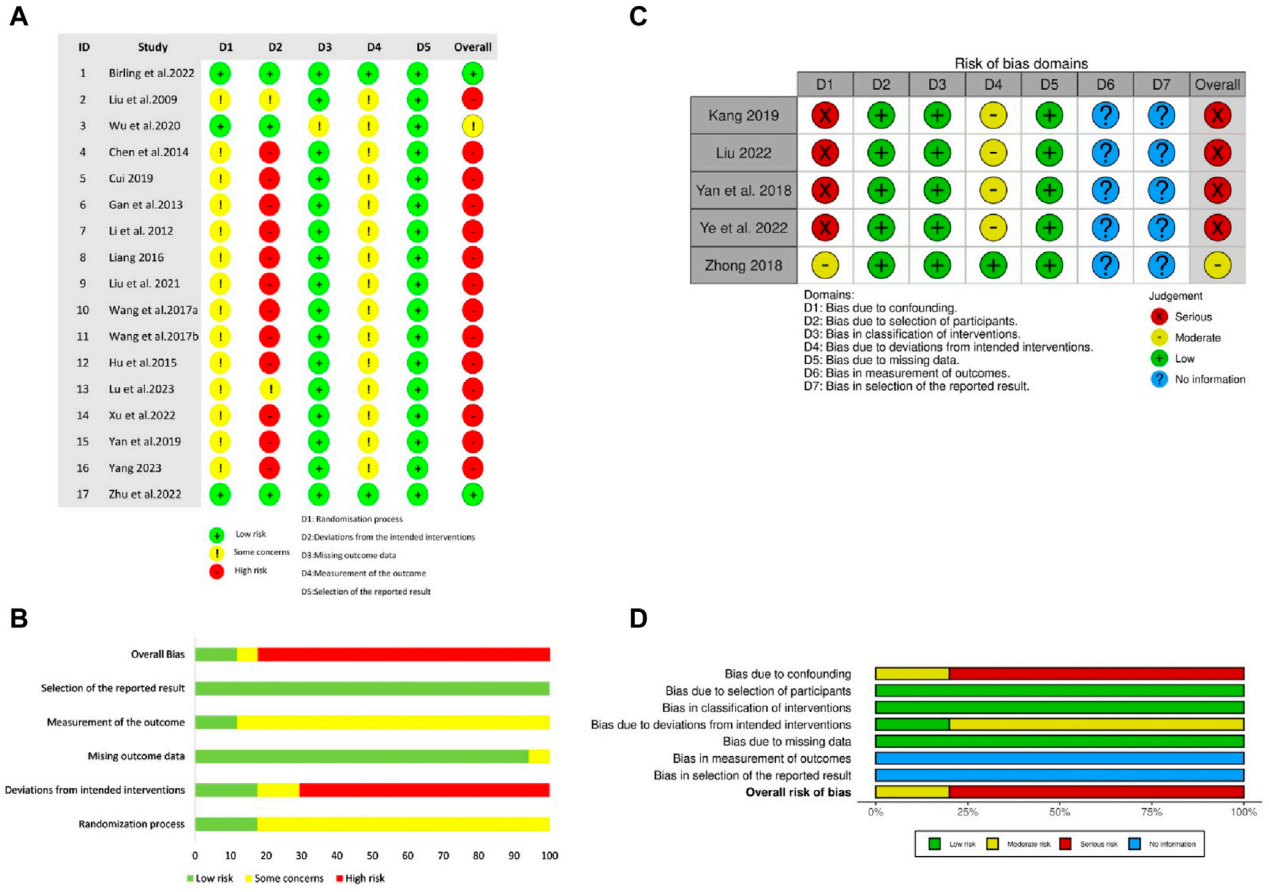

FIGURE 2
The risk of methodological bias in the included studies **(A)** Risk of Bias summary for RCTs **(B)** Risk of Bias graph for RCTs. The methodological quality of RCTs was appraised using Revised Cochrane Risk of Bias tool for randomised trials (ROB 2.0) **(C)** Risk of Bias graph for NRCTs **(D)** Risk of Bias summary for NRCTs. The methodological quality of NRCTs was appraised using Cochrane Risk of Bias in Non-randomized Studies-of Interventions (ROBINS-I).

pursuance of the GRADE system, the quality of evidence ranged between very low and low ratings, with six outcomes rated as "Very low" and one rated as "Low." The predominant factor contributing to degradation was the risk of bias within the trials included. Furthermore, some of the included trials were NRCT rather than RCT designs, contributing to initial degradation.

6 Discussion

6.1 Summary of findings

Our review reflected the current knowledge state regarding using ZRAS for PI. ZRAS was comparable to benzodiazepines but superior to Z-drugs in reducing PSQI global scores. Compared to hypnotics alone, the addition of ZRAS further reduced PSQI scores by 2.2–3.2 points, which was clinically significant (Zhao et al., 2022). Such addition of ZRAS also further extended total sleep time, shortened sleep onset latency and reduced number of awakenings. The cumulative sample size for meta-synthesis was sufficient. Nevertheless, the evidence supporting these positive results had very-low-to-low quality due to insufficient blinding and underuse of RCT design. Comparative efficacy between ZRAS and CBT-i, or combined ZRAS and CBT-i versus CBT-i alone, could not be determined due to lack of available data. ZRAS demonstrated good tolerability with AEs markedly lower than those associated with hypnotics; all ZRAS-related AEs were under 3%, except for fatigue, which reached 6.2%. Overall, ZRAS is safe for management of PI, while its efficacy cannot be definitely concluded due to quality shortfalls in most of the included trials.

6.2 Strengths, limitations, and comparison with previous systematic reviews

We noticed four Chinese (Ji et al., 2019; Yuan, 2019; Wang et al., 2020; Zhu and Wang, 2020) and two English (Birling et al., 2020; Chen et al., 2020) available systematic reviews and/or meta-analyses with similar theme. Five of them only reviewed ZRAS capsules, omitting other dosage forms (Ji et al., 2019; Yuan, 2019; Chen et al., 2020; Wang et al., 2020; Zhu and Wang, 2020). In these reviews, inappropriate inclusion of trials on compounded ZRAS capsule, those lacking valid diagnostic criteria, and/or those without internationally recognized sleep scales/questionnaires for

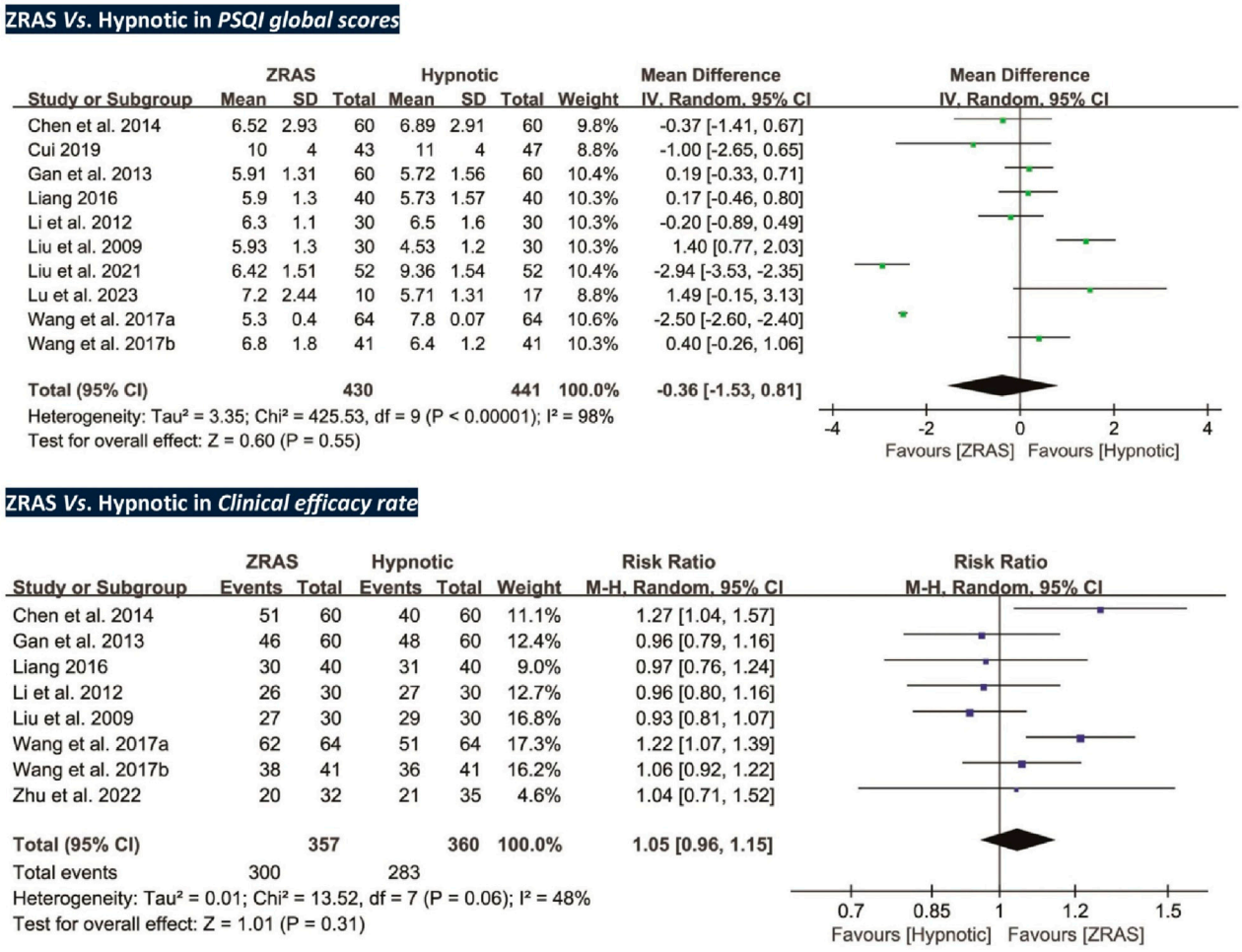

FIGURE 3
Forest plots of PSQI global scores and clinical efficacy rate (ZRAS *vs.* Hypnotic).

quantitative synthesis also introduced extra variability, making it difficult to interpret the results. The remaining one review even included studies targeting insomnia secondary to schizophrenia and hypertension (Birling et al., 2020), further complicating interpretation. All six reviews were conducted at least 5 years ago, whereas our study incorporated the latest evidence from recent years.

Our review, with updated retrieval and stricter selection criteria, included more trials and reduced variability. Other strengths included: 1) we provided evidence that ZRAS enhanced objective sleep parameters, a critical clinical issue inadequately addressed in previous reviews; 2) we appraised the methodology in different types of trial using the eligible tools, an aspect ignored in two earlier reviews (Ji et al., 2019; Birling et al., 2020); and 3) the GRADE framework, employed in our review, was not previously used in any ZRAS-related reviews to assess evidence quality.

However, limitations exist. First, the poor methodological quality of included trials seriously undermines evidence reliability. Second, high heterogeneity could not be fully explained despite use of subgroup, meta-regression and sensitivity analyses. This, however, does not imply that low-quality trials should be excluded. A previous methodological study highlighted that there was a danger with any approach to excluding trials from systematic review because there was no clear-cut distinction between high- and low-quality trials, and reviewers can only know that they are susceptible to bias but can never know for sure whether a trial is biased (Harvey and Dijkers, 2019). Furthermore, TSA analysis demonstrated that the sample size for meta-synthesis was sufficient; and subgroup, meta-regression, and sensitivity analyses confirmed the robustness of the results, i.e., none of the potential influencing factors altered the overall findings. Finally, 21/22 (95.5%) trials included were conducted in China, possibly inflating effectiveness of TCM therapies due to cultural confidence among patients (Zhao et al., 2022). Chinese researchers might also be more inclined to report positive findings. This is evidenced by the significant publication bias we identified (Supplementary Appendix S14). The applicability of present findings beyond Chinese communities is limited. ZRAS has been listed with the Australian Registry of Therapeutic Goods under the name "Zao Ren An Shen" (AUST L301484) (Birling et al., 2022), providing a foundation for studying its applicability to diverse consumer populations.

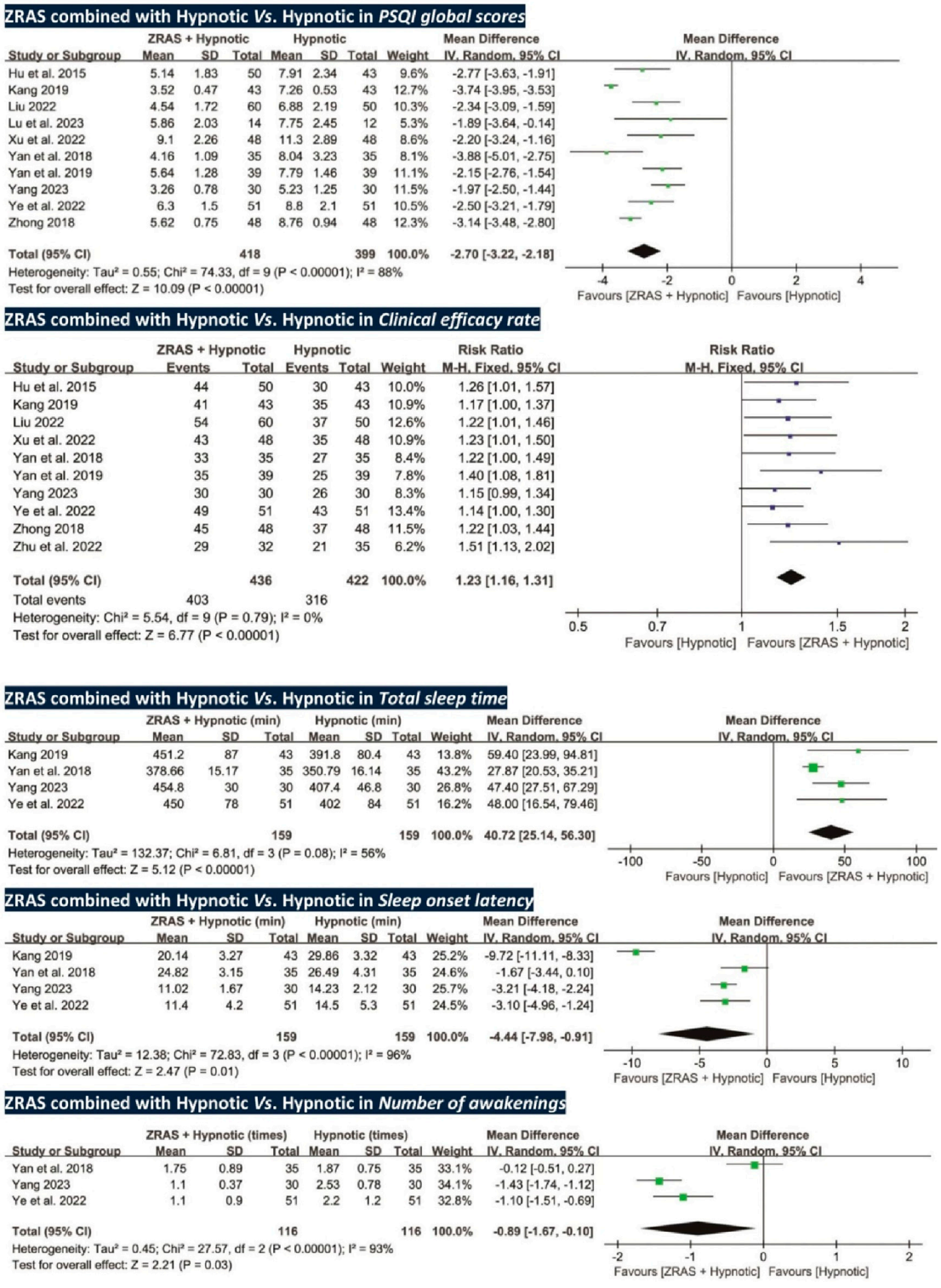

FIGURE 4
Forest plots of PSQI global scores, clinical efficacy rate, and polysomnography -recorded total sleep time, sleep onset latency and number of awakenings (ZRAS$^+$ Hypnotic vs. Hypnotic).

6.3 Interpretation of findings

CBT-i is the front-line treatment for PI (Zhao et al., 2021), yet its limited accessibility makes CAM remedies more appealing in some regions where traditional medicine practices are widely accepted (Ell et al., 2023). Chinese medicines have been used to manage insomnia for over 2,000 years (Ni et al., 2015). A National Health Interview Survey estimates that over 1.6 million adults in United States use

CAM to manage insomnia or trouble sleeping (Pearson et al., 2006). Furthermore, the use of botanical drugs was found to be the most prevalent and popular, with 49.2% users reporting significant improvement (Pearson et al., 2006). No studies available compared ZRAS with CBT-i, warranting further investigation into their comparative efficacy. Benzodiazepines and Z-drugs, most commonly prescribed for insomnia, raise concerns regarding dependency and safety (Leach and Page, 2015). Benzodiazepines may cause dizziness, headaches, lethargy, nightmares, daytime fatigue, ataxia, nausea, and/or falls; and Z-drugs are associated with dizziness, somnolence, falls, headaches and migraine, nausea and emesis, and/or diarrhea (Leach and Page, 2015; Madari et al., 2021). Some of these AEs were confirmed in our review (Supplementary Appendix S5). CCPP, as a CAM product, also has some adverse drug reactions (Chan et al., 2015). However, the reported AEs caused by even combined ZRAS and hypnotics were fewer than those of hypnotics, implying a clinically valuable option of adding ZRAS to benzodiazepines/Z-drugs to optimize effectiveness while minimizing AEs. Such co-administration of conventional and Chinese medicines has already been established as a routine modality in modern China for managing sleep disturbances (Ni et al., 2015). While this model remains less prevalent in Western nations, our findings provide valuable insights from China's experience for policymakers of these countries to seriously consider the WHO's recommendation to integrate traditional medicine into the national healthcare system (von Schoen-Angerer et al., 2023). Moreover, standardization in preparation endows CCPP with advantages such as stable quality, heightened safety, rapid absorption, and enhanced bioavailability (Zhang et al., 2020). Compared to Chinese medicinal decoctions, CCPP not only offer greater convenience for patients in terms of administration, transportation, and storage (Zhang et al., 2020), but they also facilitate prescription issuance for physicians (Chen et al., 2012). These advantages render CCPP more suitable for promotion in Western countries, including the United States and European Union member states (Chen et al., 2012).

By using the sleep-deprived animal models, the possible mechanisms of ZRAS in improving PI have been investigated. Studies have found that ZRAS reduced the sleep onset latency (Chen and Shan, 2020) and wake after sleep onset (Wang et al., 2023), while prolonged the total sleep time (Wang et al., 2023) of sleep-deprived rodents. This improvement in sleep quality was associated with reduced dopamine (Li, 2020), increased serotonin (Li, 2020), and/or increased adenosine A1/A2 receptor levels (Chen and Shan, 2020) in the brain of the rodents. Additionally, network pharmacology analyses demonstrated that ZRAS improved sleep through multiple targets and pathways, including the modulation of neurotransmitters (Yang et al., 2020; Zhu, 2021), protein phosphorylation (Zhu, 2021), and tryptophan metabolism (Yang et al., 2020).

Studies have also focused on the individual metabolites that ZARS contains. For example, jujubosides, jujuboside A, and jujuboside B, which are saponins from Ziziphi spinosae semen, have been reported to have sedative and hypnotic effects (Bian et al., 2021). The hypnotic effect of jujubosides is believed to be mediated through the serotonergic system (Cao et al., 2010). Extracts from Salviae miltiorrhizae radix et rhizoma have also been documented to exert sedation effect (Lobina et al., 2018). Tanshinones, the principal bioactive metabolites of Salviae miltiorrhizae radix et rhizoma (Yang et al., 2019), demonstrate significant sedative-hypnotic effects (Fang et al., 2010). In addition, animal studies demonstrated that schizandrin, a major metabolite of Schisandrae chinensis fructus, could pass through the blood-brain barrier and exhibited sedative and hypnotic bioactivity, potentially mediated by its modulation of the serotonergic system (Zhang et al., 2018). Zheng *et al.* had investigated the synergistic mechanism of multiple metabolites in the combination of Ziziphi spinosae semen and Schisandrae chinensis fructus for insomnia treatment. Utilizing network pharmacology-based and molecular docking approach, the research group identified 41 target-disease related genes of this combination and found that neuroactive ligand-receptor interactions is a key mechanism underlying the efficacy of this drug combination in treating insomnia (Zheng et al., 2022). Similarly, another animal study revealed a synergistic effect of combining Ziziphi spinosae semen and Salviae miltiorrhizae radix et rhizoma. This would enhance sleep duration and reduce sleep onset latency in rodent models (Fang et al., 2010).

Only three reviewed trials included follow-ups (two- or 4-week) (Birling et al., 2022; Zhu et al., 2022), hindering the determination of the medium-to-long-term efficacy of ZRAS. Investigating issues commonly occur in hypnotics—such as dependence, withdrawal symptoms, and rebound insomnia (Voshaar et al., 2004; Schifano et al., 2019)—when using ZRAS is essential. While few trials included suggested that ZRAS alleviated concomitant symptoms of PI, such as fatigue (Liu and Gong, 2021), anxiety (Kang, 2019; Liu and Gong, 2021) and depression (Kang, 2019), inadequate data impedes quantitative analysis. Studying ZRAS's potential to address these accompanying symptoms is crucial, particularly for CAM users with motivation for more holistic care by addressing multivariate symptoms (Satija and Bhatnagar, 2017; Kemppainen et al., 2018). This appears to be promising because TCM tends to concentrate on the overall functional wellbeing rather than the disease defined by specific pathological changes only (Jiang, 2005). Previous clinical (Yu et al., 2022) and pre-clinical (Wang et al., 2021) studies also showed that ZRAS exerted both anxiolytic and sleep-promoting effects in patients/animal models with comorbid anxiety and insomnia.

The Australian study previously cited was the sole trial suggesting that ZRAS might not effectively improve PI (Birling et al., 2022). The investigators claimed that TCM syndrome pattern did not influence treatment outcome, as there was no statistical difference in TCM pattern scores between ZRAS and placebo-ZRAS after treatment (Birling et al., 2022). However, we hold a different perspective. Syndrome differentiation-based treatment is a fundamental principle guiding Chinese medicine prescriptions (Jiang et al., 2012), and is believed to provide better efficacy (Yeung et al., 2012). The PPRC (https://ydz.chp.org.cn/#/main) states that ZRAS is best suited for insomnia with "deficiency of *Heart*-blood" pattern. In the Australian study: 1) not all patients met this pattern; 2) the pattern was diagnosed by a single investigator and quantified using a self-reported questionnaire without psychometric properties; 3) the statistical power was insufficient to infer the pattern's impact on efficacy, considering only 38 patients with multiple patterns in the experimental group (Birling et al., 2019; Birling et al., 2022). Evidently, factors influencing the trial design for Chinese medicine may not solely stem from differences between Eastern and Western cultures, but also from disparities among scholars' fundamental understandings of TCM, particularly regarding the awareness of syndrome

differentiation. Modern western medicine only emphasizes the necessity of clear diagnosis for treatment, while the application of Chinese medicine requires not only a clear diagnosis of the disease but also of the associated TCM syndromes. Even if a patient is diagnosed with PI, for instance, different TCM syndromes may necessitate distinct treatments. For instance, The *Pharmacopoeia* (https://ydz.chp.org.cn/#/main) explicitly states that for PI (deficiency of *Heart*-blood), ZRAS are recommended, whereas for PI patients diagnosed as "deficiency of *Kidney* essence" and "stagnation of *Qi* due to depression of the *Liver*," An Shen Bu Nao solution and Jie Yu An Shen granules are respectively recommended. In addition to randomization, double-blinding and placebo-control design, future studies should also focus on participants with fixed pattern (deficiency of *Heart*-blood) and increase sample size to minimize statistical error.

Although ZRAS may be a promising alternative option for PI, the current favorable findings should be approached cautiously due to certain concerns about their reliability. First, most of the trials (n = 19, 86.4%) did not report any attrition (Figure 2), which is unusual in insomnia-related clinical trials (Birling et al., 2020). Secondly, only two included trials implemented adequate blinding. Adequate blinding in RCTs for participants, personnel and assessors is essential to minimize risk of bias, especially when primary outcomes relied heavily on subjective assessments by participants or clinicians. Even objective outcomes such as polysomnography-measured parameters can be compromised as it is unclear whether the technicians who interpreted the measurements were aware or not of the allocation (Birling et al., 2020). Unlike acupuncture trials, achieving proper blinding with placebos in drug trials is feasible. Whereas, there are also emerging perspective advocating for unblinded pragmatic trials due to their emphasis on practical applicability and generalizability, which can improve the external validity of real-world trials beyond treatment effects, although this approach is a major contributor to the risk of bias (Sox and Lewis, 2016). To reconcile these differing views, we recommend conducting blinded RCTs as well as unblinded real-world studies separately. Combining results from both research paradigms may lead to a more reliable and impartial assessment of ZRAS. Finally, the dearth of pre-registration information compromised the transparency of the trial results. This is also a primary factor contributing to publication bias risk, as it leaves open the possibility of selective reporting by authors. Therefore, pre-registration and protocol uploading in future studies are necessary.

7 Conclusion

The studies reviewed in this analysis demonstrate a preference for ZRAS over benzodiazepines and Z-drugs in terms of short-term efficacy. Moreover, combining ZRAS with benzodiazepines/Z-drugs has also yield greater therapeutic effect with fewer AEs. However, the reliability of these findings is significantly compromised by the methodological drawbacks of the included studies. Furthermore, the limited number of trials providing follow-up data and/or assessing accompanying symptoms of PI, such as fatigue, depression, anxiety, etc., precludes quantitative analysis. Therefore, the medium-to-long-term efficacy of ZRAS in treating PI and associated symptoms remains uncertain. Given the potential benefits of ZRAS, well-designed RCTs with extended follow-up periods and comprehensive outcomes are warranted. Nevertheless, the current body of evidence does not offer sufficient support to conclusively endorse the use of ZRAS for PI treatment.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

Author contributions

F-YZ: Conceptualization, Formal analysis, Investigation, Methodology, Writing–original draft. PX: Data curation, Software, Writing–original draft. GK: Conceptualization, Writing–review and editing. ZZ: Methodology, Writing–review and editing. W-JZ: Formal analysis, Writing–review and editing. J-YZ: Investigation, Writing–review and editing. Y-SH: Supervision, Writing–review and editing. L-PY: Validation, Writing–review and editing. Q-QF: Formal analysis, Methodology, Visualization, Writing–review and editing. RC: Project administration, Writing–review and editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was supported by Health and Medical Research Fund [20212801], Health Bureau, Hong Kong SAR to YS-H; Shanghai Key Laboratory for Pharmaceutical Metabolite Research Project [SHZYDX 2023–01] to FY-Z; TCM Research Project, Yangpu District Health Commission [YPZYQ202301] to JY-Z; and TCM Research Project, Shanghai Municipal Health Commission [2022CX007] to WJ-Z.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2024.1376637/full#supplementary-material

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TYPE Review
PUBLISHED 26 June 2024
DOI 10.3389/fphar.2024.1359890

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RECEIVED 22 December 2023
ACCEPTED 29 May 2024
PUBLISHED 26 June 2024

CITATION
Ain QT, Saleem N, Munawar N, Nawaz R, Naseer F and Ahmed S (2024), Quest for malaria management using natural remedies. *Front. Pharmacol.* 15:1359890. doi: 10.3389/fphar.2024.1359890

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Quest for malaria management using natural remedies

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Malaria, transmitted through the bite of a *Plasmodium*-infected *Anopheles* mosquito, remains a significant global health concern. This review examines the complex life cycle of *Plasmodium*, emphasizing the role of humans and mosquitoes in its transmission and proliferation. Malarial parasites are transmitted as sporozoites to the human body by biting an infected female *Anopheles* mosquito. These sporozoites then invade liver cells, multiply, and release merozoites, which infect red blood cells, perpetuating the cycle. As this cycle continues, the affected person starts experiencing the clinical symptoms of the disease. The current treatments for malaria, including chloroquine, artemisinin-based combination therapy, and quinine, are discussed alongside the challenges of drug resistance and misdiagnosis. Although efforts have been made to develop a malarial vaccine, they have so far been unsuccessful. Additionally, the review explores the potential of medicinal plants as remedies for malaria, highlighting the efficacy of compounds derived from *Artemisia annua*, *Cinchona* species, and *Helianthus annuus* L., as well as exploration of plants and phytocompounds like cryptolepine, and isoliquiritigenin against drug-resistant *Plasmodium* species. Moreover, studies from Pakistan further highlight the diverse vegetal resources utilized in malaria treatment, emphasizing the need for further research into natural remedies. Despite the advantages of herbal medicines, including cost-effectiveness, and fewer side effects; their limitations must be taken into account, including variations in potency and potential drug interactions. The review concludes by advocating for a balanced approach to malaria treatment and prevention, emphasizing the importance of early detection, accurate diagnosis, and integrated efforts to combat the disease in the endemic regions.

KEYWORDS

malaria, natural products, *Artemisia*, *Cinchona*, drug resistance

1 Introduction

Malaria is one of the ancient diseases of humanity. Its historical records can be traced back as early as 2700 BC. By the early 1800s, malaria had become a global scourge, afflicting people across the world (Garcia, 2010). Despite scientific and medicinal advancements, malaria is still endemic in over 90 countries, and around 2 billion people are at risk of developing this infection annually (Finnigan, 2023). In the malaria hotspots, it is one of the leading causes of mortality, causing more than 1 million deaths worldwide (Pradines and Robert, 2019).

Malaria is a parasitic infection transmitted to humans by the *Anopheles* mosquito. When a mosquito bites a human, the parasite responsible for causing malaria is transmitted to the human body. *Plasmodium* exists in different forms, but only *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, and *Plasmodium malariae* are considered infectious. Among them, *P. falciparum* causes the most severe disease (Talapko et al., 2019). Malaria symptoms include headache, fever, myalgia, vomiting, and diarrhea. A severe infection can result in life-threatening conditions such as encephalopathy, high-grade fever, and gastroenteritis (Bartoloni and Zammarchi, 2012).

In endemic areas, malaria significantly impacts not only the health of individuals but also their socioeconomic profile. Due to poor living conditions and nutrition status, malaria disproportionately affects impoverished communities, exacerbating the challenges faced by vulnerable social groups (Suh, Kain, and Keystone, 2004). Malaria stands as one of humanity's most fatal afflictions. The horrors unleashed by this ancient disease persist to the present day. Unfortunately, an effective and potent cure for the infection continues to elude us. The challenges span from complexities of early detection of malaria to obstacles to formulating a suitable remedial strategy, collectively presenting a formidable hurdle in its treatment.

Quinine was the first drug for malaria treatment (Weinke et al., 1992). However, with advancements in medicine, treatment options have also evolved. Currently, chloroquine, artemisinin, proguanil, primaquine, mefloquine, and sulfonamides are used to manage malaria (Gilles and Warrell, 1996). However, there is still no empirical drug for it, as these drugs are plagued by resistance and undesirable side effects (Castelli, Tomasoni, and Matteelli, 2012).

Furthermore, homology modeling and molecular dynamics simulations provide valuable insights into *P. falciparum* chloroquine resistance transporter (*PfCRTs*) and its interactions with chloroquine (Patel and Roy, 2021). *In vitro* assays reveal increased IC_{50} (a measure of potency) values for mefloquine, suggesting diminished parasite susceptibility. Moreover, the concurrent emergence of resistance to artesunate, coupled with mefloquine resistance, underscores the urgent requirement for alternative approaches to malaria treatment (Rogers et al., 2009b).

For centuries, natural products have been utilized in the treatment of various conditions. This is particularly true for malaria because many anti-malaria drugs are derived from plants, including quinine and artemisinin, which are crucial in malaria control (Kingston and Cassera, 2022). However, the reduced effectiveness of the old therapies due to various reasons including the emergence of resistance in the parasite necessitates the search for newer agents to treat malaria. This article aims to review natural remedies that can be used to treat malaria including the resistant strains while emphasizing the challenges faced by the empirical class of drugs.

2 Epidemiology

According to the World Health Organization's annual report on malaria, there were more than 240 million malaria cases worldwide. From 2000 to 2021, around 2 billion people were estimated to have suffered from the infection. However, The number of total malaria cases globally increased in 2021, from 245 million in 2020 to 247 million in 2021 (WHO, 2023a; 2022). Most cases are concentrated in the African region (95%), with around 2% in the Southeast Asian region. Approximately 29 countries accounted for 96% of malarial deaths (Figures 1,2; Table 1).

A significant burden of malaria cases is shared by four countries, namely, Nigeria (27%), the Democratic Republic of Congo (12%), Uganda (5%), and Mozambique (4%) (Commission, 2023). Similarly, around 96% of deaths due to malaria occurred in four countries, i.e., Nigeria, the Democratic Republic of Congo, Niger, and the United Republic of Tanzania (WHO, 2023a). According to the WHO, more than 94% of malaria cases (233 million) and 95% of deaths occurred in the African region (Organization, 2023).

However, the epidemiology of malaria in India is distinct due to several features of the *Plasmodium* parasites, *Anopheles* vectors, ecoepidemiological aspects conducive to disease transmission, and susceptible humans living in rural and forested areas (Khan MuhammadImran et al, 2023). Notably, in 2021, India accounted for 79% of malaria cases and about 82.4% of all malaria deaths in the South-East Asia Region (Organization, 2022).

Lastly, a systematic review for finding out the prevalence of malaria (from 2001 to 2021) estimated pooled malaria prevalence in Pakistan to be 23.3%, with *P. vivax*, *P. falciparum*, and mixed infection rates of 79.13%, 16.29%, and 3.98%, respectively. Moreover, the analysis marked Karachi as the hotspot for malaria with a prevalence rate of 99.79% (Khan Nikhat et al., 2023). This data suggests that malaria is still endemic in most parts of the world. Although the infection rates have decreased over the years, it remains one of the most challenging health problems faced by the global south.

3 Pathophysiology of malaria

The life cycle of *Plasmodium* involves humans and mosquitoes, where the human body acts as a site for asexual reproduction, while mosquitoes are involved in the sexual stage of their lifecycle (Figure 3). Mosquitoes serve as vectors and transmit *Plasmodium* to humans, where it causes malaria (Giribaldi et al., 2015). *Plasmodium* is transmitted to humans by the female *Anopheles* mosquito. There are more than 450 species of *Anopheles*, but about 68 are involved in the transmission of the disease (Giribaldi et al., 2015).

Once it bites a human, the sporozoite form of the parasite is transmitted to the host.

The sporozoite form circulates via the bloodstream and resides in the liver, leading to malaria infection (Menard, 2005). The sporozoites undergo asexual reproduction, resulting in merozoites. This stage is categorized as the "liver stage" with no clinical symptoms. In the case of *P. falciparum* and *P. malariae*, the merozoites are released into the bloodstream, leading to the erythrogenic stage, where malaria infection symptoms start appearing. In the case of *P. vivax* and *P. ovale*, the parasite may enter a dormant hypnozoite stage, significantly increasing the risk of reinfection (Markus, 2011). As the merozoites invade RBCs, they rapidly grow and develop into ring-like forms called "trophozoites" that can be identified via Giemsa staining (Giribaldi et al., 2015).

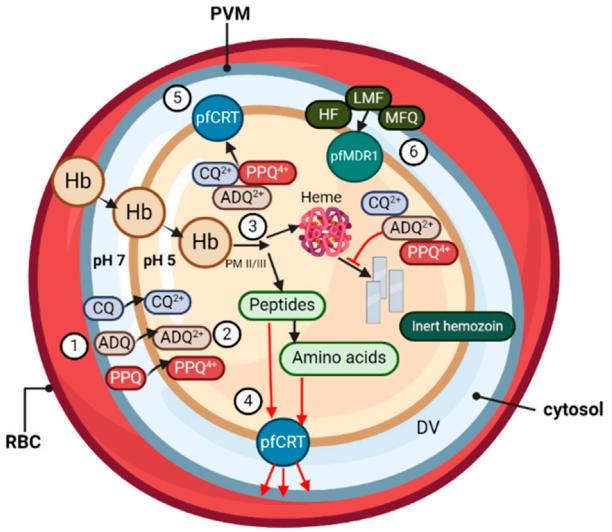

FIGURE 1
During its asexual blood stage, the *P. falciparum* parasite, surrounded by its parasitophorous vacuole membrane (PVM), develops within the host RBCs. (❶) Quinoline-based antimalarials, including chloroquine (CQ), amodiaquine (ADQ), and piperaquine (PPQ), concentrate from the parasitic cytosol (pH ~7) into the digestive vacuole (DV) (pH ~5.2). (❷) Once inside the DV, weak-base drugs are protonated, unable to passively diffuse out through the DV membrane. (❸) Protonated drug molecules bind to toxic heme by-products and grooves on hemozoin crystals in the DV, resulting in >1,000-fold drug accumulation due to pH trapping and heme-binding. (❹) The DV membrane protein *PfCRT* is believed to be involved in transporting peptides released from hemoglobin digestion into the parasite cytosol. (❺) In drug-resistant parasites, mutations in *PfCRT* enable the efflux of protonated drug molecules out of the DV, away from their heme target. (❻) DV membrane transporter *PfMDR1* mutations influence parasite susceptibility by redirecting drugs such as halofantrine (HF), lumefantrine (LMF), and mefloquine (MFQ) into the DV, away from their primary site of action.

4 Current treatments

The treatment and management of malaria depend upon accurate and timely diagnosis since its symptoms can differ significantly from one person to another (Bartoloni and Zammarchi, 2012). The clinical objectives of treating uncomplicated malaria are to cure the infection as rapidly as possible and to prevent progression to severe disease. "Cure" is defined as the elimination of all parasites from the body (WHO, 2023b). Current guidelines recommend that non-*falciparum* malaria should be treated with a 3-day course of oral chloroquine and oral analgesics (Chiodini et al., 2007). Primaquine is considered the most effective drug to eliminate *P. vivax* and *P. ovale*, even for the dormant hypnozoites in the liver (Wångdahl et al., 2022). Artemisinin-based combination therapy is used to treat uncomplicated *P. falciparum* malaria infection and the artemisinin-lumefantrine combination is a drug of choice for its treatment (McIntosh et al., 1996). Severe *falciparum* infection is treated with intravenous (IV) drugs until the patient can be switched to oral drugs. The treatment for severe malaria in adults and children is IV artesunate (Trampuz et al., 2003). The chemical structures of some of these drugs are provided in Figure 4.

Furthermore, treatment options for pregnant women and children afflicted with malaria are different and should be used with caution to avoid undesirable side effects (Whitty, Edmonds, and Mutabingwa, 2005). In addition, severe malaria infections in pediatrics should be treated with empirical broad-spectrum antibiotics. In case of uncomplicated infection, a combination of artemether-lumefantrine can be utilized as the first line of treatment (Chiodini et al., 2007). In the first trimester of pregnancy, uncomplicated malarial infection should be treated with quinine-clindamycin combination therapy. In the second and third trimesters of pregnancy, artemether-lumefantrine treats uncomplicated *P. falciparum* malaria (Whitty, Edmonds, and Mutabingwa, 2005). For severe malaria, in any trimester of pregnancy, artesunate is usually preferred over quinine. Severe malarial infection by *P. falciparum* in pregnancy can potentially result in stillbirth or early delivery, so it's advisable to start the treatment as early as possible, under the supervision of a specialist doctor (Nunes and Scherf, 2007).

Furthermore, in patients co-infected with HIV/AIDS and uncomplicated *P. falciparum* malaria, artesunate + sulfadoxine-pyrimethamine is not recommended if they are being treated with co-trimoxazole. Additionally, artesunate + amodiaquine is not recommended if they are being treated with efavirenz or zidovudine (WHO, 2023a; Obeagu et al., 2024). Another critical treatment case is malaria-tuberculosis co-infection. Severe drug interaction, for example, rifampicin-induced liver injury may be worsened by amodiaquine-containing artemisinin-based combination therapy, complicating the treatment plan (Adedeji et al., 2018). Currently, there is insufficient evidence to modify the existing dosing recommendations based on mg/kg body weight. However, due to the higher risk of recrudescent malaria infections in these patients, close monitoring is advised (World Health, 2023).

Moreover, the November 2022 update to treatment recommendations for malaria includes several key points. Firstly, it suggests the use of artesunate-pyronaridine as an artemisinin-based combination therapy option for treating uncomplicated *P. falciparum* malaria, despite low-certainty evidence. Additionally, pregnant women diagnosed with uncomplicated *P. falciparum* malaria during the first trimester are advised to undergo treatment with artemether-lumefantrine, again supported by a strong recommendation despite low certainty evidence. Additionally, to prevent relapse in cases of *P. vivax* or *P. ovale* malaria in children and adults (with specific exceptions), primaquine at a dosage of 0.5 mg/kg/day for 7 days was strongly recommended. Conversely, the update advises against using primaquine at a dosage of 1.0 mg/kg/day for 7 days to prevent relapse in *P. vivax* or *P. ovale* malaria, based on a conditional recommendation and very low-certainty evidence (Organization, 2023).

In addition to antipyretics, like acetaminophen, and anti-emetics, anti-seizure medications are also advised. Importantly, generalized seizures are more common in children with *P. falciparum* malaria than in those with malaria caused by other species. Usually, parenteral or rectal benzodiazepines or intramuscular paraldehyde are given. However, prophylactic use of anti-convulsants has no firm empirical foundation in otherwise uncomplicated malaria and therefore is not recommended (World Health, 2023).

5 Challenges associated with current treatments

One of the main challenges faced by clinicians in malaria treatment is drug resistance developed by *Plasmodium*. *P.*

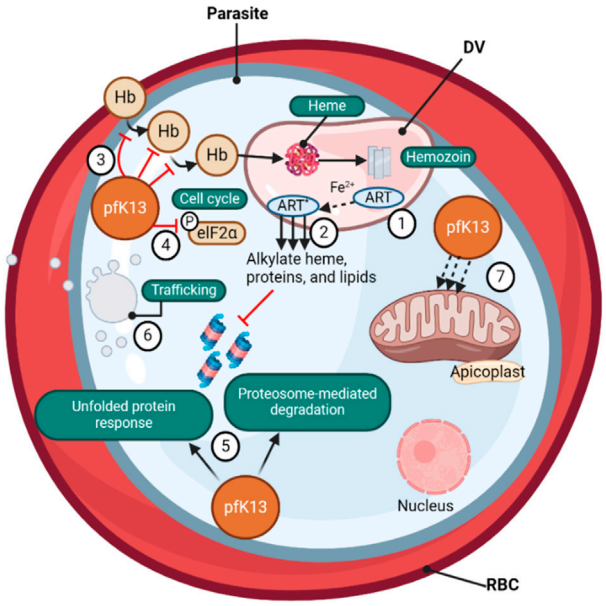

FIGURE 2
(❶) *Artemisinin*-based (ART) drugs are activated by cleavage of their endoperoxide by iron protoporphyrin IX (Fe^{2+}-heme), a product of parasite-digested hemoglobin. (❷) The Fe^{2+}-heme- ART carbon-centered radicals alkylate and damage a multitude of parasite proteins, heme, and lipids and inhibit proteasome-mediated protein degradation. *PfK13* mutations, located primarily in the β-propeller kelch domain, confer ART resistance. (❸) The loss of *PfK13* function provided by mutations has been shown to cause reduced endocytosis of host Hb and (❹) to extend the duration of ring-stage development, perhaps via PK4-mediated eIF2α phosphorylation, resulting in lowered levels of Hb catabolism and availability of Fe^{2+}-heme as the drug activator, reducing ART activation (❺) *PfK13* mutations activate the unfolded protein response, maintain proteasome-mediated degradation of polyubiquitinated proteins in the presence of ART, (❻) remove drugs and damaged proteins through an increase in PI3K-mediated vesicular trafficking. (❼) *PfK13* may also help regulate mitochondrial physiology and maintain membrane potential during drug-induced ring-stage quiescence. The asterisk signifies the activated form of ART (Wicht, Mok, and Fidock, 2020).

falciparum, responsible for severe malarial infection, has developed resistance to many drugs, including chloroquine, mefloquine, and quinine (Vestergaard and Ringwald, 2007). Chloroquine is considered the most cost-effective drug for malaria management, but recent data suggests that the treatment failure rate with chloroquine is more than 70% due to rapid drug-resistance development (Vestergaard and Ringwald, 2007). Amodiaquine, an essential part of the drug combinations used for treating malaria, is also facing the same threat.

Moreover, malaria misdiagnosis is another major challenge in combatting the epidemic. Accurate use of diagnostic procedures may not only lead to early infection treatment but also help select the correct antimalarial drug regimen against a particular pathogen (Shahbodaghi and Rathjen, 2022).

Vector control is one of the bigger challenges in the fight against malaria. In addition to outdoor transmission, growing levels of insecticide resistance in targeted vectors threaten the efficacy of long-lasting insecticidal nets and indoor residual spraying. These methods reduce malaria incidence but generally have little impact on malaria prevalence. Larvicidal treatments can be useful but are not recommended for rural areas. The research needed to improve the quality and delivery of mosquito vector control (Benelli and Beier, 2017). Due to the drug-drug interaction of antimalarial drugs, caution is always needed before prescribing. When treating malaria in someone who is also taking anti-tuberculosis medications, one must be careful to prevent harmful drug interactions. This is because the anti-tuberculosis drugs, such as rifampicin and isoniazid, can cause liver damage, and this risk could be heightened by using artemisinin-based combination therapy with amodiaquine to treat malaria, potentially leading to more severe outcomes (Adedeji et al., 2018).

6 Mechanisms of resistance

There is a plethora of factors behind anti-malaria drug resistance. The mechanisms of resistance to chloroquine in malaria parasites often involve mutations in the *PfCRT* gene, which reduce the drug accumulation within the parasite, diminishing its efficacy and facilitating parasite survival (Patel and Roy, 2021). In mosquitoes, exposure to chloroquine can lead to alterations in innate immunity, potentially affecting their ability to treat *Plasmodium* infections. Moreover, chloroquine disrupts normal apoptotic processes in mosquito midgut cells, potentially facilitating parasite survival and development within the mosquito host. These changes in parasite susceptibility and mosquito physiology can significantly influence malaria transmission dynamics and the proliferation of drug-resistant strains (Abrantes et al., 2008). The resistance mechanisms to mefloquine in *P. falciparum* involve mutations in the *pfmdr1* gene, impacting drug transport and efficacy. Amplification of *pfmdr1* correlates with heightened mefloquine resistance and treatment failure rates. Prolonged parasite clearance post-mefloquine treatment signifies potential resistance development. *In vitro* assays indicated elevated IC_{50} values for mefloquine, indicative of decreased parasite susceptibility. Furthermore, emerging resistance to artesunate, alongside mefloquine resistance, highlights the pressing need for alternative malaria treatment approaches (Rogers et al., 2009a).

Studies regarding molecular mechanisms involved in anti-malaria drug resistance found the involvement of variation in *P. vivax* multidrug resistance gene 1 (*PvMDR1*) (Reed et al., 2000; Chaorattanakawee et al., 2017; Li et al., 2020), *P. vivax* chloroquine resistance transporter (*PvCRT*) (Sá et al., 2019), *P. vivax* dihydrofolate reductase-thymidylate synthase (*PvDHFR-TS*), dihydropteroate synthase (*PvDHPS*) (Hawkins et al., 2007; Auburn et al., 2018; Auburn et al., 2019), and *P. vivax* multidrug resistance protein 1 (*PvMRP1*) (Bright et al., 2013; Flannery et al., 2015) in mediating drug resistance (Buyon, Elsworth, and Duraisingh, 2021). Furthermore, chemoprevention or chemoprophylaxis strategies may also inadvertently exacerbate the resistance problem. Malaria chemoprevention interventions do not always lead to favorable outcomes; counter-intuitively, they may increase the prevalence of resistance-associated genetic mutations (Plowe, 2022).

Drugs that are effective in adults cannot be used to treat malaria in pregnancy and children. This is another area of concern as many times, the same drugs are prescribed in all age groups leading to

increased risk of adverse reactions and potentially life-threatening consequences during pregnancy (Phillips-Howard and Wood, 1996). Moreover (Acosta et al., 2020), assessed the relationship among drug dose, timing, and drug resistance in the malaria model. The analysis revealed that the higher dose administration may lead to high-level resistance. In addition, altering treatment timing can also influence the risk of resistance emergence. The study concluded that identifying the "right" time and dose was crucial for maximizing clinical goals (Acosta et al., 2020). In addition, an investigation focusing on the correlation between anti-malarial market characteristics and the emergence of arteminisin revealed that there was an urgent need for tight regulation to delay the emergence and spread of arteminisin (Guissou et al., 2023).

7 A glance at malaria vaccine-related advancements and development

About half of the world's population is still at risk of contracting malaria, so making an effective malaria vaccine is the need of the hour, a goal that remains elusive for now. In 2021 the WHO approved the first malaria vaccine, RTS,S/AS01 vaccine (Mosquirix™), for widespread use. However, development continues on promising candidates such as R21, PfSPZ, and *P. vivax* vaccines (El-Moamly and El-Sweify, 2023).

R21/Matrix-M™ Malaria Vaccine is a product of collaboration between the University of Oxford and Novavax. By reaching 77% effectiveness in Phase 2 clinical trials, R21 demonstrated remarkable efficacy. Moreover, it is designed to be low-dose, accessible, and cost-effective, for use in children under 3 years of age (Aderinto et al., 2024). In addition to these developments, many researchers have emphasized the need for developing asexual blood-stage malaria vaccine development against *Plasmodium* (Takashima et al., 2024).

Thus far, two types of vaccines have been developed. One type, called subunit vaccines, like RTS,S, and R21, target a specific part of the parasite, but they have not been very effective at stimulating the immune system to protect against malaria for a long time (MacMillen et al., 2024). Another type of vaccine called whole-organism vaccines, such as Radiation Attenuated Sporozoites (RAS) exhibited better protection, but they are difficult and expensive to make since they need to be grown inside mosquitoes. Moreover, these vaccines require a series of IV doses administered over multiple clinic visits (Sissoko et al., 2022). To overcome this, researchers proposed a novel strategy called a "prime-and-trap" approach, involving using two different vaccines together. *In vivo*, the first vaccine (the priming dose) a single dose of a self-replicating RNA encoding full-length *P. yoelii* CS protein was delivered using advanced Nano carriers called LION™. The second vaccine (the trapping dose) consists of an attenuated malaria parasite, delivered as a whole-organism vaccine (WO RAS). Interestingly, an accelerated regimen, i.e., either 5-day or same-day immunization induced a strong immune response. Notably, mice vaccinated on the same day experienced a 2-day delay in the appearance of parasites in their blood and achieved 90% protection against a 3-week spz challenge. Moreover, this same-day regimen provided 70% protection against the 2-month spz challenge. This innovative approach may aid in developing vaccines that require fewer doses and provide long-lasting protection against malaria (MacMillen et al., 2024).

Furthermore, malaria transmission-blocking vaccines, which interrupt malaria transmission from one person to another, have also been tested in malaria-endemic areas of Africa. The study found that all the tested formulations were safe and well tolerated in healthy adults. Particularly, ProC6C-AlOH/Matrix-M vaccine elicited the highest levels of functional antibodies, requiring further investigation (Tiono et al., 2024).

8 Medicinal plants as remedies for malaria

Herbal medicines have many proven advantages over synthetic drugs, as the former is more cost-effective, has fewer side effects, and is more efficient (Mohammadi et al., 2020). Vegetal species have been used for centuries for different therapeutic purposes. Researchers from all over the world have identified numerous plant-based products that are effective in managing various diseases including malaria (Lemma et al., 2017; Hassan et al., 2021; Afzal et al., 2022; Nawaz, Ishtiaq, and Anwar, 2022). Derived from the plant named *Artemisia annua*, artemisinin has revolutionized the treatment of malaria (Phillipson, 1990). Similarly, various herbal medicines are derived from *Cinchona* species, with quinine being one of them (White, 1985). Febrifugine, another antimalarial drug, is derived from the plant *Dichroea febrifuga* Lour. (Farnsworth and Soejarto, 1991). Artemisinin and quinine contain bioactive compounds/phytochemicals, alkaloids and terpenoids that are effective for the treatment of malaria infection (Moyo et al., 2020). The structural formula for artemisinin, quinine, and febrifugine is shown in Figure 5. Here we describe some of the most important plants with promising anti-malarial activities.

8.1 *Cryptolepis sanguinolenta* (Lindl.) Schltr.

Cryptolepis sanguinolenta (Apocynaceae) leaves are used in the treatment of malaria. In 1996, cryptolepine was first isolated from the roots of *C. sanguinolenta*, and it inhibits DNA synthesis, which is the main reason for its antimalarial action (Osafo, Mensah, and Yeboah, 2017). In a study, both cryptolepine and isocryptolepine exhibited effective inhibition of *P. falciparum in vitro*, regardless of the strain's resistance to chloroquine. Cryptolepine demonstrated slightly better performance in killing the parasite, with an IC_{50} ranging from 0.2 to 0.6 μM, compared to isocryptolepine's (another alkaloid from *C. sanguinolenta*) IC_{50} of about 0.8 μM. The antimalarial activity of cryptolepine was also confirmed *in-vivo* rodent malarial parasites. The results showed that cryptolepine was effective against even the resistant varieties of *Plasmodium* parasites (Grellier et al., 1996; Table 3). This plant has been used in traditional African medicine to manage malaria, showing promising results (Mohammadi et al., 2020).

8.2 *Artemisia annua* L.

In a clinical study, patients with severe malaria who responded to neither Artemisinin combination Therapy (ACT) nor IV artesunate were treated with dried leaves of *A. annua* (Asteraceae). At the dose of 0.5g, twice daily for 5 days, the *A.*

TABLE 1 Malaria cases and deaths by geographical region (WHO Annual Report, 2022).

Region	Cases (%)	Deaths (%)
Africa	95	96
Southeast Asia	2	1
Eastern Mediterranean Region	1	≤1
Others	1	≤1

annua dried-leaf oral solution. Another study investigated *A. annua* anti-malaria potentials by subjecting its aqueous and hydroalcoholic extracts to *in vitro* and *in vivo* evaluations. Interestingly, the hydroalcoholic extract was found to be more active (IC_{50} = 3.27 ± 1.42 nM) than artemisinin (IC_{50} = 5.10 ± 1.89 nM) *in vitro*. However, the *in vivo* investigations using *Plasmodium berghei* NK 173 infected mice revealed that the aqueous extract of *A. annua*, with artemisinin content of 20 mg/kg, demonstrated comparable efficacy to pure artemisinin administered at a dosage of 140 mg/kg (Zime-Diawara et al., 2015). A series of clinical trials have proven the efficacy of these compounds, making these drugs one of the preferred choices of clinicians for eradicating *Plasmodium* parasites from the body (Chiodini et al., 2007). Information about several drugs derived from *Artemisia* species is provided in Table 2.

A study was conducted by (Elfawal et al., 2015) to demonstrate the efficacy of the whole *A. annua* plant, as a malaria therapy, is more potent than a comparable dose of pure artemisinin in a rodent malaria model. For this purpose, in the rodent malaria model, *P. chabaudi* was chosen due to its susceptibility to both whole-plant *A. annua* and artemisinin treatment. *P. yoelii* was selected as an artemisinin-resistant strain for challenge experiments. The study design consisted of five animal groups, 4 serving as treatment groups and 1 as control. Treatments included dried *A. annua* plant, and pure artemisinin, administered orally. The experiment utilized two doses of pure artemisinin and two doses of whole plant. The low dose of pure artemisinin was 24 mg/kg, while the high dose was 120 mg/kg. Similarly, the low dose of whole-plant *A. annua* corresponded to 24 mg of artemisinin per kilogram of body weight, and the high dose was 120 mg/kg. The results revealed that a stable resistance to the whole plant was achieved; three times slower than stable resistance to artemisinin. *A. annua* treatment was even more resilient than the double dose of artemisinin. This resilience was attributed to the potential evolutionary refinement of the plant's secondary metabolic products into a redundant, multi-component defense system (Elfawal et al., 2015; Table 3).

8.3 *Helianthus annuus* L.

An Indonesian study investigated the *in vivo* and *in vitro* efficacy of *H. annuus* (Asteraceae). The ethanolic extracts from all parts of *H. annuus* were made separately. The root extract was more potent *in vitro* with IC_{50} values of 2.3 ± 1.4 μg/mL. In addition, leaf and flower extracts showed significant antimalarial activity with IC_{50} values of 4.3 ± 2.2 and 4.8 ± 0.0 μg/mL, respectively. *In vivo* studies in *P. berghei*-infected mice, treated with *H. annuus* root extract demonstrated the highest percentage inhibition, while leaf extract also showed significant inhibition (63.6 ± 8.0). Notably, the root

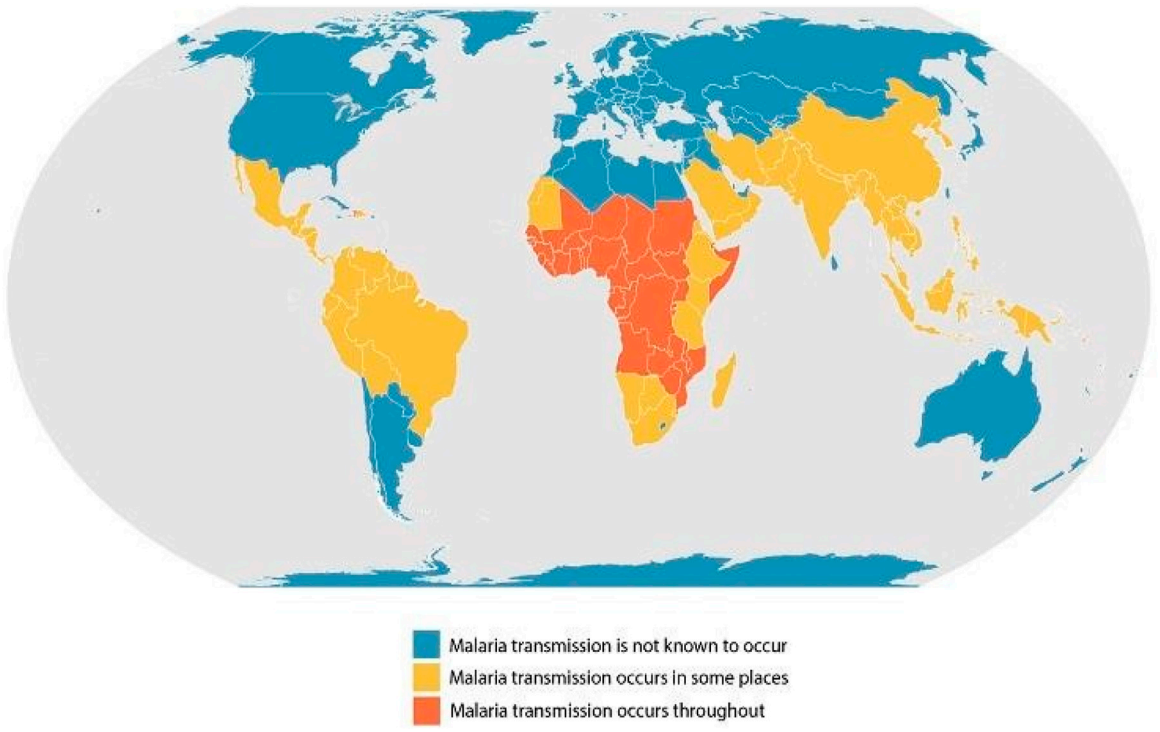

FIGURE 3
Geographical distribution of malaria (Centre for Disease Control and Prevention).

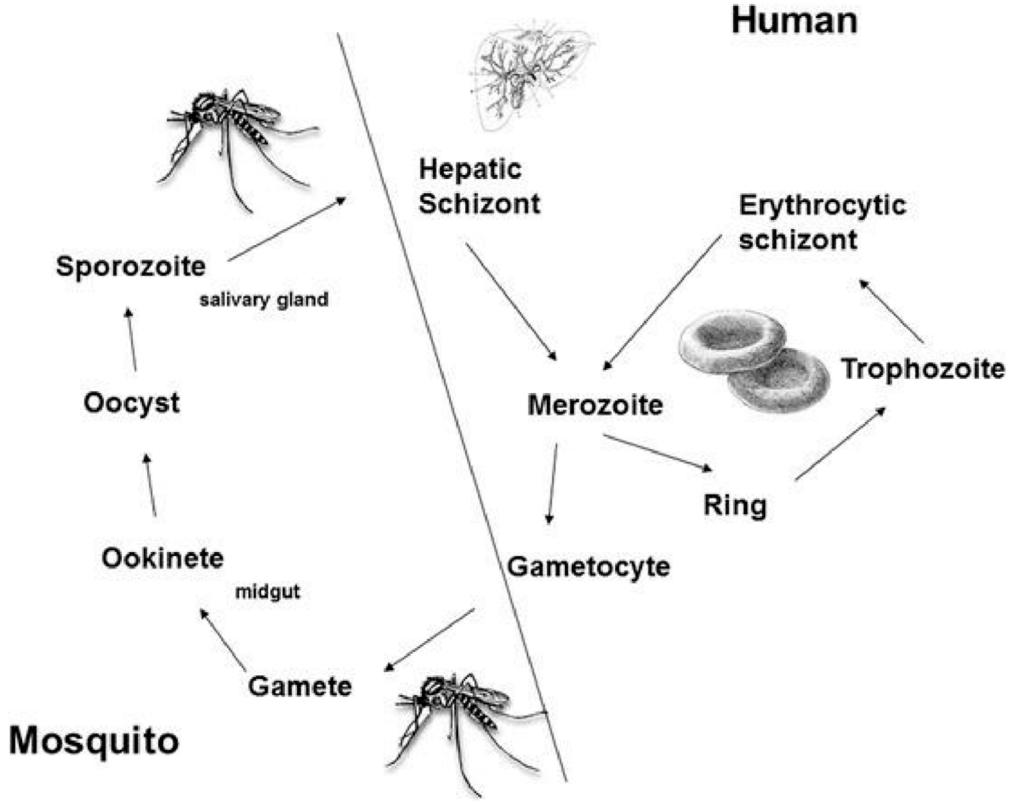

FIGURE 4
The life cycle of *Plasmodium* in humans and *Anopheles* mosquito.

extract *in vivo* curative assay showed a significant median effective (ED_{50}) value of 10.6 ± 0.2 mg/kg. At 400 mg/kg dose, the group showed the highest prophylactic inhibition (79.2%) on day 3. Overall, the *H. annuus* extracts exhibited high activity against malaria (Ekasari et al., 2019; Table 4).

8.4 *Cyperus articulates* L.

A study focusing on the volatile oil obtained from rhizomes of the *C. articulates* (Cyperaceae) plant, commonly found in the Amazon region was carried out in Brazil. In this study, *P. falciparum* W2 (chloroquine-resistant) and 3D7 (chloroquine-sensitive) strains in erythrocytes were exposed to *C. articulates* volatile oils at different concentrations. The oil showed high activity against the two *P. falciparum* strains, with IC_{50} = 1.21 μg/mL for W2, and 2.30 μg/mL for 3D7. Furthermore, *in vivo,* antimalarial activity was tested in *P. berghei*-infected BALB/c mice. Treatment with *C. articulates* was a success in all 18 ACT-resistant cases (Daddy et al., 2017; Table 3).

The discovery of artemisinin has played a critical role in treating malaria, as it is not only active against various plasmodial forms, resistant to older drugs like chloroquine but also has fewer side effects. Its combination with other medications, such as lumefantrine, is crucial in combatting severe malarial infection (Marwa et al., 2022). With the development of different commercial forms of artemisinin-based drugs, there has been some improvement in the control over malaria endemics in the African and Mediterranean regions (Marwa et al., 2022). *C. articulates* volatile oil also significantly reduced parasitemia and anemia in animals treated with 100 and 200 mg/kg doses (Silva et al., 2019).

8.5 *Hypoestes forskaleii* (Vahl) R

Hypoestes forskalei (Acanthaceae) holds an eminent place in ethnopharmacology. In addition to its use as an anti-malaria, it is used as a cytotoxic, antimicrobial, larvicidal, antioxidant, antipyretic, antileismanial, and antitrypanosomal agent. To test *H. forskalei* anti-malaria effects, its leaf extracts were studied in both *in vitro* and *in vivo* settings. Notably, all the test doses of the crude extract and the fractions significantly reduced parasitemia and prolonged mean survival time ($p < 0.001$) as compared to their negative control groups. At 600 mg/kg dose of the crude extract during the 4-day suppressive test, maximum parasitemia suppression effect was observed (56%). At the same dose, the n-butanol, chloroform, and aqueous *H. forskalei* fractions revealed a percentage suppression of about 50, 38, and 19, respectively. Moreover, the n-butanol fraction, at a dose of 600 mg/kg, exhibited a significant curative effect ($p < 0.001$) in Rane's test with a suppression of about 49% (Misganaw, Amare, and Mengistu, 2020; Table 4).

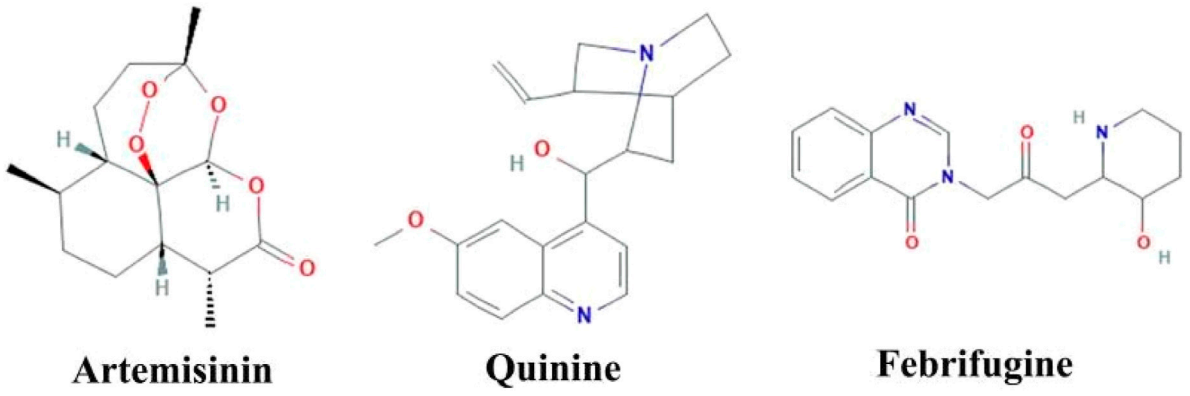

FIGURE 5
Structure of 3 common plant-derived anti-malaria drugs.

TABLE 2 Important commercial drugs derived from *Artemisinin*.

Artemisinin commerical derivatives	Brand name	Manufacturer	Pharmaceutical dosage form
Artemether	Artenam	Arenco	Ampoule
	Artem	Hilton	Ampoule
Artemether plus lumefanterine	Coartem	Novartis	Tablet
	Lumartem	Cipla	Tablet
Artesunate	Asunate	Adley	Vial for injection
	Falcigo	Zindus cadila	Vial for injection
Artesunate plus amodiaquine	Co-artesun	Guilin	Tablet
Dihydroartemisinin	Alaxin	GVS labs	Tablet
Artesunate plus mefloquine	Artequin	Acino Switzerland	Tablet

8.6 *Angelica keiskei* (Miq.) Koidz.

Chalcones is a renowned antimalarial phytocompound. *A. keiskei* (Apiaceae) is one of the plants that are rich in chalcone. A study aiming to determine the antimalarial activity of *A. keiskei* root extract by *in vitro* assay used the *P. falciparum* strain 3D7. The root extract, with an IC_{50} value of 16.091 μg/mL, exhibited an inhibitory effect as compared to chloroquine as a positive control with an IC_{50} value of 0.007 μg/mL. The study concluded that *A. keiskei* root extracts could be categorized as one of the antimalarial agents (Wardani, Wahid, and Rosa, 2020; Table 4).

8.7 *Quercus infectoria* G.Olivier

Similarly, a study conducted in Malaysia evaluated the anti-malaria potential of *Quercus infectoria* (Fagaceae). For this purpose, acetone, ethanol, methanol, and aqueous extracts were made from the *Q. infectoria* galls. Among these extracts, the acetone extract showed the highest antimalarial effects (IC_{50} = 5.85 ± 1.64 μg/mL), followed by the methanol extract (IC_{50} = 10.31 ± 1.90 μg/mL) against chloroquine-sensitive *P. falciparum*. Moreover, the study established the safety of these extracts, both acetone and methanol extracts were found to be non-toxic to the normal cell lines and statistically significant to artemisinin ($p < 0.05$) (Zin et al., 2020; Table 4).

8.8 *Pogostemon cablin* (Blanco) Benth

Another study conducted in Thailand found that the ethanol extract of *Pogostemon cablin* (Lamiaceae) carries the potential to become a more effective and safer anti-malarial agent. The efficacy and safety of the extract was tested by both *in vitro* and *in vivo* methods. *P. cablin* extract showed a significant IC_{50} of 24.49 ± 0.01 μg/mL against chloroquine-resistant *P. falciparum* K1. In comparison, the cytotoxic analyses revealed a nontoxic effect of the extract on Vero cells at a concentration of 80 μg/mL. In *P. berghei*-infected ICR mice, the ethanolic extract showed no toxic effect on mice at a dose of 2,000 mg/kg body weight. Notably, treatment with *P. cablin* extracts significantly suppressed parasitemia in mice by 38.41%, 45.12%, and 89.00% at doses of 200, 400, and 600 mg/kg body weight, respectively (Phuwajaroanpong et al., 2020; Table 3).

TABLE 3 Plants against resistant *Plasmodium* species.

Scientific name and family	Common name	Part used and extract	Dosing	Study type	Citation
Cryptolepis sanguinolenta (Apocynaceae)	Nibima, Yellow-dye root	**Part:** Root **Extract:** Aqueous	Cryptolepine IC_{50}: 0.2–0.6 μM Isocryptolepine IC_{50}: 0.8 μM	*In vitro*	Grellier et al. (1996)
Artemisia annua (Asteraceae)	Sweet wormwood	**Part:** Whole plant **Extract:** Aqueous	24mg/kg-120 mg/kg	*In vivo*	Elfawal et al. (2015)
Cyperus articulates (Cyperaceae)	Jointed flatsedge	Volatile oil	IC_{50} against W2 (resistant strain): 1.21 μg/mL	*In vitro*	Silva et al. (2019)
Pogostemon cablin (Lamiaceae)	Patchouli	**Part:** Dried Ariel parts **Extract:** Ethanol	IC_{50} against K1 (resistant) 24.49 μg/mL	*In vitro*	Phuwajaroanpong et al. (2020)
• *Toddalia asiatica* (Rutaceae) • *Rhamnus prinoides* (Rhamnaceae) • *Vernonia lasiopus* (Compositae)	• Orange Climber • Gesho • -	**Part:** • Root bark • Leaves • Root bark **Extract:** Aqueous	Dose: 500 mg/kg	*In vivo*	Muregi et al. (2007)
• *Maytenus senegalensis* (Celastraceae) • *Rhamnus staddo* (Rhamnaceae)	• Spike thorn • Staddo	**Part:** • Root bark • Leaves and root bark **Extract:** Aqueous	Dose: 500 mg/kg	*In vivo*	Muregi et al. (2007)
• *Citrullus colocynthis* (Cucurbitaceae) • *Physalis alkekengi* (Solanaceae) • *Solanum nigrum* (Solanaceae)	• Bitter apple • Chinese lantern • Black nightshade	**Part:** • Fruits • Leaves and fruit • Fruits **Extract:** Methanolic	IC_{50} • 6.9 μg/mL • 18.67 μg/mL • 13.08 μg/mL	*In vitro*	Haddad et al. (2017)
Glycyrrhiza glabra (Fabaceae)	Licorice	**Part:** Root **Extract:** Ethyl acetate and chloroform	Isoliquiritigenine IC_{50} 7.2 μg/mL Isoliquiritigenine + Chloroquine IC_{50} 1.90 μg/mL Isoliquiritigenine + Artemisinin IC_{50} 1.75 ng/mL	*In vitro*	Kumar et al. (2024)
Cuscuta reflexa (Convolvulaceae)	Giant dodder	**Part:** Whole plant **Extract:** Methanol	IC_{50} <10 to 2.2 μg/mL	*In vitro*	Ojha et al. (2023)
Caesalpinia bonducella (Fabaceae)	Gray Nicker	**Part:** Root **Extract:** Dichloromethane	Norcaesalpin D IC_{50} 0.98–2.13 μg/mL	*In vitro*	Nondo et al. (2017)
• *Momordica charantia* (Cucurbitaceae) • *Diospyros monbuttensis* (Ebenaceae)	• Bitter melon • Yoruba ebony	**Part:** • Leaves • Leaves **Extract:** Alcohol	IC_{50} • 3.2 nM • 12.5 nM	*In vitro*	Olasehinde et al. (2014)
Costus afer (Costaceae)	Ginger lily	**Part:** Stem **Extract:** Aqueous	IC_{50} 10.3–15.05 μg/mL	*In vitro*	Jimoh et al. (2019)

(Continued on following page)

10.3389/fphar.2024.1359890

TABLE 3 (*Continued*) Plants against resistant *Plasmodium* species.

Scientific name and family	Common name	Part used and extract	Dosing	Study type	Citation
Rubia cordifolia (Rubiaceae)	Indian madder	**Part:** Aerial **Extract:** (crude extraction) • Hexane • Methanol • Water	Dose: 50 µg/mL IC_{50} • 0.551 µg/mL • 1.23 µg/mL • 5.34 µg/mL	*In vitro*	Jeremiah et al. (2023)

8.9 *Kniphofia foliosa* Hochst.

K. foliosa (Asphodelaceae) is indigenous to the Ethiopian highlands. Traditionally, its rhizomes are used for abdominal cramps, wound healing, as well as malaria management. To empirically establish *K. foliosa* as an anti-malaria agent, an investigation was carried out. In this study, two compounds (knipholone and dianellin) were isolated from the 80% methanolic extract of *K. foliosa* rhizomes. Upon evaluation by using Peters' 4-day suppressive test against *P. berghei* in mice, the hydroalcoholic extract (400 mg/kg) and knipholone (200 mg/kg) demonstrated the highest activity with chemo suppression values of 61.52% and 60.16%, respectively. The dose-response plot analysis revealed the ED_{50} doses of knipholone and dianellin were 81.25 and 92.31 mg/kg, respectively. A molecular docking study revealed that knipholone had a strong binding affinity to the *P. falciparum* l-lactate dehydrogenase target (Alebachew et al., 2021; Table 4).

8.10 *Physalis angulata* L., *Jatropha curcas* L., *Alstonia spectabilis* R.Br.

To empirically understand the malaria treatment practices and evaluate the anti-plasmodial activity and phytochemicals of several plants used by the Tetun ethnic people in West Timor Indonesia, an experimental study was designed. In this study, ethanolic extracts from the whole *P. angulate* plant (family: Solanaceae), stem barks of *A. spectabilis* (family: Apocynaceae), and *Jatropha curcas* (family: Euphorbiaceae) were tested among others. Among the 11 plants studied in this investigation, *P. angulata, J. curcas,* and *A. spectabilis* extracts showed strong anti-plasmodial activity against the *P. falciparum* 3D7 strain *in vitro*, with IC_{50} values of 0.22, 0.22, and 1.23 µg/mL, respectively (Maximus et al., 2021) (Table 4).

8.11 *Allium paradoxum* (M.Bieb.) G.Don

A study aimed to examine the inhibitory effects of *A. paradoxum* (Amaryllidaceae) on *P. falciparum* and *P. berghei*. The highest efficacy of *A. paradoxum* extract was observed at 80 µg/mL in *P. falciparum* culture, resulting in 60.43% growth inhibition compared to control groups. The significantly highest parasite growth inhibition with 88.71% was seen in the mice infected with *P. berghei* when administered with 400 mg/kg extract compared to control groups. However, no significant changes in the liver and kidney cells were observed between the experimental and control groups (Elmi et al., 2021; Table 4).

8.12 *Toddalia asiatica* (L.) Lam., *Rhamnus prinoides* L'Hér., and *Vernonia lasiopus* O. Hoffm.

Extracts from various plant species were collected, authenticated, and processed using hot water extraction to mimic traditional methods. The activities of these extracts were tested against a chloroquine-resistant strain of *P. berghei* NK65 in mice. At the dose of 500 mg/kg, twice a day for 4 days, aqueous extract of *V. lasiopus* (root bark) and *R. prinoides* (leaves) showed remarkable

TABLE 4 Plants with anti-malaria properties.

Scientific name and family	Common name	Part used and extract	Dosing	Citation
Artemisia annua (Asteraceae)	Sweet wormwood	**Part:** Leaves **Extract:** Hydroalcohol	IC_{50} *In vitro*: 3.27 ± 1.42 nM *In vivo*: 20 mg/kg	Zime-Diawara et al. (2015)
Helianthus annuus (Asteraceae)	Common sunflower	**Part:** Whole plant **Extract:** Ethanol	IC_{50} *In vitro*: **Roots:** 2.3 ± 1.4 μg/mL **Leaf:** 4.3 ± 2.2 μg/mL **Flower:** 4.8 ± 0.0 μg/mL *In vivo*: 400 mg/kg ED50: 10.6 ± 0.2 mg/kg	Ekasari et al. (2019)
Hypoestes forskaleii (Acanthaceae)	White ribbon bush	**Part:** Leaves **Extract:** Crude, n-butanol	*In vivo*: 600 mg/kg	Misganaw etal. (2020)
Angelica keiskei (Apiaceae)	Tomorrow's leaf	**Part:** Root	IC_{50} *In vitro*: 16.09 μg/mL	Wardani et al. (2020)
Quercus infectoria (Fagaceae)	Manja-kani	**Part:** Galls **Extract:** Acetone, methanol	IC_{50} *In vitro*: **Acetone extract:** 5.85 ± 1.64 μg/mL **Methanol extract:** 10.31 ± 1.90 μg/mL	Zin et al. (2020)
Kniphofia foliosa (Asphodelaceae)	Lella	**Part:** Rhizome **Extract:** Hydroalcohol	*In vivo*: **Hydroalcohol extract:** 400 mg/kg **Knipholone (phytocompound):** 200 mg/kg	Alebachew et al. (2021)
• *Physalis angulata* (Solanaceae) • *Jatropha curcas* (Euphorbiaceae) • *Alstonia spectabilis* (Apocynaseceae)	• Cutleaf groundcherry • Physic nut • Bitterbark	**Part:** • Whole plant • Stem bark • Stem bark **Extract:** Ethanol	IC_{50} *In vitro*: • *P. angulate* 0.22 μg/mL • *J. curcas* 0.22 μg/mL • *A. spectabilis* 1.23 μg/mL	Maximus et al. (2021)
Allium paradoxum (Amaryllidaceae)	Few-flowered garlic	**Part:** Whole plant **Extract:** Hydroalcohol	*In vitro*: 80 μg/mL *In vivo*: 400 mg/kg	Elmi et al. (2021)

suppression of parasitemia on day 4 post-infection (p.i.) with values of 51% and 54%, respectively. Moreover, the group treated with *Toddalia asiatica* (root bark) showed 100% survival up to day 15 p.i. Other extracts, including *R. prinoides* (leaves and root bark), showed prolonged survival with 40% of mice surviving beyond day 9 p.i. (Muregi et al., 2007; Table 3).

8.13 *Maytenus senegalensis* (Lam.) Exell, and *Rhamnus staddo* A.Rich.

In the same study by (Muregi et al., 2007), some plant extracts performed well when given in combination with chloroquine, particularly, extracts of *M. senegalensis, R. staddo, T. asiatica,* and *V. lasiopus,* in combination with chloroquine at a dose of 20 mg/kg, once a day for 2 days, and plant aqueous extracts at a dose of 500 mg/kg, twice a day for 4 days exhibited a remarkable suppression of parasitemia ranging from 51% to 66%. Survival analysis on day 14 p.i. showed that chloroquine in combination with certain plant extracts resulted in 40%–60% survival rates, with some mice surviving beyond day 14 p.i. Notably, combinations with *R. prinoides* (root bark) and *V. lasiopus* (root bark) showed 60% survival on day 14 p.i., with the last mice surviving beyond day 24 and day 30 p.i., respectively (Muregi et al., 2007; Table 3).

8.14 *Citrullus colocynthis* (L.) Schrad., *Physalis alkekengi* L., and *Solanum nigrum* L.

Drawing inspiration from Iranian traditional medicine, a study tested methanolic extracts from these plants against multi-drug resistant (K1) strains of *P. falciparum*. For the resistant strain, the study found that methanolic extracts of *C. colocynthis* exhibited promising *in vitro* anti-plasmodial activity, with an IC_{50} value of 6.9 μg/mL. Similarly, *Solanum nigrum* and *P. alkekengi* also showed activity against the resistant strain, with IC_{50} values of 18.67 and 13.08 μg/mL, respectively (Haddad et al., 2017; Table 3).

8.15 *Glycyrrhiza glabra* L.

In an *in vivo* investigation, the root extract of *Glycyrrhiza glabra* (Fabaceae) demonstrated anti-plasmodial potential. This activity was attributed to its phytomolecule: Isoliquiritigenin. Moreover, *in-vivo* antimalarial efficacy was evaluated through a 4-day suppression test in a mouse model. The phytocompound, *in vitro*, exhibited moderate anti-plasmodial activity against the multidrug-resistant strain (K1) of *P. falciparum*. Notably, the ethyl acetate extracts performed moderately against the resistant strain of *P. falciparum* (K1), with an IC_{50} value of 7.2 μg/mL. *In vivo*, mice infected with a chloroquine-resistant strain of

P. yoelli nigeriensis were administered with the ethyl acetate extract at a dose of 500 mg/kg, which showed the highest inhibition of *P. yoelii* nigeriensis growth (82.73%), followed by doses of 250 mg/kg (64.40%) and 100 mg/kg (46.09%) compared to the control group. Also, the ethyl acetate extracts increased the mean survival time of the mice to 18.2 days, 13.5 days, and 16.5 days, respectively (Kumar et al., 2024; Table 3).

Interestingly, isoliquiritigenin was found to synergize with chloroquine and artemisinin against the multidrug-resistant strain (K1) of *P. falciparum*. The IC_{50} of chloroquine in the presence of isoliquiritigenin was found to drop from 0.132 to 0.013 μg/mL (10-fold). Importantly, the IC_{50} of isoliquiritigenin itself was found to drop from 5.2 to 1.90 μg/mL (up to 2.73-fold) in the presence of chloroquine. Similarly, with the isoliquiritigenin-artemisinin combination, it was found that the IC_{50} of artemisinin dropped from 3.9 to 1.75 ng/mL (2.22 times reduction). However, since all values fell within the range of >01 and ≤2, it indicated that the combination had an additive effect (Kumar et al., 2024; Table 3).

8.16 *Cuscuta reflexa* Roxb.

C. reflexa (Family: Convolvulaceae) has traditionally been used by the indigenous people of Odisha, India for the treatment of malaria. An *in vitro* evaluation was carried out to provide an empirical basis for this practice. *In vitro*, fractions of methanol extract from the shed-dried whole plant of *C. reflexa* exhibited promising effects (IC_{50} ranging from < 10.0 to 2.2 μg/mL) against *P. falciparum* R539T (artemisinin resistance strain) and RKL (chloroquine resistance strain) strains. Notably, no *in vitro* cytotoxicity was recorded. Moreover, *C. reflexa* fraction showed high *in vivo* parasite suppression, with a mean survival time similar to that of artesunate (19.3 vs 20.6 days) at a dose of 20 mg/kg in *P. berghei* ANKA-infected male Swiss-albino mice (Ojha et al., 2023; Table 3).

8.17 *Caesalpinia bonducella* (L.) Fleming

Another study explored the anti-malaria qualities of norcaesalpin D obtained from dichloromethane root extract of *C. bonducella* (Fabaceae). Additionally, crude extracts, fractions, and isolated compounds were evaluated for anti-plasmodial activity against chloroquine-resistant *P. falciparum* (Dd2, K1) and artemisinin-resistant *P. falciparum* strains. The results indicated the fractions from *C. bonducella* roots were found to be highly effective against K1, Dd2, and artemisinin-resistant parasites. Moreover, norcaesalpin D from *C. bonducella* root extract was active with IC_{50} of 0.98, 1.85, and 2.13 μg/mL against 3D7, Dd2, and IPC 4912-Mondolkiri parasites, respectively (Nondo et al., 2017; Table 3).

8.18 *Momordica charantia* L., and *Diospyros monbuttensis* Gürke

A Nigerian study tested the sensitivity of resistant *P. falciparum* species to plant extracts of *M. charantia, D. monbuttensis*, and *M. lucida* by using an *in vitro* micro-test (Mark III), based on assessing the inhibition of schizont maturation. Among the isolated parasites, all were sensitive to quinine, mefloquine, and artesunate; however, 51% of the isolates were resistant to chloroquine, 13% to amodiaquine, and 5% to sulphadoxine/pyrimethamine. The study found that the highest activity was obtained with an extract of *D. monbuttensis* with an IC_{50} of 3.2nM, while *D. monbuttensis* produced inhibitory activity with IC_{50} = 12.5 nM. However, the least satisfactory activity was obtained from *M. lucida* extract (IC_{50} = 25 nM) (Olasehinde et al., 2014; Table 3).

8.19 *Costus afer* Ker Gawl.

C. afer (Costaceae) was investigated for anti-malaria potentials *in vitro* activity by using its methanol stem extract and its residual aqueous fraction against chloroquine-resistant, and artemether-resistant *P. falciparum* strains. The extracts demonstrated significant and dose-dependent inhibitions of schizont growth in the resistant *Plasmodium* strains with IC_{50} values of 11.27 and 15.05, and 10.30 and 11.23 μg/mL against, chloroquine-resistant and artemether-resistant strains, respectively (Jimoh et al., 2019; Table 3).

8.20 *Rubia cordifolia* L.

R. cordifolia is part of Kenyan traditional medicine for malaria treatment; however, there is limited scientific evidence supporting this practice. To begin addressing this gap, a study aimed to assess the plant-extract efficacy, taken from the aerial part of *R. cordifolia*, against chloroquine-resistant (W2) *P. falciparum* strains. Major findings of the study included a significant inhibition of parasite growth, in a dose-dependent manner. At 50 μg/mL, inhibition was (84.07%, 77.94%, and 66.08%) for hexane, methanol, and water extracts, respectively, against the resistant strain. Hexane extract showed an IC_{50} 0.551 μg/mL, while methanol extract gave encouraging results with an IC_{50} 1.231 μg/mL. However, the aqueous extract showed moderate activity (IC_{50} = 5.348 μg/mL) (Jeremiah et al., 2023; Table 3).

9 Medicinal plants used for malaria treatment in Pakistan

The Himalayan region of Pakistan is renowned for its diverse herbal remedies the local communities use to treat various health problems. A study on these plants found that Asteraceae (11.9%) was the most cited vegetal family against malaria. Other plant families being used included Lamiaceae (5.9%), Solanaceae, Verbenaceae (4.7%), and Violaceae (3.5%). A study conducted by (Shah and Rahim, 2017), in the Soon Valley of Pakistan, indicated the use of more than fifty varieties for malaria treatment in the region. The researchers identified that anti-malarial properties of seven plant species were reported for the first time. These plant species included *Withania coagulans* (Stocks), *Fagonia cretica* L. (Zygophyllaceae), *Carthamus oxyacanthus* M.Bieb. (Asteraceae), *Ehretia obtusifolia* Hochst. ex A.DC. (Boraginaceae), *Helianthus*

annuus L. (Asteraceae), *Olea ferruginea* (Aiton) Steud. (Oleaceae), and *Vitex trifolia* L. (Lamiaceae).

Another study focused on three local plants for their anti-malarial activity. The plant species analyzed included *P. kurroa*, *C. bonducella*, and *A. absinthium*. The investigators used cold alcohol, hot alcohol, and aqueous preparations of extracts obtained from the plants to ascertain their antimalarial activity. The study found that *P. kurroa* was more effective than the other two plants against malaria (Irshad, Mannan, and Mirza, 2011).

10 Discussion

One of the strengths of this review is its emphasis on the use of natural products for malaria treatment. Natural remedies for treating malaria have several advantages over mainstream drugs. First, natural products offer a vast array of chemical structures with diverse pharmacological properties (Dias, Urban, and Roessner, 2012). This chemical diversity provides a rich source of compounds with potential anti-malarial activity, allowing for the discovery of novel drugs or drug leads. This chemical diversity also helps in delaying the emergence of drug resistance. The prevention or at least delay of drug resistance may also be helped by the fact that many natural products exhibit activity against multiple stages of the malaria parasite's life cycle, including the blood stages (schizonts), liver stages (hypnozoites), and transmission stages (gametocytes) (Roberts et al., 2017). This multifactorial action may help prevent the development of drug resistance and improve treatment outcomes. Natural products often contain multiple bioactive compounds that can act synergistically to enhance their anti-malarial activity. This synergism may result in greater efficacy and lower risk of drug resistance compared to single-compound therapies. Most natural products exhibit anti-malarial activity with minimal toxicity to human cells, making them potentially safer alternatives to synthetic drugs (Amelo, Nagpal, and Makonnen, 2014). This is particularly important in malaria-endemic regions where access to healthcare and monitoring for adverse drug reactions may be limited. This review not only focuses on the use of natural products for treating malaria but emphasizes that combining treatment with a hybrid approach of vector control methods with rigorous hygiene measures can be an effective strategy for combating malaria. Especially, the distribution of insecticide-treated bed nets and indoor residual spraying with long-lasting insecticides can significantly reduce mosquito populations and prevent malaria transmission indoors (Hellewell et al., 2021). Targeted application of larvicides to mosquito breeding sites, such as stagnant water bodies, can prevent the development of mosquito larvae and reduce adult mosquito populations (Lardeux et al., 2002). Implementing an integrated approach that combines multiple vector control methods, tailored to local epidemiological and ecological conditions, can enhance the effectiveness and sustainability of malaria control efforts. Establishing robust surveillance systems to monitor malaria transmission dynamics, vector populations, and insecticide resistance can inform evidence-based decision-making and guide the adaptation of control strategies over time.

11 Limitations of the herbal treatments

An important limitation of herbal medicine is the variation in the potency and effectiveness that may occur due to several factors, including geographic variations in malaria parasite strains, drug resistance patterns, and differences in local healthcare infrastructure and practices. For example, *P. falciparum* is the most prevalent and deadliest of the malaria parasite species, and resistance to anti-malarial drugs, such as chloroquine and sulfadoxine-pyrimethamine, has been widely reported in many regions, particularly in sub-Saharan Africa (Crider et al., 2022). National and international treatment guidelines for malaria may vary based on epidemiological data, drug resistance patterns, and healthcare resources. Treatment recommendations may be tailored to specific regions or populations to optimize treatment outcomes and minimize the risk of drug resistance. For example, ACTs are recommended as first-line treatment for uncomplicated *P. falciparum* malaria in most endemic regions, but the specific ACT regimen may vary based on local drug resistance patterns (Saito et al., 2023).

Another limitation of herbal treatments is the lack of standardized dosing for malaria which can have several implications, including variability in treatment efficacy, safety concerns, and challenges in comparing research findings across studies. Natural product treatments often contain complex mixtures of bioactive compounds, which may vary in concentration and composition depending on factors such as plant species, growing conditions, harvesting methods, and processing techniques (Santos-Zea et al., 2016). This variability makes it difficult to establish standardized dosing regimens across different preparations of the same natural product. Many natural products lack comprehensive pharmacokinetic data, including information on absorption, distribution, metabolism, and excretion (ADME) in humans. Without this data, it is challenging to determine optimal dosing regimens that achieve therapeutic levels of active ingredients while minimizing the risk of toxicity or suboptimal efficacy. Natural products may contain toxic compounds or interact with other medications, leading to adverse effects or drug interactions (Fugh-Berman, 2000). Without standardized dosing and rigorous safety evaluation, there is a risk of overdosing, under-dosing, or unintended adverse reactions, particularly in vulnerable populations such as children, pregnant women, or individuals with underlying health conditions.

The potential interactions of natural products such as for malaria with other medications are an important consideration, as they can impact treatment efficacy, safety, and patient outcomes, thus limiting their use (Fugh-Berman, 2000). While natural products are often perceived as safe, they contain bioactive compounds that may interact with conventional medications that may alter their pharmacokinetics or pharmacodynamics. Natural products may affect the ADME of other medications, leading to alterations in their blood levels and therapeutic effects. For example, certain natural products may inhibit or induce drug-metabolizing enzymes in the liver (e.g., cytochrome P450 enzymes), influencing the metabolism of co-administered medications and potentially leading to drug toxicity or treatment failure (Yang, Wang, and Zeng, 2002; Parvez and Rishi, 2019). Furthermore, natural products may have additive, synergistic, or antagonistic effects when combined with other medications, altering their pharmacological

actions. For instance, some natural products may enhance the anticoagulant effects of blood-thinning medications or potentiate the sedative effects of central nervous system depressants. Similarly, St. John's wort (*Hypericum perforatum* L.), a herbal remedy sometimes used for its antidepressant effects, could induce drug metabolizing enzymes and reduce the efficacy of medications, including certain anti-malarial drugs, oral contraceptives, and immunosuppressants (Yang, Wang, and Zeng, 2002).

12 Conclusion

Malaria remains a formidable global health challenge, causing significant morbidity and mortality, particularly in impoverished regions. Despite numerous diagnostic and medicinal breakthroughs, the *Plasmodium* resistance development to anti-malaria drugs will undo most of this progress. This threat has become an impetus behind the race for novel, effective, and affordable drugs and vaccines. Natural products derived from plants like *A. annua, C. sanguinolenta, H. impetiginosus, P. kurroa,* and *A. hexapetalus* are among the noteworthy sources. Interestingly, *A. annua* is the precursor of artemisinin, one of the most significant drugs considered a mainstay of treatment against malaria. Moreover, plants like *V. lasiopus*, *R. prinoides*, *T. asiatica, C. colocynthis,* and phytocompounds like isoliquiritigenin, and norcaesalpin D should be further studied for effects against drug-resistant *Plasmodium* species. However, while herbal medicines have advantages like cost-effectiveness and fewer side effects, they also have limitations. These limitations include variations in potency, lack of standardized dosing, and potential interactions with other medications. Therefore, either modern or herbal medicines should be used after conducting a cost-benefit analysis associated with the treatment plan. Only a balanced approach can have an effectual impact. In addition, an emphasis on early detection, accurate diagnosis, and integrated efforts for disease prevention is also crucial. Additionally, implementing stringent hygiene measures and promoting community awareness will also contribute to reducing the burden of malaria in endemic areas.

Author contributions

QA: Data curation, Formal Analysis, Investigation, Resources, Writing–original draft. NS: Data curation, Investigation, Methodology, Software, Writing–original draft, Writing–review and editing. NM: Data curation, Formal Analysis, Funding acquisition, Writing–review and editing. RN: Data curation, Formal Analysis, Funding acquisition, Writing–review and editing. FN: Data curation, Formal Analysis, Methodology, Project administration, Resources, Validation, Visualization, Writing–review and editing. SA: Formal Analysis, Methodology, Project administration, Resources, Supervision, Visualization, Writing–review and editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. The authors thank the United Arab Emirates University for financing this research under UPAR project G00003696, grant code 12S094.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 03 July 2024
DOI 10.3389/fphar.2024.1365639

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OPEN ACCESS

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RECEIVED 04 January 2024
ACCEPTED 03 June 2024
PUBLISHED 03 July 2024

CITATION
Fu C-F, Li J-L, Chen J-W, Liang H, Zhao W-R, He S-Y, Ma X-W, Yang X-F and Wang H-L (2024), Mechanism and therapeutic potential of traditional Chinese medicine extracts in sepsis. *Front. Pharmacol.* 15:1365639. doi: 10.3389/fphar.2024.1365639

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Mechanism and therapeutic potential of traditional Chinese medicine extracts in sepsis

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Sepsis is a complex syndrome characterized by multi-organ dysfunction, due to the presence of harmful microorganisms in blood which could cause mortality. Complications associated with sepsis involve multiple organ dysfunction. The pathogenesis of sepsis remains intricate, with limited treatment options and high mortality rates. Traditional Chinese medicine (TCM) has consistently demonstrated to have a potential on various disease management. Its complements include reduction of oxidative stress, inhibiting inflammatory pathways, regulating immune responses, and improving microcirculation. Traditional Chinese medicine can mitigate or even treat sepsis in a human system. This review examines progress on the use of TCM extracts for treating sepsis through different pharmacological action and its mechanisms. The potential targets of TCM extracts and active ingredients for the treatment of sepsis and its complications have been elucidated through molecular biology research, network pharmacology prediction, molecular docking analysis, and visualization analysis. Our aim is to provide a theoretical basis and empirical support for utilizing TCM in the treatment of sepsis and its complications while also serving as a reference for future research and development of sepsis drugs.

KEYWORDS

sepsis, traditional Chinese medicine, extracts, pharmacological action, mechanism

1 Introduction

The management of sepsis has emerged as a critical scientific challenge, necessitating urgent attention as a significant global public health crisis (Groote and Meersch-Dini, 2022). The extensive heterogeneity observed in host, pathogen, and the environment contributes significantly to disease development (Jones et al., 2021). Despite recent advancements in sepsis treatment and advanced life support, mortality rate from septic shock remains approximately is 50% (Vincent et al., 2019). The ongoing pursuit of improved treatment resulted in research for development of alternative therapies to manage sepsis.

In traditional Chinese medicine, sepsis is commonly classified as one of the exogenous febrile diseases documented in ancient Chinese medical literature, including the Treatise on Febrile Diseases, Treatise on Epidemic Febrile Diseases, and other relevant publications (Fan et al., 2020). The theory of traditional Chinese medicine (TCM) emphasizes that the treatment of sepsis should focus on clearing heat and detoxification, restoring normal bowel movement, promoting blood circulation and removing blood stasis, as well as

strengthening immune system. According to the concept of entirety and the method of treatment with syndrome differentiation of TCM, the clinical efficacy of sepsis was significantly improved and the case fatality rate effectively reduced (Zhao et al., 2017). The collection, processing, and preparation of Chinese herbal medicine adhere to the fundamental principles of TCM, which boasts into a rich history for both theory and practice when it comes to treating critically ill patients. The practitioners of TCM have expertise for identification of symptoms related to acute and critical illnesses, as well as formulating appropriate TCM treatments. Traditional Chinese medicine can serve as an adjunctive therapy in the management of sepsis due to its advantageous characteristics, including a complex composition, multiple targets of action, and involvement in various signaling pathways (Usmani et al., 2021). It exhibits diverse effects such as anti-inflammatory properties, improvement of microcirculation, alleviation of gastrointestinal dysfunction, and enhancement of the immune system. Moreover, it may even confer protection against organ damage caused by sepsis (Usmani et al., 2021). The efficacy and safety of employing this evidence-based TCM treatment in conjunction with intensive sepsis therapy increasingly acknowledged by the medical community and patients. Traditional Chinese medicine has played a crucial role in the management of COVID-19 patients, providing us novel insights into the merits and significance of employing traditional Chinese medicine for sepsis treatment (Lyu et al., 2021). Traditional Chinese medicine has been incorporated into the standard treatment for sepsis (Wang X.-H. et al., 2022), including medications such as Xuebijing injection (XBJ), Shenfu injection, Qingwen Baidu decoction, Xuanbai Chengqi decoction (XBCQ), and Qingmai decoction (QWBD). The significant advancements have been made in the exploration of the efficacy and mechanism of action for TCM to sepsis treatment, with an increasing number of studies corroborating the prominent role in the prevention and management of sepsis (Zheng et al., 2023). Although there have been review articles discussing the research progress of traditional Chinese medicine compounds and monomer extracts in the treatment of sepsis and multi-organ injury (Song et al., 2023), this review merely presents a superficial list of various monomer extracts used for sepsis treatment, lacking a comprehensive analysis and discussion on their therapeutic mechanisms. It is noteworthy that our review commences with an exploration of the etiology and pathogenesis of sepsis, subsequently delving into the therapeutic strategies for sepsis utilizing traditional Chinese medicine extracts based on their respective mechanisms of action. This approach facilitates a comprehensive understanding of TCM's therapeutic process for sepsis, as TCM typically addresses diseases through multiple pathways and targets. Categorizing interventions based on their mechanism rather than solely relying on TCM extracts enhances our comprehension and cognition of TCM's efficacy in treating sepsis. Our review establishes correlations between the mechanisms of traditional Chinese medicine extracts for treating sepsis and its pathogenesis, thereby emphasizing the logical basis behind TCM disease management and bolstering its persuasiveness. Consequently, this review serves as a valuable reference for future development of sepsis medications.

2 Overeview of sepsis

Sepsis is a life-threatening syndrome of multiple organ dysfunction caused by dysregulation in the body's immune response to infection (Sygitowicz and Sitkiewicz, 2021). The mortality rate of patients with sepsis can exceed 30%–35% in the absence of timely and effective intervention, as a result of an increased inflammatory response caused by immunosuppression or primary infection (Vincent et al., 2019). The complications induced by sepsis involve multi-organ dysfunction such as pulmonary impairment, cerebral injury, renal impairment, hepatic impairment, and myocardial damage (Sun et al., 2020; Zhou and Liao, 2021; Chang et al., 2022; Sekino et al., 2022; Zhao et al., 2022). The high mortality rate resulting from sepsis is primarily attributed to the occurrence of multi-organ failure induced by the disease. The pathogenesis of sepsis is highly intricate at the cellular and molecular level, which encompasses various pathophysiologic processes such as inflammation imbalance, immune dysfunction, mitochondrial damage, coagulation disorders, neuroendocrine immune network abnormalities, endoplasmic reticulum stress, and autophagy, ultimately resulting in multi-organ dysfunction (Huang et al., 2019a). According to the conventional perspective, following an initial phase of excessive inflammation, a subsequent phase of diminished inflammation, partially attributed to the release of anti-inflammatory cytokines, leads to profound immunosuppression (Sheth et al., 2019). Currently, preliminary results indicated that the stages of inflammation promotion and immune suppression may occur concurrently (Zhong and Yin, 2023), rendering the interaction between host and pathogen conducive for disease development. The high-risk factors for sepsis patients encompass advanced age, gender, presence of immunosuppressive diseases, medications, history of cancer, diabetes mellitus, alcohol misuse, and the utilization of indwelling urinary catheters (Tokuda et al., 2023). The severity of sepsis depends on the host's age, comorbidities, and immune status, as well as pathogenic factors such as toxicity, microbial species, and infection burden (Olinder et al., 2022). In addition to pathogen-related factors, there may also be host genetic factors that could increase the risk of developing sepsis (Mukherjee et al., 2019). The latest guidelines released by the "Surviving Sepsis Campaign" in October 2021 further emphasize the implementation of sepsis screening for standardized treatment, enhance infection control and optimize antimicrobial drug utilization, as well as recommend novel treatment techniques and concepts. The current international clinical guidelines primarily include rapid screening and early diagnosis, accurate identification and control of infection sources and pathogenic microorganisms, timely and effective antibiotic treatment and fluid resuscitation, management of hemodynamics, and mechanical ventilation (Fitridge and Thompson, 2007).

Despite some advancements in the research and treatment of sepsis in recent years, a comprehensive understanding of its pathogenesis and key mechanisms is still lacking, necessitating further improvements in targeted therapies. However, lack of effective treatment methods hinders the management of cellular and tissue organ damage resulted from sepsis. The treatment of sepsis remains a global challenge, involving the prevention of

multiple infections, management of inflammatory responses and immune-mediated damage, as well as addressing gastrointestinal dysfunction and coagulation disorders. (Arefian et al., 2017; Cecconi et al., 2018; Marik, 2018).

3 Chinese medicine theory guides sepsis treatment

The fundamental principle of TCM is rooted in the "holistic concept," which serves as a guiding principle, while the "syndrome differentiation" acts as the diagnostic and therapeutic method. These two theories direct the management of sepsis with TCM (Li and Xu, 2011). The "holistic concept" posits that the human body functions as an integrated organic entity, emphasizing the synergistic interplay among multiple organs to safeguard against disease and maintain homeostasis. The integration of "syndrome differentiation" and "disease differentiation" involves understanding the location, etiology, nature, and interplay between "positive" and "evil" factors, reflecting the pathological changes. The aforementioned statement also aligns with the concept of "personalized medicine" in contemporary medical practice, which holds immense significance in the realm of diagnosis and treatment. Western medicine research has shifted from a one-sided focus on eradicating disease-causing microorganisms to regulating the body's immune response. It now emphasizes the intrinsic connection between organs in the whole body, as evidenced by theories such as liver-kidney syndrome, heart-kidney syndrome, and lung-intestinal axis. Traditional Chinese medicine is used to dynamically adjust multiple systems in patients, aligning with the holistic concept of Chinese medicine (Liu et al., 2022). Due to variations in individual characteristics, pathogenic factors, and disease duration, patients exhibit diverse responses to drug therapy. Additionally, each sepsis patient presents distinct clinical manifestations, disease progression levels, and alterations in disease mechanisms. Therefore, it is essential to employ diverse methods and treatment modalities tailored to the individual, timing, and location, aligning with the diagnostic and therapeutic approach of Chinese medicine known as "syndrome differentiation."

The TCM treatment of sepsis can be summarized as "Four Syndromes and Four Methods" based on the etiology and pathogenesis. The "Four Syndromes" include blood stasis, toxic heat, viscera and qi obstruction as well as acute deficiency. Correspondingly, the "Four Methods" are activating blood circulation to remove stasis, clearing heat and detoxifying, regulating the lower energizer while nourishing the root (Xu and Lu, 2018). The improvement of microcirculation corresponds to the traditional Chinese medicine approach of "activating blood circulation to resolve stasis." In sepsis, microcirculatory disorders are a significant pathological change characterized by inadequate capillary perfusion and sluggish blood flow. The objective of the "activating blood circulation to resolve stasis" in TCM is to enhance blood circulation, particularly microcirculation, thereby alleviating tissue hypoxia and ischemia, preventing the formation of microcirculatory thrombosis, as well as halting further progression of disseminated intravascular coagulation (DIC). The inhibition of inflammatory mediator release aligns with the treatment principle of "clearing heat and detoxifying." A significant amount of inflammatory mediators such as tumor necrosis factor and interleukins are released during sepsis, resulting in systemic inflammation of the body. Traditional Chinese medicine's approach to "clearing heat and detoxification" can mitigate inflammatory response through the utilization of specific TCM possessing heat-clearing and detoxifying properties, thereby suppressing excessive release of inflammatory mediators. Promoting the elimination of endotoxins is akin to the principle of "regulating the lower energizer" in traditional Chinese medicine. In sepsis, bacterial endotoxins accumulate within the body, exacerbating the condition. For septic patients presenting with symptoms such as constipation and abdominal distension, TCM that facilitate bowel movement and heat expulsion can be employed for sepsis treatment. Modern medicine acknowledges that the intestines serve as a major reservoir of bacteria in the human body. During severe infections like septicemia, stress weakens or damages intestinal barrier function, allowing a significant influx of bacteria and endotoxins into circulation via the portal vein and intestinal lymphatic system, resulting in gut-derived endotoxemia and bacterial translocation. The principle underlying both immune regulation and the "nourishing the root" method in traditional Chinese medicine is similar. Sepsis often leads to suppression or disruption of the body's immune function. In traditional Chinese medicine, the emphasis on nourishing the spleen, kidneys, and other organs aims to enhance immune function, which can be seen as analogous to modern medical immunomodulatory treatments.

Combined with the clinical manifestations, the "method of supporting and consolidating the essence" should be considered as a fundamental treatment approach for "septic shock" or "sepsis recovery," predominantly relying on TCM that promote and consolidate vital energy. "Activating blood circulation to remove stasis" is a treatment method used when shock is combined with DIC or to prevent the occurrence of DIC, primarily using traditional Chinese medicine that promotes blood circulation and resolves stagnation. "Clearing heat and detoxifying" and "regulating the lower energizer" are therapeutic approaches employed to combat pathogenic infections, while also serving as preventive measures against septic shock. These methods primarily focus on utilizing TCM with heat-removing properties to clear heat and toxins from the body's lower internal organs (Li and Wang, 2017; Ling et al., 2023). The Chinese herbal medicine types that clear heat and detoxify, promote blood circulation, remove blood stasis, and strengthen the foundation of treatment are effective in treating sepsis. The "Four Syndromes and Four Methods" provide a convenient way to identify infectious shock in TCM. The four syndromes can simultaneously correspond to the various stages of pathology in Western medicine, thereby enhancing the precision in drug identification and utilization (Jing et al., 2022). The treatment of sepsis in TCM aims not only to eliminate pathogenic factors, but also to restore internal balance and coordination within the organism (Liu et al., 2022). It regulates the functions of internal organs, promoting harmony between the five viscera and six bowels, allowing the body to achieve an optimal state through medication for self-regulation. This ultimately restores internal homeostasis and realizes the delicate interplay of yin and yang.

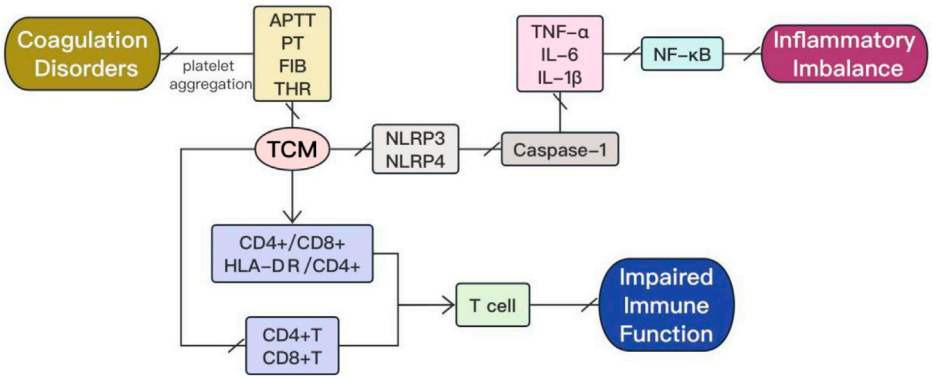

FIGURE 1
The mechanism actions in treatment of sepsis with TCM extracts involve the modulation of inflammatory imbalance, impaired immune function, and coagulation disorders.

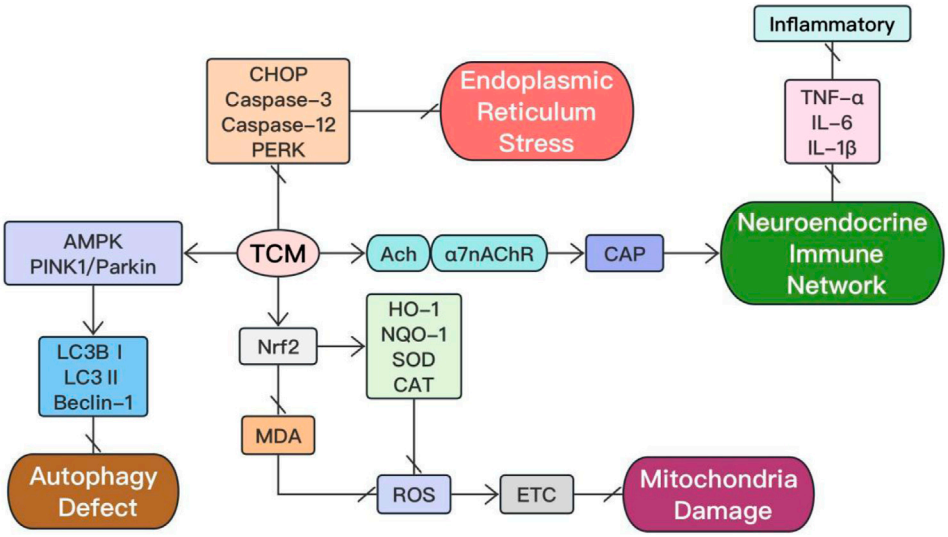

FIGURE 2
The other mechanism actions in treatment of sepsis with TCM extracts.

4 Mechanism action of TCM extracts in treatment of sepsis

Traditional Chinese medicine extracts and its active components possess distinct chemical structures, precise therapeutic effects, and facilitate quality control. These compounds offer "individualized therapies" for different stages of sepsis development through multiple pathways and targets, thereby promoting body equilibrium (Han and Wu, 2010; Li et al., 2013). This review provides a comprehensive overview of the advancements in sepsis treatment research, focusing on the extraction and isolation of bioactive compounds from natural sources. These compounds are obtained through inhibiting the release of inflammatory mediators, facilitating endotoxin elimination, enhancing microcirculation, and modulating immune responses.

The mechanisms action of TCM extracts in the treatment of sepsis are systematically summarized and presented in Figures 1, 2. The extracts of traditional Chinese medicine regulate diverse mechanisms through various signaling pathways to enhance sepsis management, exemplifying the multifaceted approaches and targets characteristic of TCM in disease treatment.

4.1 Inflammatory imbalance

Inflammatory imbalance serves as the fundamental basis for the pathogenesis and progression of sepsis. The pathogenic microorganisms, including bacteria, fungi, parasites, and viruses, elicit responses throughout the course of sepsis. The initial acute response triggered by the host in response to an invasive pathogen typically involves phagocytosis of the pathogen by macrophages and production of a variety of pro-inflammatory cytokines. This leads to the onset of a cytokine storm and activation of the intrinsic immune system (Lin et al., 2022). The activation of the intrinsic immune

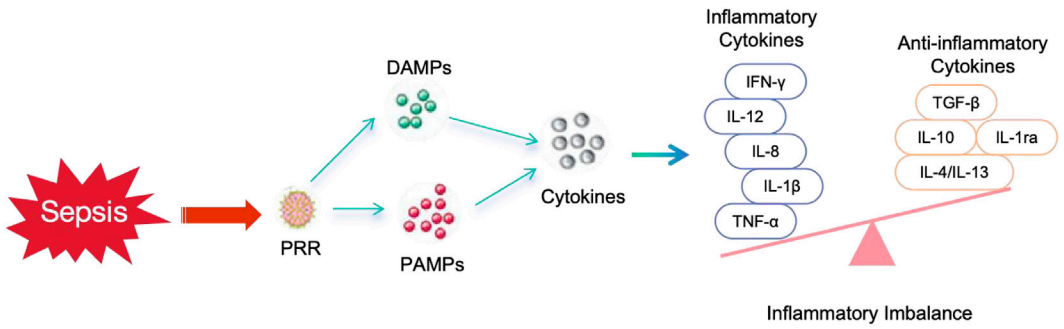

FIGURE 3
The inflammatory imbalance process during sepsis.

system is clearly mediated by pattern-recognition receptor, which detect damage-associated molecular pattern (DAMP) or pathogen-associated molecular pattern (PAMP) and subsequently upregulate inflammation-related genes, initiating a cascade of immune cell activations (Raymond et al., 2017). In the immune response to sepsis, exogenous factors derived from pathogens, such as lipopolysaccharides, and endogenous factors released by injured cells both play a crucial role. Figure 3 shows the inflammatory imbalance process during sepsis. The findings of various studies have demonstrated the effective inhibition of inflammatory factor release, regulation of pro-inflammatory and anti-inflammatory responses, antagonism against endotoxins, inhibition of hyperfibrinolysis, as well as modulation of the expression of inflammatory signaling pathways by herbal monomers (Li et al., 2013). These mechanisms contribute to the protection of septic organs' function and improvement in patient prognosis. The characteristics of TCM extracts by the modulation of inflammatory imbalance on sepsis are summarized in Table 1.

The active components salvianolic acid and tanshinone, derived from the Chinese herbal medicine *Salvia miltiorrhiza* Bunge, possess biological effects such as enhancing circulation, exerting anti-inflammatory and antioxidant effects (Wang J. et al., 2019). These components can potentially serve as therapeutic agents against the release of inflammatory and oxidative mediators during sepsis and microcirculatory disorders in shock (Yuan J. et al., 2021). Xu (2022) discovered that the incorporation of salvianolic acid into conventional fluid resuscitation can effectively suppress the inflammatory and stress responses during sepsis combined with shock. Additionally, it has been shown to significantly enhance blood oxygen metabolism in pediatric patients with sepsis combined with shock. Wu S. et al. (2020) utilized lipopolysaccharide (LPS)-induced macrophage RAW264.7 and a murine sepsis model to assess the impact of metabolites isolated from extracts of the aerial parts of *S. miltiorrhiza*, specifically salvianolic acid A (administered via intraperitoneal injection at concentrations of 125, 25, 5, and 1 μg/mL), on sepsis. The cellular activity was evaluated using the methyl thiazolyl tetrazolium (MTT) assay, while the levels of inflammatory factors were measured in both cells and mouse livers through enzyme-linked immunosorbent assay (ELISA) analysis. The results demonstrated that salvianolic acid A increased cellular activity in all dosage groups. Moreover, the levels of inflammatory factors TNF-α and IL-6 were significantly or markedly reduced, while the level of IL-10 was significantly elevated in all dosage groups (except for the 5 μg/mL group). These findings suggest that salvianolic acid A effectively mitigated the impact of LPS on cellular activity. The effects of intraperitoneal administration of salvianolic acid A at a dosage of 100 mg/kg on liver injury in rats with cecal ligation and puncture (CLP) were analyzed and investigated (Wei et al., 2021). The expression levels of inflammatory markers TNF-α, IL-1, IL-6, as well as liver injury markers aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were detected using ELISA. Additionally, liver tissues from the rats were subjected to pathological observation. The results demonstrated that compared to the sepsis control group, the salvianolic acid experimental group exhibited a significant reduction in levels of both liver injury markers and inflammatory markers. Zhang et al. (2017) investigated the interventional effects of tanshinone IIA (10 mg/kg) on cerebral microcirculation and neuroinflammatory responses in LPS-induced septic mice. Utilizing the BI2000 microcirculation image processing system, researchers observed that blood flow velocity of microvessels and microarteries in the soft meninges at different phases of each group's mice through an open cranial window. Additionally, ELISA was employed to detect the levels of inflammatory factors in mouse brain hippocampal tissue. The results demonstrated a significant acceleration in both micro-artery and micro-vein blood flow velocity within the drug group compared to that of the model group, along with significantly reduced levels of TNF-α and IL-1β in cerebral hippocampus tissue when compared to those found in the model group. These findings indicated that tanshinone IIA could enhance cerebral microblood flow velocity, mitigate neuroinflammatory reactions, and exert protective effects on the brain.

Lei and Li (2021) reported that polydatin significantly reduced serum levels of inflammatory factors in SAKI mice. Wang B. et al. (2020) demonstrated that resveratrol exerts regulatory effects on downstream signaling molecules of Toll-like receptor 4 (TLR-4), sphingosine kinase 1 (SphK1), extracellular signal-regulated kinase 1/2 (ERK1/2), and NF-κB, leading to a reduction in cytokine production by human primary monocytes stimulated with LPS through phospholipase D (PLD). Moreover, it downregulates myeloid differentiation factor 88 (MyD88), which inhibits the LPS/Toll-4/NF-κB signaling pathway, thereby attenuating the

TABLE 1 Characteristics of TCM extracts by the modulation of inflammatory imbalance on sepsis.

Name	Source	Validation model	Administration	Effect	Organ	Positive control	Ref.
Salvianolic acid	*Salvia miltiorrhiza* Bunge	Children with sepsis and shock	200 mg/time	↓: CRP, PCT, NF-κB, HMGB1, sTREM-1	NA	NA	Xu (2022)
Salvianolic acid A	*Salvia miltiorrhiza* Bunge	LPS-induced C57 mice	125, 25, 5, and 1 μg/mL	↓: TNF-α、IL-6 ↑: IL-10	Lung	Dexamethasone (200 μg/mL)	Wu et al. (2020b)
Salvianolic acid A	*Salvia miltiorrhiza* Bunge	CLP-induced SD rats	100 mg/kg	↓: TNF-α, IL-1, IL-6, AST, ALT, ALP	Liver	NA	Wei et al. (2021)
Tanshinone IIA	*Salvia miltiorrhiza* Bunge	LPS-induced mice	10 mg/kg	↓: TNF-α, IL-1β	Brain	NA	Zhang et al. (2017)
Polydatin	*Polygonum cuspidatum* Siebold & Zucc.	CLP-induced SAKI mice	30 mg/kg	↓: MDA, TNF-α, IL-1β, IL-6 ↑: GSH/GSSG, SOD, T-AOC	Kidney	SIRT3 inhibitor 3-TYP (5 mg/kg)	Lei and Li (2021)
Resveratrol	*Veratrum album* L.	Patients undergoing TRUS biopsy CLP-induced male C57/BL6 mice	1 mg/kg body weight	↓: PLD, SphK1, ERK1/2, NF-κB, LPS/Toll-4/NF-κB, HMGB1, MyD88	NA	NA	Wang et al. (2020a)
Astragalus polysaccharides	*Astragalus membranaceus* (Fisch.) Bge. or A. membranaceus (Fisch.) Bge. var. *mongholicus* (Bge.) *Hsiao*	CLP-induced SD rats	100 mg/kg	↓: IL-6, TNF-α, ESM-1, TLR4/NF-κB ↑: HSP70	Lung	NA	Xu et al. (2023)
Isoliquiritigenin	*Glycyrrhiza uralensis* Fisch. ex DC.	LPS-induced male C57BL/6 mice	50 μM or 100 μM	↓: HMGB1 ↑: NCOA4	Kidney	Ferrostatin-1	Tang et al. (2021)
Murrayanine	*Murraya paniculata* (L.) Jack	LPS-induced RAW 264.7 cells and female Balb/c mice	10 mg/kg	↓: iNOS, COX -2, NO, PGE2, TNF-α, IL-6, NF-κB	Liver, Lung, Kidney	Dexamethasone	Gupta et al. (2019)
Ginsenoside Rg2 and Rh1	*Panax ginseng* C.A.Mey.	LPS-induced RAW 264.7 cells and ICR mice	20 mg/kg	↓: TLR4, NF-κB, TNF-α, IL-1β, IFN-β ↑: p38-STAT1	Liver, Kidney	Dexamethasone	Huynh et al. (2020)
Ginsenoside Rg1 and Re	*Panax ginseng* C.A.Mey.	LPS-induced RAW 264.7 and RAW-Blue cells LPS-induced male SD rats and BALB/c mice	30, 5, 70 μg/mL Rg1 and 50, 70 μg/mL Re 10 mg/kg Rg1 and 20 mg/kg Re	↓: TNF-α, IL-1β, IL-6, COX-2, iNOS, TLR4	NA	TAK-242 (1 mg/kg)	Su et al. (2015)
Phillyrin	*Forsythia suspensa* (Thunb.) Vahl	LPS-induced EA.hy926 cell and AKI mice	20 μM	↓: ROS, cathepsin L, heparinase, NF-κB/MAPK, IL-1β, IL-6, TNF-α	Kidney	Cathepsin L inhibitor	Zhang et al. (2020)
Puerarin	*Pueraria lobata* (Willd.) Ohwi	LPS-induced male BALB/C mice	50 mg/kg	↓: ICAM-1, VCAM-1, E-selectin, TNF-α, IL-1β	Lung	NA	Wang et al. (2019b)
Ethanol extract of *Cinnamomum cassia*	*Cinnamomum cassia* (L.) J.Presl	LPS-induced sepsis mouse model and MSU-induced gout model using male C57BL/6 mice	50, 100 mg/kg	↓: NLRP3, NLRC4, AIM2, IL-1, caspase-1	NA	zVAD	Shin et al. (2017)
Ligustrazine	*Ligusticum chuanxiong* S.H.Qiu, Y.Q.Zeng, K.Y.Pan, Y.C.Tang & J.M.Xu	CLP-induced male C57BL/6 mice Patients with congenital non-cyanotic heart disease undergoing open heart surgery	4 mg/kg, 40 mg/kg 3 mg/(kg·BW)	↓: MDA, MPO, IL-6, IL-8, TNF-α, ICAN-1	Lung Heart	NA	Lu et al., 2007; Zeng (2010)

(Continued on following page)

TABLE 1 (*Continued*) Characteristics of TCM extracts by the modulation of inflammatory imbalance on sepsis.

Name	Source	Validation model	Administration	Effect	Organ	Positive control	Ref.
Astragalus polysaccharides	*Astragalus membranaceus* (Fisch.) Bge. or *A. membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao	LPS-induced male C57BL/6 mice	100, 200, 400 mg/kg	↑: IL-2, IL-10, TGF-β ↓: IL-4, IL-17, TNF-α, Th1/Th2, Th17/Treg	Lung	NA	Xu and Liu (2022)
Curcumin	*Curcuma longa* L.	CLP-induced SD rats	50, 100, 200 mg/kg	↓: IL-6, IL-10, TNF-α	Lung	NA	Jiang et al. (2022)
Baicalin	*Scutellaria baicalensis* Georgi	LPS-induced male BALB/C mice	75, 150, 300 mg/kg	↓: W/D, $PaCO_2$, Th17/Treg, TNF-α, IL-6, IL-17, MDA, TLR4/NF-κB ↑: TGF-β, SOD, OI, PaO_2, Treg/Th17	Lung	Dexamethasone (7.2 mg/kg)	Xu et al. (2022b)
Salidroside	*Rhodiola crenulate* (Hook.f. & Thomson) H.Ohba	CLP-induced male BALB/C mice	5, 10, 20 mg/kg	↓: α-SMA, FN, Vimentin, Col Ⅰ, p-JAK2, p-STAT3, iNOS, IL-6 ↑: IL-4, IL-10	Lung	NA	Guo et al. (2022)
Kukoamine B	*Lycium chinense* Mill.	LPS-induced KM mice	1.25, 2.5, 5 mg/kg	↓: TLR4, TLR9, iNOS, COX-2, NF-κB, TNF-α, IL-6	Lung, liver, kidney, heart	NA	Liu et al. (2011)
Berberine	*Coptis chinensis* Franch.	LPS-induced male KM mice LPS-induced male C57BL/6 mice	25, 50, 100 mg/kg 10 mg/kg	↓: TNF-α, IFN-γ, NO, NF-κB, IL-1β, IL-6 ↑: IL-10	Lung	NA	Li et al. (2006), Wang et al. (2020b)

production of cytokines, chemokines, and high mobility group protein B1 (HMGB1) by human primary monocytes stimulated with LPS and exerting anti-inflammatory effects.

Xu et al. (2023) discovered that *Astragalus* polysaccharides (APS), a kind of polysaccharides obtained from the roots of *A. membranaceus* (Fisch.) Bge. or *A. membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao, mitigated the acute inflammatory response in septic rats. Tang et al. (2021) observed that the utilization of isoliquiritigenin reduced the expression of HMGB1 and inhibited ferroptosis in a sepsis-induced model of AKI, thereby attenuating the inflammatory response.

The major compound isolated from *Murraya paniculata* (L.) Jack is murrayanine, which has been reported to possess significant antioxidant (Abu Bakar et al., 2007), anti-inflammatory (Gupta et al., 2010), and immunomodulatory activities (Shah et al., 2008). Gupta et al. (2019) discovered that murrayanine inhibited the expression of inducible nitric-oxide synthase (iNOS) and cyclooxygenase (COX-2), reduces the production of nitric-oxide (NO), prostaglandin E2 (PGE2), TNF-α, and IL-6, decreases NF-κB activity, ameliorates LPS-induced lung, liver and kidney injuries, as well as increases survival rates in mice with LPS-induced sepsis models. These findings demonstrated that murrayanine was an ideal natural compound and a new chemical entity for treating sepsis and other inflammatory diseases.

The anti-inflammatory effects of ginseng have been demonstrated in a variety of diseases (Kim et al., 2018; Xu H. et al., 2022). Ginseng contains a diverse range of bioactive compounds, and its therapeutic potential has been associated with neuromodulation, anticancer activity, lipid modulation, and antithrombotic activity (Ru et al., 2015). However, there is currently no definitive study comparing the relative efficacy of different components of ginseng in the treatment of sepsis. A study suggested that treatments with ginsenoside Rg2 and Rh1 might exhibit greater efficacy compared to single-component treatments (Huynh et al., 2020). Similarly, another study demonstrated that the combination of ginsenoside Rg1 and Re in the treatment of septic mice effectively reduced LPS-induced hyperthermia, leukocyte counts, and serum levels of pro-inflammatory mediators. Furthermore, this combination even exhibited a 90% increase in survival rate among lethally septic shocked mice. Subsequent analysis revealed that the effective anti-inflammatory effect could be attributed to the different distribution locations of these components (Rg1 being located both intracellularly and extracellularly while Re is located extracellularly), suggesting that combining various components of ginseng might yield superior therapeutic outcomes (Su et al., 2015).

Phillyrin exhibited inhibitory effects on reactive oxygen species (ROS) production, reduced the expression levels of histone L and heparinase both *in vitro* and *in vivo*, and suppressed the secretion of inflammatory cytokines to ameliorate renal function in AKI mice (Zhang et al., 2020). The isoflavone compound puerarin has demonstrated its ability to dilate coronary blood vessels, enhanced myocardial metabolism, exhibited antioxidant and anti-inflammatory activities, protect vascular endothelial function, and

improve microcirculation (Wei et al., 2014). Puerarin pretreatment at a dose of 50 mg/kg for 4 days significantly attenuated the serum levels of intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin, as well as TNF-α and IL-1β in mice with LPS-induced endotoxemia (Wang S. et al., 2019). These findings suggested that puerarin effectively suppressed the release of inflammatory factors and adhesion molecules, thereby ameliorating LPS-induced vascular endothelial cell injury.

Modern studies have confirmed the therapeutic effect of *Cinnamomum cassia* (L.) J.Presl in sepsis by inhibiting the activation of inflammatory vesicles. Shin et al. (2017) utilized bone marrow-derived macrophage BMDMs to investigate the modulatory effect of 50 and 100 mg/kg *C. cassia* ethanol extract on inflammatory vesicle activation. They also established a mouse model of LPS-induced sepsis and a monosodium urate-induced gout model, observing that the compound increased the survival rate of mice with LPS-induced septic shock while inhibiting the activation of nucleotide-binding domain, leucine-rich repeat, and pyrin domain-containing protein 3 (NLRP3), NLRP4, and melanoma-deficiency factor 2 inflammatory vesicles through inhibition of apoptosis-associated granulocyte-like proteins oligomerization within CARD structural domains, consequently suppressing IL-1β and caspase-1 secretion.

A study replicating a mouse model of sepsis by CLP demonstrated that ligustrazine significantly attenuated the pulmonary inflammatory response during sepsis, leading to a significant reduction in plasma levels of IL-6 in ligustrazine-treated septic mice (Zeng, 2010). Clinical studies have also revealed that ligustrazine effectively decreased peripheral blood levels of IL-8 and TNF-α in patients undergoing extracorporeal circulation, potentially contributing to the management of systemic inflammatory response syndrome during extracorporeal circulation (Lu et al., 2007).

The relevant studies have demonstrated that *Astragalus* polysaccharide (APS) exhibits diverse pharmacological activities, including anti-inflammatory, antioxidant, anti-atherosclerotic, apoptosis inhibition, and ischemia-hypoxia protection (Zhou et al., 2018). Consequently, it is extensively employed in clinical settings as a crucial immunomodulator or antioxidant (Zhang et al., 2019). The sepsis mouse model was created using LPS and divided them into groups, with APS administered at different doses and saline interventions respectively (Xu and Liu, 2022). The results demonstrated a significant improvement in the imbalance of Th1/Th2 and Th17/Treg cells in septic mice treated with APS. Additionally, there was an increase in the levels of anti-inflammatory factors such as IL-2, IL-10, and TGF-β, while pro-inflammatory factors including IL-4, IL-17, and TNF-α were downregulated. Furthermore, a significant dose-dependent effect was observed.

Curcumin exhibited significant potential in reducing the levels of IL-6, IL-10, and TNF-α within the lung tissue of septic rats (Jiang et al., 2022). This suggested that curcumin effectively ameliorated inflammation levels, alleviated immune suppression, and mitigated damage to lung tissues in septic rats.

Baicalin (BA), a flavonoid compound found in the root of *Scutellaria baicalensis* Georgi, exhibits various pharmacological effects such as lipid-lowering, anti-inflammatory, antibacterial, antioxidant and immunomodulatory activities. The mice were induced with sepsis through intraperitoneal injection of LPS, followed by treatment with baicalin to mitigate lung injury (Xu L. et al., 2022). The results demonstrated that BA inhibited TLR4/NF-κB pathway activation, suppressed inflammatory response, promoted Treg/Th17 balance shift towards Treg cells, reduced lung tissue damage and improved lung function. The effect was dose-dependent.

Salidroside, the active component found in *Rhodiola crenulate* (Hook.f. & Thomson) H.Ohba, exhibits pharmacological effects that include anti-aging, anti-inflammatory, immunomodulatory, and antioxidant properties. A murine model of acute lung injury with sepsis was established through CLP (Guo et al., 2022). The findings demonstrated that salidroside attenuated the pulmonary response in CLP-induced septic mice by downregulating the expression of JAK2 and STAT3, while significantly reducing the levels of iNOS and IL-6 in lung tissue. Moreover, there was a significant increase in IL-4 and IL-10 levels, indicating that salidroside ameliorates lung injury in CLP-induced septic mice by modulating inflammatory factors within the lung tissue.

Kukoamine B is an alkaloid component primarily derived from the Chinese medicinal plant *Lycium chinense* Mill. In a study, the affinity of kukoamine B towards LPS and CpG DNA was investigated, along with its inhibitory effect on the release of TNF-α and IL-6 from RAW264.7 cells both independently and in combination (Liu et al., 2011). The findings demonstrated that kukoamine B exhibited significant neutralization of multiple pathogenic molecules and effectively suppressed the induced inflammatory response. Notably, kukoamine B represents the first reported natural product-derived inhibitor targeting both LPS and CpG DNA.

Berberine, a bioactive alkaloid isolated from Chinese herbal medicine *Coptis chinensis* Franch., has been shown to reduce plasma levels of TNF-α, IFN-γ and NO in mice with sepsis (Li et al., 2006). Recent domestic research also confirmed that berberine could inhibit the activation of NF-κB signal transduction and further supports its use as a single drug for treating sepsis patients along with other drugs (Wang Y. et al., 2020).

In conclusion, TCM extracts with anti-inflammatory properties effectively suppress the release of inflammatory factors, regulate pro-inflammatory/anti-inflammatory balance, and modulate the expression of inflammatory signaling pathways in organs affected by sepsis (show Figure 1). Therefore, these TCM extracts hold tremendous potential for treating sepsis.

4.2 Impaired immune function

The dysfunction of immune function is a crucial factor contributing to the occurrence and progression of sepsis. During the inflammatory response in sepsis, neutrophils interact with and migrate across endothelial cells under the influence of chemokines that are attracted to the site of inflammation. At this location, they recognize and engulf pathogens, release various active factors and proteolytic enzymes, ultimately eliminating the pathogen (Shen et al., 2017). Severe impairment of the immune system is a common pathological change observed in sepsis. During the initial cytokine storm phase, a significant number of sepsis patients may succumb to death. While survivors may experience

TABLE 2 Characteristics of TCM extracts by the modulation of impaired immune function on sepsis.

Name	Source	Validation model	Administration	Effect	Organ	Positive control	Ref.
Astragalus injection	*Astragalus membranaceus* (Fisch.) Bge. or *A. membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao	Patients with traumatic splenic rupture after splenectomy	20 mL in 250 mL 5% glucose	↑: $CD3^+$, $CD4^+$, CD4 +/CD8+ ↓: $CD8^+$	NA	NA	Gao et al. (2018)
Astragalus granule	*Astragalus membranaceus* (Fisch.) Bge. or *A. membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao	Patients with sepsis	1.0, 2.0, 3.0 g/time/d	↑: $CD4^+$, CD4 +/CD8+ ↓: $CD8^+$, NK cell	NA	NA	Xu et al. (2022c)
Rhubarb preparation	*Rheum palmatum* L.	Patients with sepsis	50 g in 50 mL Physiological saline (2 times/d)	↓: TNF-α, IL-6, IL-1β ↑: IL-10, CD4+/CD8+, HLA-DR/CD4+	NA	NA	Yin and Guo (2015)
Artesunate	*Artemisia annua* L.	CLP-induced male BALB/c mice	10 mg/kg	↓: CD4+/CD8+, PD-1, CTLA-4, BTLA, caspase-9 ↑: TNF-α, CD4+T, CD8+T, MAPK/ERK	Lung, spleen	NA	Yuan et al. (2023)
Ginsenoside Rg1	*Panax ginseng* C.A.Mey.	CLP-induced male 57BL/6 mice	20 mg/kg	↓: TNF-α, IL-6, IL-10, NO ↑: neutrophil	Spleen, thymus, liver, lung	NA	Zou et al. (2013)
Salidroside	*Rhodiola crenulate* (Hook.f. & Thomson) H.Ohba	CLP-induced male C57BL mice	20 mg/kg	↓: IL-6, IL-2, IL-10, CXCL-10, CD4+T, CD8+T	Thymus	NA	Pan et al. (2022)
Baicalin	*Scutellaria baicalensis* Georgi	Endotoxin-induced SD rats	100, 200 mg/kg/d	↓: TNF-α, IL-6, caspase-1, caspase-11 ↑: CD3+T/CD4+T, CD4+/CD8+, Th1/Th2	Lung	Ulinastatin (200,000 U/kg)	Yang et al. (2022)

immune suppression, leading to an inability to clear primary infections and reactivation of latent viruses. Sepsis-induced immunosuppression affects both innate and adaptive immunity. The immunosuppressive response following sepsis has been characterized as a compensatory anti-inflammatory response syndrome, which is regulated by co-stimulatory molecules like CD80/B7-1 that are produced through activation of the TLR signaling pathway. Additionally, this response involves the conversion of naive T cells into cytokine-induced regulatory T cells, resulting in decreased expression of antigen-presenting associated transcription factors (e.g., CD80/B7-1) and IRF4/MUM1 (Honma et al., 2005; Negishi et al., 2005; Pepin et al., 2018). Traditional Chinese medicine has the ability to regulate the functions of immune cells, including their composition, differentiation, activation, secretion, and killing (Yang and Fan, 2021). This enables it to enhance the body's innate resistance against pathogens and prevent cytokine storms. Specifically, it can interrupt and reverse the immunosuppressive state associated with severe sepsis, thereby improving prognosis and preventing further damage caused by pathogenic toxins (Wang and Chen, 2017). The characteristics of TCM extracts by the modulation of impaired immune function on sepsis are summarized in Table 2.

The advanced study have demonstrated that *Astragalus* injection exhibits a significant improvement effect on T lymphocyte subsets in patients with traumatic splenectomy, while maintaining high levels of drug safety (Gao et al., 2018). This suggested that *Astragalus* injection possessed the ability to modulate various immune active cells, thereby ameliorating immune imbalance through metabolic regulation and exerting dual regulatory effects on immune function. The immunomodulatory effects of high-dose Huangqi granules was further validated by promoting lymphocyte proliferation and regulating the proportion of lymphocyte subsets through rigorous investigations (Xu X. et al., 2022).

Yin and Guo (2015) utilized *Rhubarb* preparation for small intestine perfusion via naso-intestinal tube and observed a significant reduction in the levels of TNF-α, IL-6, and IL-1β among sepsis patients. Simultaneously, there was an increase in the levels of IL-10, as well as improvements in CD4+/CD8+ ratios and human leukocyte antigen-DR (HLA-DR)/CD4+ expression. These findings provided evidence supporting the immunomodulatory effects of *Rhubarb* preparation on sepsis patients.

An immunosuppressive mouse model of CL-induced sepsis-related secondary bacterial infection was established,

demonstrating that artesunate could reverse immunosuppression not only through innate immune regulation but also through adaptive immune regulation (Yuan et al., 2023). These immunosuppressive effects on the adaptive immune response were achieved by increasing the number of T cells through inhibition of $CD4^+$ and $CD8^+$ T cell apoptosis. Artesunate inhibited the expression of programmed cell death protein-1 (PD-1), cytotoxic T lymphocyte antigen-4 (CTLA-4), and B and T lymphocyte attenuator (BTLA) receptors while activating the MAPK/ERK signaling pathway as its potential mechanism. Furthermore, it was found that artesunate can inhibit caspase-9 expression and activation, potentially specifically targeting the mitochondrial apoptosis pathway in T cells. These findings suggested that artesunate had the potential to function as a modulator for both T cell number and function, making it a promising candidate for treating immunosuppressive diseases.

The intravenous administration of 20 mg/kg ginsenoside Rg1 to CLP mice resulted in an increase in peritoneal neutrophil counts (Zou et al., 2013). Additionally, the application of ginsenoside demonstrated inhibitory effects on thymus and spleen lymphocyte apoptosis, thereby enhancing bacterial clearance rate and survival rate. These findings suggested that ginsenoside Rg1 had the potential to enhance innate immunity, promote maintenance of adaptive immunity, and provide effective protection against sepsis.

Salidroside is derived from the dried roots and rhizome of *R. crenulate* (Hook.f. & Thomson) H.Ohba, possessing anti-cancer, antioxidant, anti-inflammatory, and immunomodulatory properties (Magani et al., 2020). Researches indicated its significant role in improving neuroinflammation (Wang et al., 2018), inhibiting renal fibrosis (Li et al., 2019), ameliorating fatty liver conditions (Zheng et al., 2018), and suppressing cancer cell proliferation (Zhu et al., 2020). Pan et al. (2022) established a sepsis mouse model induced by CLP and divided the mice into groups. They were treated with either salidroside or saline solution. The administration of salidroside significantly reduced the expression of inflammatory factors TNF-α, IL-6, IFN-γ, as well as inhibitory factor IL-10 in the spleen of septic mice. This suggested that salidroside could inhibit the inflammatory response in the spleen of septic mice and subsequently regulate their splenic immune function. Additionally, salidroside was found to inhibit CXC chemokine ligand-10 (CXCL-10) expression in the spleen of mice with sepsis, thereby reducing infiltration by immune cells and improving splenic immune status.

After baicalin intervention, there were a decrease in the elevated levels of TNF-α and IL-6 in the serum of septic rats, as well as an increase in the percentages of $CD3^+$ T lymphocytes, $CD4^+$ T lymphocytes, CD4+/CD8+ ratio, and Th1/Th2 ratio in peripheral blood (Yang et al., 2022). No significant difference between the high-dose baicalin group and ulinastatin group was observed when compared to each other. These findings suggested that baicalin had the potential to downregulate inflammatory factors and improve immune disorders in septic rats.

Traditional Chinese medicine extracts can regulate the body's immune function and improve immune disorders (show Figure 1). They play a crucial role in reversing the immunosuppressive state of severe sepsis and enhancing the body's own resistance.

4.3 Coagulation disorders

The current research has identified the interaction between inflammation and coagulation as a pivotal mechanism in the pathogenesis of sepsis. The inflammatory response in sepsis triggers a coagulation reaction and initiates coagulation, while the ensuing coagulation response further amplifies the inflammatory process (Ma et al., 2019). Under normal circumstances, three key physiological factors regulate the activation of coagulation through anticoagulant pathway systems: the tissue factor pathway inhibitor system, the activated protein C (APC) system, and the antithrombotic system that controls coagulation activation (Petäjä, 2011). The three pathways in sepsis exhibit varying degrees of disruption. Owing to impaired protein synthesis, the coagulation inhibitor pathways demonstrate sustained depletion and protein degradation at low levels. The downregulation of thrombomodulin (TM) and endothelial protein C receptor expression is attributed to the conversion of protein C to APC under inflammatory conditions (Levi and Poll, 2015). The maximal activation of endogenous fibrinolysis and coagulation is significantly attenuated in sepsis, wherein the production of fibrinogen activators (i.e., tissue plasminogen activator (t-PA)) and urokinase-type plasminogen activator (u-PA) by vascular endothelial cell storage sites leads to an augmented stimulation of fibrinogen activation and release of subquantified plasmin, while this effect is counteracted by the sustained increase inhibitor-1 (PAI-1) (Biemond et al., 1995). The Chinese medicines extracts can exert their effects on various components including blood vessels, endothelial cells, platelets, and coagulation factors. They possess the ability to inhibit platelet aggregation and activation, thereby improving sepsis-associated coagulation disorders and reducing the incidence of DIC, ultimately enhancing the quality of life for sepsis patients (Jin et al., 2022). The characteristics of TCM extracts by the modulation of coagulation disorders on sepsis are summarized in Table 3.

Persicae semen extract possessed inhibitory effects on platelet aggregation, the release of various inflammatory cytokines, inflammation-induced vascular permeability, and pulmonary vascular endothelial damage reduction (Yi et al., 2016). Huangqi (*Astragalus*) and Danggui (*Angelica*) were revealed the ability to induce relaxation in airway smooth muscle, scavenge free radicals, inhibit platelet aggregation, and suppress the release of inflammatory mediators such as thromboxane (Li et al., 2015). These actions effectively counteract microthrombosis and confer a protective effect on the vascular endothelium. In another study, breviscapine effectively enhanced the regulation of cellular coagulation and fibrinolysis (Li Z. et al., 2017).

A sepsis model was established by inducing CLP in SD rats (Li and Jin, 2016). Simultaneously, safflower yellow A was administered at various doses, revealing that a low dose of safflower yellow A effectively attenuated the prolongation of prothrombin time (PT) and activated partial thromboplastin time (APTT). Furthermore, it significantly suppressed the expression levels of serum TF, IL-1β, and TNF-α, while enhancing the expression level of serum tissue factor pathway inhibitor (TFPI). These findings suggested that a low dose of safflower yellow A could alleviate the reciprocal amplification between coagulation and inflammation during sepsis, ameliorate sepsis-induced coagulation dysfunction,

TABLE 3 Characteristics of TCM extracts by the modulation of coagulation disorders on sepsis.

Name	Source	Validation model	Administration	Effect	Organ	Positive control	Ref.
Persicae semen extract	*Prunus persica* (L.) Batsch	SD rats model of stasis-heat syndrome	2, 4, 8 g/kg	↓: CD31 ↑: NF-κB, IκB	Kidney	*Salvia miltiorrhiza* (14 g/kg)	Yi et al. (2016)
Huangqi (*Astragalus*) and Danggui (*Angelica*)	*Astragalus membranaceus* (Fisch.) Bge. or *A. membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao *Angelica sinensis* (Oliv.) Diels	Male ICR mice with lung fibroblasts model	0.1 mL/10 g (the dosage of Huangqi and Danggui is 50 g: 10 g, 30 g:30 g, 10 g:50 g, 25 g: 5 g, 15 g:15 g, 5 g:25 g)	↓: Hyp	Lung	NA	Li et al. (2015)
Breviscapine	*Erigeron breviscapus* (Vaniot) Hand.-Mazz.	SD rats with OGD model	10, 30, 90 mg/L	↓: LDH, TNF-α, IL-6, IL-1β, PAI-1, ET-1 ↑: t-PA, PGI_2	Brain	NA	Li et al. (2017b)
Safflower yellow A	*Carthamus tinctorius* L.	CLP-induced male SD rats	20 mg/kg	↓: PT, APTT, TFPI ↑: IL1-β, TNF-α, TF	NA	NA	Li and Jin (2016)
Emodin	*Rheum palmatum* L.	Male Tuck-Ordinary mice with cerebrovascular thrombosis model	4 mg/kg	↓: TNF-α, IL-1β ↑: SOD, PT, aPTT	Heart	NA	Nemmar et al. (2015)
Emodin-6-*O*-b-D-glucoside	*Rheum palmatum* L.	CLP-induced male C57BL/6 mice	0.1, 0.2, 0.5, 1, 2, 5, 10 μM	↓: TACE, EPCR, p38, ERK1/2, JNK	NA	NA	Lee et al. (2013)
Emodin	*Rheum palmatum* L.	CLP-induced male SD rats	50 mg/(kg·d)	↓: APTT, MA, CI, FLEV	NA	NA	Lin et al. (2018)
Baicalin	*Scutellaria baicalensis* Georgi	Endotoxin-induced SD rats	100, 200 mg/kg/d	↓: APTT, PT, FIB	Lung	Ulinastatin (200,000 U/kg)	Yang et al. (2022)

regulate inflammatory factors' expression, delay organ dysfunction syndrome onset, and exert protective effects against lung injury in septic conditions.

The therapeutic effects of emodin on cardiovascular responses induced by diesel exhaust particles (DEP) were investigated in a mouse model (Nemmar et al., 2015). They discovered that emodin significantly reduced the levels of inflammatory factors such as leukocytes, TNF-α, and IL-1. Moreover, it effectively inhibited platelet aggregation *in vitro*, prevented DEP-induced shortening of APTT and PT, while improved the pre-thrombotic state of small cerebral arteries and veins. The intravenous administration of emodin-6-*O*-b-D-glucoside in CLP model mice effectively attenuated the release of endothelial cell protein c receptor (EPCR) induced by CLP through inhibition of TNF-α converting enzyme (TACE) expression, thereby exerting a modulatory effect on anticoagulation (Lee et al., 2013).

CLP-introduced rats with sepsis exhibited a hypocoagulable tendency attributed to the attenuation of coagulation factors and fibrinogen function (Lin et al., 2018). Moreover, it was observed that the endogenous coagulation factor function and fibrinogen function of CLP rats improved, leading to a reduction in 24-h mortality rate following intervention with emodin (50 mg/kg.d). After baicalin intervention, a significant reduction in the levels of APTT, PT, and fibrinogen (FIB) in the sepsis group of rats was observed, particularly in the high dose group of baicalin (Yang et al., 2022). These findings suggested that baicalin effectively ameliorated coagulation disorders in septic rats and disrupted the coagulation-inflammation vicious cycle that mutually exacerbated each other.

Therefore, TCM extracts hold back platelet aggregation and activation, improve coagulation dysfunction in sepsis, as well as reduce the incidence of disseminated intravascular coagulation and multiple organ dysfunction syndrome, enhancing the prognosis of septic patients (show in Figure 1).

4.4 Other mechanisms

4.4.1 Neuroendocrine immune network

The homeostatic regulation involved in the interaction between the neuroendocrine and immune systems constitutes a crucial component of the host response during septic shock (Muscatell et al., 2015). The central nervous system's response to sepsis relies on three primary mechanisms: (1) circulatory inflammatory mediators that communicate with the central nervous system through the choroid plexus and ventricular organs; (2) involvement of the autonomic nervous system, where primary afferent nerves and sensory nerves interact with PAMPs, thereby amplifying inflammatory cytokine activation; (3) activation of endothelial cells in the blood-brain barrier, leading to the release of inflammatory mediators like NOS metabolites

TABLE 4 Characteristics of TCM extracts by the modulation of neuroendocrine immune network, endoplasmic reticulum stress, autophagy defect, and mitochondria damage on sepsis.

Name	Source	Validation model	Administration	Target mechanism	Effect	Organ	Positive control	Ref.
Scutellaria baicalensis extract	*Scutellaria baicalensis* Georgi	LPS-introduced male SD rats	1.5, 3.0 g/kg	Neuroendocrine immune network	↓: NO, TNF-α, IL-1β, IL-6, IL-18 ↑: ACh	Lung	Galantamine (5 mg/kg)	Cui et al. (2012)
Puerarin	*Pueraria montana* var. *lobata* (Willd.) Maesen & S.M.Almeida ex Sanjappa & Predeep	D-galactose model ICR mice	60, 120 mg/kg	Neuroendocrine immune network	↑: ChAT, ACh	Brain	NA	Pan et al. (2008)
Paeoniflorin	*Paeonia lactiflora* Pall.	MCAO model SD rats	2.5, 5, 10 mg/kg	Neuroendocrine immune network	↓: M2 receptor ↑: M1 group receptor, ACh, Kir6.2/Kir6.1	Brain	NA	Wang et al. (2012)
Paeoniflorin	*Paeonia lactiflora* Pall.	Human umbilical vein endothelial cells after ischemia reperfusion	0.078, 0.156, 0.312 mg/mL	Neuroendocrine immune network	↓: ICAM-1, VCAM-1, p-p38 MAPK	NA	NA	Wang et al. (2016)
2′-Hydroxycinnamaldehyde (HCA), 2'-Benzoyloxycinnamaldehyde (BCA)	*Cinnamomum cassia* (L.) J.Presl	LPS-stimulated microglial cultures and microglia/neuroblastoma cocultures	HCA (2 μM), BCA (1 μM)	Neuroendocrine immune network	↓: ERK1/2, JNK, p38 MAPK, NF-κB, iNOS, IL-1β, TNF-α, NO	NA	NA	Hwang et al. (2011)
Genipin	*Gardenia jasminoides* J.Ellis	CLP-introduced male C57BL/6J mice	2.5 mg/kg	Endoplasmic reticulum stress	↓: CHOP, caspase-3, GRP78, p-eIF2α, PERK	Spleen	NA	Luo et al. (2023)
Resveratrol	*Veratrum album* L.	LPS-introduced male New Zealand white rabbits	250 mg/kg	Endoplasmic reticulum stress	↓: PERK, ATF4, CHOP, caspase9, Bax ↑: Bcl-2	Lung	NA	Han et al. (2020)
Ginsenoside Rb1	*Panax ginseng* C.A.Mey.	CLP-introduced female C57BL/6 mice	20 mg/kg	Endoplasmic reticulum stress	↓: GRP78, caspase-12	Heart, lung	NA	Huang et al. (2019b)
Allicin	*Allium sativum* L.	CLP-introduced male Balb/c mice	30 mg/kg	Autophagy defect	↑: SOD, LC3B, Beclin-1 ↓: TNF-α, IL-6, MDA	Lung	3-MA (15 mg/kg)	Peng et al. (2017)
Allicin	*Allium sativum* L.	CLP-introduced male Balb/c mice	30 mg/kg	Autophagy defect	↑: SOD, LC3II/I, Beclin-1 ↓: GPT, GOT, TNF-α, IL-6, MDA	Liver	NA	Gao et al. (2019)
Artesunate	*Artemisia annua* L.	LPS-introduced wide type male BALB/c mice	20 μg/mL	Autophagy defect	↑: LC3II/I, LC3B, NF-κB p65, p-Ser317/p-Ser637 ↓: TNF-α, IL-6, AMPK	Spleen	3-MA, ULK-101, SKF-96365, Bafilomycin A1	Liu et al. (2020)
Genipin	*Gardenia jasminoides* J.Ellis	CLP-introduced male imprinting control region mice	1, 2.5, 5 mg/kg	Autophagy defect	↓: calpain 1 ↑: Atg12-Atg5, Atg3, Rab7	Liver	NA	Cho et al. (2016)
Ginsenoside Rg3	*Panax ginseng* C.A.Mey.	CLP-introduced male C57BL/6 mice	6.25, 12.5, 25 μM	Autophagy defect	↑: OCR, MTP, AMPK, Beclin-1 ↓: ROS, AMPK	Liver	NA	Xing et al. (2017)

(Continued on following page)

10.3389/fphar.2024.1365639

TABLE 4 (*Continued*) Characteristics of TCM extracts by the modulation of neuroendocrine immune network, endoplasmic reticulum stress, autophagy defect, and mitochondria damage on sepsis.

Name	Source	Validation model	Administration	Target mechanism	Effect	Organ	Positive control	Ref.
Ginsenoside Rg1	*Panax ginseng* C.A.Mey.	LPS-introduced C57BL/6 mice	40 mg/kg	Autophagy defect	↓: cleaved caspase 3, cleaved caspase-8 ↑: LC3II/LC3I	Lung	NA	Ji et al. (2019)
Ginsenoside Rg1	*Panax ginseng* C.A.Mey.	CLP-introduced male C57BL/6 mice	40, 200 mg/kg	Autophagy defect	↓: Iba1, TNF-α, IL-6, IL-1β, cleaved caspase 3, LC3-II, p62	Brain	NA	Li et al. (2017a)
Radix hedysari alcohol extract	*Hedysarum polybotrys* Hand.-Mazz.	CLP-introduced male SD rats	1.0, 2.0, 4.0 g/kg/d	Mitochondria damage	↑: HDL, IL-10, pNrf2/Nrf2, HO-1, NQO-1 ↓: AST, ALT, TC, TG, IL-6, MCP-1, TNF-α	Liver	NA	Yuan et al. (2021a)
Resveratrol	*Veratrum album* L.	CLP-introduced male Wistar albino rats	100 mg/kg	Mitochondria damage	↓: MDA, TNF-α ↑: GSH, SOD, GPX	Kidney, liver	NA	Aydın et al. (2016)
Resveratrol	*Veratrum album* L.	LPS-introduced male ICR mice	0.3 mg/kg	Mitochondria damage	↓: iNOS, NO, MDA, H_2O_2 ↑: GSH/GSSG, T-AOC, SOD, CAT	Lung	NA	Zhang et al. (2014)
Polydatin	*Polygonum cuspidatum* Siebold & Zucc.	LPS-introduced wild-type C57 mice	30 mg/kg	Mitochondria damage	↓: ROS ↑: SIRT3, SOD2, CypD	Lung, kidney, liver	NA	Wu et al. (2020a)
Polydatin	*Polygonum cuspidatum* Siebold & Zucc.	CLP-introduced C57BL/6 mice	30 mg/kg	Mitochondria damage	↓: ROS ↑: CLS1, CL, ATP, MMP	Kidney	3-TYP (5 mg/kg)	Lei et al. (2022)
Astragalus polysaccharides	*Astragalus membranaceus* (Fisch.) Bge. or *A. membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao	CLP-introduced SD rats	100, 200 mg/kg	Mitochondria damage	↓: ROS ↑: Na^+ -K^+ -ATP, SIRT1, PGC-1α, p-AMPK/AMPK	Kidney	Dexamethasone (10 mg/kg)	Wang et al. (2021b)
Ligustrazine	*Ligusticum chuanxiong* S.H.Qiu, Y.Q.Zeng, K.Y.Pan, Y.C.Tang & J.M.Xu	Patients with severe sepsis with myocardial injury	80 mg + GS/NS 250 mL	Mitochondria damage	↓: cTnT, MDA, ROS ↑: SOD	Heart	NA	Chen (2011)
Danhong injection	*Salvia miltiorrhiza* Bunge and *Carthamus tinctorius* L.	CLP-introduced male Wistar rats	5 mL/kg	Mitochondria damage	↓: MDA, HMGB1 ↑: SOD	Heart	NA	Hu and Lou (2016)

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(Sonneville et al., 2013). Additionally, dysfunction of the hypothalamic-pituitary-adrenal axis results in reduced serum levels of adrenocorticotropin, adrenocorticotropin, and adrenocortisol in sepsis patients, leading to adrenal insufficiency syndrome (Kanczkowski et al., 2015). The evidence suggests that norepinephrine responds to LPS by exerting inhibitory effects on the expression of pro-inflammatory factors, such as TNF-β and IL-12, while simultaneously promoting the expression of anti-inflammatory cytokines like IL-10 (Tynan et al., 2012). There is limited research on monomer treatment of neuroendocrine immune network; however, recent studies have proposed the concept of the cholinergic anti-inflammatory pathway (CAP) in elucidating the involvement of the vagus nerve in sepsis regulation. The CAP represents a crucial neuro-immune regulatory pathway that activates α7 nicotinic ACh receptors (α7nAChR) through vagal stimulation, leading to acetylcholine (ACh) release and subsequent inhibition of pro-inflammatory cytokine synthesis and release. Consequently, targeting this inflammatory response offers a promising therapeutic approach for sepsis (Fujii et al., 2017). The field of traditional Chinese medicine encompasses a diverse range of plant species, with its active constituents primarily consisting of polysaccharides, glycosides, and alkaloids. It is plausible that certain components within traditional Chinese medicine exert their effects through cholinergic receptor modulation. Its anti-inflammatory and immunomodulatory properties are associated with the inhibition of inflammatory cytokine production, while also exhibiting bidirectional regulatory effects on cytokine synthesis and secretion by inflammatory cells. Thus, it exerts its actions via CAP (Deng et al., 2017). The characteristics of TCM extracts by the modulation of neuroendocrine immune network on sepsis are summarized in Table 4.

The administration of *S. baicalensis* extract in rats with acute lung injury resulted in an increase in serum ACh levels and a decrease in serum TNF-α and NO levels, effectively inhibiting the progression of inflammatory response (Cui et al., 2012). However, there were no significant effects observed on choline acetyltransferase (ChAT) and acetylcholinesterase (AChE) activities related to ACh synthesis and decomposition. Therefore, it can be inferred that the cholinergic anti-inflammatory pathway modulated by *S. baicalensis* extract may be attributed to its ability to enhance ACh release, which subsequently binds to nicotinoid α7 receptors or M receptors on monocytes, leading to a significant inhibition of TNF-α through intracellular signal transduction pathways. This mechanism also suppresses the release of proinflammatory factors IL-1β, IL-6, and IL-18, thereby regulating the overall inflammatory response.

Puerarin was found to enhance choline acetyltransferase activity and increase ACh content in the cerebral cortex of D-galactose rats (Pan et al., 2008). However, it did not exert a significant effect on cholinesterase activity. These findings suggest that puerarin's anti-inflammatory effect may be attributed to its ability to activate the vagus nerve. Experimental studies have demonstrated that paeoniflorin exerts a protective effect on inflammatory cells through the regulation of cholinergic M receptor signaling pathway, modulation of cholinergic M receptor activity, and activation of the M-receptor-G protein-KATP channel (Wang et al., 2012). Paeoniflorin was postulated to exert its inhibitory effects on adhesion factors and the p38MAPK signaling pathway through the regulation of the cholinergic anti-inflammatory pathway, as demonstrated in experimental models (Wang et al., 2016). This regulatory mechanism ultimately leads to an amelioration of inflammatory responses in endothelial cells, alleviation of tissue damage, and a protective role in HUVECs.

2′-hydroxycinnamaldehyde (HCA) and 2′-benzoyloxycinnamaldehyde (BCA) in LPS-stimulated microglia cultures and microglia/neuroblastoma cocultures were observed to have the potential anti-neuroinflammatory effects, which protected neuroblastoma cells from microglia-mediated cell death (Hwang et al., 2011). It is postulated that low density lipoprotein receptor-related protein 1 (LRP1) serves as a promising molecular target for HCA in modulating microglial responses. The findings demonstrated that treatment with HCA at a concentration of 2 μmol/L and BCA at a concentration of 1 μmol/L effectively suppressed the expression of iNOS, proinflammatory cytokines IL-1β and TNF-α, as well as NO production by inhibiting the activation of ERK1/2, JNK, p38 MAPK, and NF-κB signaling pathways. These results highlight the therapeutic potential of HCA and BCA in managing neuroinflammatory diseases.

In summary, TCM extracts may inhibit the release of pro-inflammatory cytokines through the cholinergic anti-inflammatory pathway, playing an anti-neuroinflammatory effect and ultimately reducing tissue damage in sepsis (show in Figure 2).

4.4.2 Endoplasmic reticulum stress

The endoplasmic reticulum is an intracellular organelle involved in the translocation, folding, post-translational modifications, and subsequent transport of intracellular organelles to the Golgi apparatus (Khan et al., 2015). In sepsis, accumulation of unfolded or misfolded proteins in the endoplasmic reticulum disrupts its homeostasis and leads to oxidative stress and severe calcium disturbances, resulting in endoplasmic reticulum stress (Khan et al., 2015). During endoplasmic reticulum stress, the unfolded protein response sensor may undergo a unique signal switch to induce cell death. The registration signaling mechanism involves several sequential steps: Firstly, the transcriptional activation of the C/EBP homologous protein (CHOP) gene is mediated by pkr-like endoplasmic reticulum kinase (PERK), inositol-requiring enzyme 1 (IRE1), and transcriptional activator 6 (ATF6). Secondly, there is an activation of the IRE-mediated JNK pathway, followed by TNF receptor-associated activator 2 and apoptosis signal-regulated kinase 1. Finally, activated caspase-12 associated with caspase-12 activation migrates from the endoplasmic reticulum to the cytoplasm, leading to cleavage of caspase-9 and ultimately activating caspase-3 (Li et al., 2014; García de la Cadena and Massieu, 2016; Zhang et al., 2018). In animal models of sepsis, increased markers of endoplasmic reticulum stress [e.g., glucose-regulated protein 94 (GRP94), CHOP, and caspase-12] have been observed in various organs including the heart and liver. Additionally, a direct correlation between these markers and the degree of organ dysfunction suggests that they may play a significant role in causing multi-organ failure in sepsis (Jiao et al., 2017). The induction of abnormal apoptosis due to endoplasmic reticulum stress in septic animals indicates that targeting endoplasmic reticulum stress-mediated apoptosis could be a promising avenue for research on clinical

prevention and treatment strategies for sepsis. Traditional Chinese medicine has shown potential in attenuating lymphocyte apoptosis induced by sepsis through inhibition of the endoplasmic reticulum stress pathway (Xi et al., 2017). The characteristics of TCM extracts by the modulation of endoplasmic reticulum stres on sepsis are summarized in Table 4.

Genipin, derived from *Gardenia jasminoides* J.Ellis fruit, is a well-known traditional Chinese medicine with antipyretic and detoxifying properties. It has been demonstrated to possess anti-inflammatory, antioxidant, and bacteriostatic activities (Ishiguro et al., 1983; Luo et al., 2019). Recent studies have indicated that genipin can enhance the prognosis of sepsis while reducing sepsis-related liver damage and lung injury (Kim et al., 2012).

The impact of genipin on apoptosis induced by endoplasmic reticulum stress in spleen cells following CLP was further investigated (Luo et al., 2023). The findings demonstrated that genipin significantly attenuated the expression levels of splenic CHOP and caspase-3 proteins in CLP mice. Moreover, genipin markedly reduced the number of tunel-positive splenocytes. These results indicated that genipin possessed potential protective effects against sepsis. Furthermore, it was revealed that the underlying protective mechanism of genipin against sepsis involves inhibiting endoplasmic reticulum stress, downregulating CHOP protein expression, and mitigating splenocyte apoptosis.

Curcumin is a crucial active ingredient in *Curcuma longa* L. that exerts pharmacological effects. (Marchiani et al., 2014). Numerous animal experiments have consistently demonstrated that curcumin exhibits a broad spectrum of pharmacological activities including anti-inflammatory, anti-oxidative stress, anti-apoptotic, and immunomodulatory effects (Shehzad et al., 2010). Moreover, an extensive body of research has confirmed the protective effect of curcumin against sepsis and septic shock (Karimi et al., 2019; Wang S. et al., 2021; Vieira et al., 2023).

Resveratrol is a small non-flavonoidal polyphenolic compound present in various plants. Pharmacological studies have indicated that resveratrol exhibits diverse biological activities, such as anti-inflammatory, antioxidant properties, oxygen free radical scavenging abilities, and antitumor effects (Hiramatsu et al., 2015; Lu et al., 2018). Han et al. (2020) utilized the LPS method to induce the establishment of a rabbit model of acute lung injury (ALI). The rabbits were grouped and intervened with resveratrol and sodium chloride injection. The findings from this study demonstrated that resveratrol effectively reduced the protein level and mRNA expression of PERK, ATF4, CHOP, caspase-9, Bax while increasing the protein and mRNA expression level of Bcl-2 in ALI-induced lung tissues. Additionally, resveratrol attenuated histopathological injury induced by LPS in ALI rabbit lungs. These results suggest that resveratrol exerts a protective role against LPS-induced ALI in rabbits by mitigating endoplasmic reticulum stress and apoptosis.

The sepsis mouse model using the CLP method was successfully replicated by Huang et al. (2019b), and their experimental study demonstrated significant improvement in the overall condition of mice treated with ginsenoside Rb1 compared to those in the CLP group. Additionally, the alveolar tissues exhibited clear and intact structures with reduced infiltration of inflammatory cells, resulting in uniform-sized alveoli. Myocardial tissues showed decreased infiltration of inflammatory cells, visible transverse striations of the myocardium, and a reduction in necrotic cells. The expression levels of GRP78 and caspase-12 proteins in myocardial and lung tissues of the ginsenoside group exhibited a significant reduction, with highly statistically significant differences ($p < 0.01$). These findings suggest that ginsenoside Rb1 can attenuate the severity of infection and exert a protective effect on heart and lung tissue damage in septic mice. This mechanism may be attributed to the inhibition of excessive endoplasmic reticulum stress by ginsenoside Rb1.

Therefore, TCM extracts inhibit endoplasmic reticulum stress and reduce the expression of CHOP apoptotic protein, thereby alleviating the degree of cell apoptosis and achieving improvement in organ dysfunction caused by sepsis (show in Figure 2).

4.4.3 Autophagy defect

Autophagy is a natural process in which cytoplasmic material or pathogens are phagocytosed and subsequently degraded through fusion with lysosomes. It serves as a crucial defense mechanism employed by the host to combat external pathogens and danger signals, while also playing an integral role in inducing and regulating the inflammatory response of innate immune cells - a key determinant influencing sepsis development (Qiu et al., 2019). Autophagy plays a protective role in sepsis, potentially through the following mechanisms: pathogen elimination, neutralization of microbial toxins, regulation of cytokine release, reduction of apoptotic cancer targets, and promotion of antigen expression (Nakahira et al., 2011; Ryter et al., 2014; Maurer et al., 2015). Chinese medicines can prevent sepsis by modulating autophagy-related signaling pathways, primarily involving the regulation of the AMPK pathway and PINK1/Parkin pathway. Additionally, they inhibit autophagy-related signaling pathways such as the JNK pathway, mTOR pathway, NF-κB pathway, as well as apoptosis to achieve anti-inflammatory effects and mitigate organ damage (Chang et al., 2023). The characteristics of TCM extracts by the modulation of autophagy defect on sepsis are summarized in Table 4.

Recently, there is mounting evidence indicating that autophagy defects may contribute to the development of sepsis-induced immunosuppression (Ren et al., 2017; Venet and Monneret, 2018). In a study, the expression of autophagy marker proteins LC3B and Beclin-l in sepsis-associated acute lung injury (ALI) was examined, along with the impact of allicin treatment on these markers (Peng et al., 2017). The findings revealed an increase in the expression of LC3B and Beclin-1 in lung tissues of mice with sepsis ALI, suggesting an upregulation in autophagy levels during septic ALI. Furthermore, treatment with allicin further augmented the expression of LC3B and Beclin-1 in lung tissue, indicating its ability to enhance autophagy levels and exert a protective effect against septic ALI. Another study showed that allicin enhanced the expression of LC3-B and Beclin-1 in hepatic tissues of septic mice, with a direct correlation to the level of autophagy (Gao et al., 2019). This study suggested that allicin's hepatoprotective effect might be attributed to its modulation of autophagic activity.

The administration of artesunate upregulated autophagy and reversed the tolerant state in LPS-tolerant mice (Liu et al., 2020). Their findings unveiled a novel immunopharmacological effect of artesunate in reversing LPS tolerance by restoring autophagy. It has

been demonstrated that artesunate restores cytokine production and enhances bacterial clearance through the induction of autophagy.

The mice were induced sepsis in through CLP and observed a decrease in hepatic expression of autophagy-associated protein Atg3, which was attenuated by genipin. CLP impaired autophagic flux, resulting in increased expression of hepatic microtubule-associated protein-1 light chain 3-II and sequestoome-1/p62 proteins; however, genipin restored the impaired autophagic flux and attenuated the CLP-induced decrease in hepatic lysosome-associated membrane protein-2 and Rab7 proteins as well as elevated expression of calpain 1 protein. These findings suggest that genipin protects against septic injury by restoring impaired autophagic flux through inhibition of the calpain system (Cho et al., 2016).

Xing et al. (2017) conducted *in vivo* and *in vitro* models of sepsis using CLP rats and LPS-treated human primary hepatocytes, respectively. They discovered that ginsenoside Rg3 can alleviates mitochondrial autophagy by activating the AMPK signaling pathway, increasing the levels of autophagy-associated proteins LC3BI, LC3II, and Beclin-1. The role of mitochondrial autophagy was investigated in rat liver and human primary hepatocytes. The effect of LPS on autophagy in mouse lung epithelial cells was observed a gradual increase in the expression of LC3II/LC3I (an autophagosome membrane protein) from 0 to 16 h followed by a decrease at 24 h (Ji et al., 2019). This suggested that autophagy initially increased but then decreased during the process induced by LPS. In addition, this study revealed that ginsenoside Rg1 administration increased autophagy and mitigated LPS-induced lung epithelial cell modulation (modulation rate decreased from 9.33% to 6.14%) after 24 h of LPS exposure in mouse lung epithelial cells, thereby attenuating sepsis-induced lung injury. Ginsenoside Rg1 was demostrated to protect against sepsis-induced brain injury by reducing the levels of autophagy-associated proteins LC3-II and p62 in the hippocampal region, inhibiting accumulation, and suppressing atypical Beclin-independent autophagy to safeguard against sepsis-induced brain injury (Li Y. et al., 2017). These studies collectively highlight the significance of modulating autophagy using ginsenosides within specific temporal and spatial parameters for effective sepsis treatment.

Traditional Chinese medicine extracts effectively modulate autophagy levels to mitigate sepsis, suppress inflammation, and attenuate multipe organ damage (show in Figure 2).

4.4.4 Mitochondria damage

Mitochondria are crucial microcellular organelles involved in energy production, protein synthesis, and catabolism (Vringer and Tait, 2023). However, sepsis-induced mitochondrial damage or dysfunction can result in cellular damage, metabolic disorders, inadequate energy production, and oxidative stress. This ultimately leads to apoptosis of organ cells and immune cells, thereby causing multiorgan immune disorders, multiorgan failure, and even death. During sepsis, the antioxidant system mechanisms are disrupted due to limited oxygen supply and incomplete oxidative reactions as well as hypoxia. Consequently, there is a significant increase in the production of free radicals (Rocha et al., 2012). When exposed to DAMP or PAMP, activated leukocytes release inflammatory cytokines that stimulate NADPH-expressing oxidases (Quoilin et al., 2014). These cytokines induce an excessive production of reactive nitrogen species (RNS), which inhibits NO through the promotion of iNOS activity. The combination of NO and ROS can lead to peroxidation, resulting in the formation of RNS, which irreversibly inhibits electron transport chain (ETC.) activity. Consequently, this dysfunction of the ETC contributes to the generation of additional ROS within mitochondria during sepsis, unfortunately leading to further mitochondrial damage such as endosomal impairment, inhibition of ETC activity, and mitochondrial DNA damage (Singer, 2014). Eventually, the mitochondrial matrix undergoes swelling, leading to rupture of the mitochondrial membrane and initiation of apoptosis. Traditional Chinese medicine has the ability to modulate apoptosis balance, stabilize mitochondrial membrane potential, and regulate other pathways in order to exert a protective effect against sepsis-induced mitochondrial damage. The characteristics of TCM extracts by the modulation of mitochondria damage on sepsis are summarized in Table 4.

Nuclear related factor 2 (Nrf2) is the most potent transcription factor that regulates the antioxidant stress response in the organism. The stimulation of cells by oxygen free radicals leads to the phosphorylation of Nrf2, its dissociation from specific receptors, translocation to the nucleus, and subsequent initiation of the expression of various downstream antioxidant genes including HO-1 and NQO-1 (Liu et al., 2016; Senthil et al., 2016). The proteins HO-1 and NQO-1 possess antioxidant, anti-apoptotic, anti-inflammatory, and microcirculation maintenance functions, which play a crucial role in the body's defense against oxidative stress (Choi, 2016; Hafez et al., 2019). The levels of Nrf2 were significantly reduced in sepsis-induced liver-injured rats, leading to an imbalance in the body's oxidative/antioxidant system. This imbalance results in the release of a substantial amount of oxygen free radicals and subsequently induces oxidative stress injury in liver tissues (Yang et al., 2018). The alcoholic extract derived from *Hedysarum polybotrys* Hand.-Mazz. (*Radix hedysari*) was discovered to significantly enhance the phosphorylation level of pNrf2/Nrf2 and the expression levels of HO-1 and NQO-1 in hepatic tissues of septic rats, indicating that *R. hedysari* alcohol extract could elevate antioxidant activity and eliminate excessive oxygen free radicals in liver tissues of septic rats, thereby mitigating inflammatory damage to hepatic tissues (Yuan C. et al., 2021).

Aydin et al. (2016); Zhang et al. (2014) demonstrated that resveratrol exhibited a reduction in iNOS and NO concentrations, an increase in the ratio of reduced glutathione to oxidized glutathione (GSH/GSSG), as well as an elevation in peroxidase activity (catalase, superoxide dismutase, and other antioxidant enzymes). Moreover, these compounds effectively mitigated organ damage in septic mice.

Polydatin could effectively activate SIRT3, leading to the deacetylation of SOD2, reduction in ROS production, stabilization of endothelial adherens junction (AJ), and ultimately improved prognosis in septic mice through the protection of endothelial permeability (Wu J. et al., 2020). Polydatin enhanced the expression of CLS1 in SAKI through SIRT3, thereby promoting CL synthesis and improving mitochondrial function (Lei et al., 2022). *Astragalus* polysaccharides could enhance the energy metabolism of renal epithelial cells, improved the mitochondrial structure of the renal cortex, and mitigated histopathological changes in septic acute kidney injury rats (Wang X. et al., 2021).

The amide alkaloid ligustrazine was obtained through isolation and purification from the traditional Chinese medicine *Ligusticum*

chuanxiong S.H.Qiu, Y.Q.Zeng, K.Y.Pan, Y.C.Tang & J.M.Xu. It acts as a novel calcium antagonist, exhibiting a wide range of pharmacological effects including antioxidant, anti-inflammatory, and antifibrotic activities (Ran et al., 2011). The compound can scavenge ROS by reducing plasma malondialdehyde (MDA) levels and increasing superoxide dismutase (SOD) activity, thereby providing protection against biofilm damage, acting as an antioxidant, and effectively improving myocardial injury in sepsis (Chen, 2011). Early administration of ligustrazine can effectively scavenge ROS, inhibit lipid peroxidation, and have other beneficial effects that contribute to improved patient prognosis (Wang, 2012; Liu, 2014). The Danhong injection exhibits a certain efficacy in combating myocardial damage induced by sepsis, as it effectively suppresses the levels of MDA and HMGB1 in the cardiac tissue of CLP septic rats while simultaneously enhancing SOD activity (Hu and Lou, 2016).

In summary, TCM extracts can exert a protective effect on sepsis-induced mitochondrial damage by inhibiting oxidative stress reactions, regulating the balance of oxidation/antioxidation, and eliminating ROS (show in Figure 2).

5 Discussion

As a significant form of complementary and integrated medicine, TCM possesses immense potential and advantages in the treatment of sepsis (Liang et al., 2015; Wang J. et al., 2022). The intervention of TCM can effectively leverage the role in addressing bacterial resistance during the diagnosis and treatment of sepsis patients, capitalizing on its distinct advantages across different stages. In the early stage of sepsis, the implementation of TCM as a complementary antibiotic therapy, particularly for patients with resistant bacterial infections, can effectively mitigate the emergence of drug-resistant bacteria, alter the resistance patterns exhibited by existing resistant or pan-resistant strains, and subsequently reduce the occurrence rate of multiple infections. The spleen and stomach play a crucial role in the development of sepsis by enhancing mental resilience and reducing the incidence of multiple organ dysfunction syndrome (MODS) through promoting clearance and minimizing turbidity. If MODS occurs, TCM can also be utilized to combat shock, dysfunction in blood coagulation, rectify the imbalance of inflammatory response, and mitigate organ damage. On the other hand, the exceptional merits of TCM are evident in its remarkable safety profile: according to a comprehensive investigation on drug-induced liver injury (DILI)-related adverse drug reactions (ADR) in China (L-ADR), Chinese medicine accounted for merely 4.5% of all reported ADR cases, while conventional drugs encompassing chemical and biological agents constituted 95.5%, with antibiotics including anti-tuberculosis medications representing the highest proportion (Wang J. et al., 2022).

In the treatment of sepsis and its complications, traditional Chinese medicine extracts and active ingredients have multiple effects such as anti-inflammatory properties, improvement of microcirculation, alleviation of gastrointestinal dysfunction, enhancement of the immune system, and prevention of organ damage. Traditional Chinese medicine extracts not only regulate the inflammation imbalance, improve impaired immune function, and enhance coagulation disorders to treat sepsis and improve patient prognosis. They also exert therapeutic effects on sepsis and multiple organ dysfunction by modulating the neuroendocrine-immune network, inhibiting endoplasmic reticulum stress, regulating autophagy defects, and improving mitochondrial damage. Given the complexity of chemical components in traditional Chinese medicine and the uncertainty of active ingredients, most studies have focused on isolating active compounds from prescriptions and single herbs for cellular or animal research. However, whether traditional Chinese medicine exacerbates multi-organ damage in sepsis patients due to potential side effects on relevant organs remains a crucial question that has not been thoroughly studied. Therefore, identifying and evaluating the potential harm of drugs on organ function is the main challenge faced by traditional Chinese medicine in terms of medication safety for sepsis treatment. In addition, there is currently no universally recognized standard for evaluating the efficacy of traditional Chinese medicine. Furthermore, the quality control of clinical trials for traditional Chinese medicine also needs improvement. In the future, a multidisciplinary collaborative effort will be necessary to elucidate the material basis of traditional Chinese medicine and provide important evidence for understanding the pathogenesis of sepsis and developing new drugs for treating sepsis. Therefore, it is imperative to introduce new systematic, standardized, and operationally strong guidelines in this research field to expedite the modernization and standardization process of traditional Chinese medicine.

6 Conclusion and future prospect

This review classifies and summarizes the efficacious monomeric constituents of traditional Chinese medicine for the treatment of sepsis based on their mechanisms of action. The findings demonstrate that the majority of potent monomeric components exert their effects by directly attenuating inflammatory factors, while some possess inhibitory properties against the NF-κB pathway or exhibit direct antagonism towards LPS. Others enhance patient survival rates by mitigating disseminated intravascular coagulation through anticoagulant activity. Furthermore, polysaccharides and saponins rectify functional abnormalities in sepsis via immunoregulatory effects, whereas specific monomers and extracts regulate autophagy to prevent and manage sepsis. This review provides valuable insights into the diverse pathways and therapeutic targets of monotherapy with TCM for sepsis treatment, facilitating a comprehensive comprehension of its underlying mechanisms.

The current research primarily focuses on the comprehensive study of TCM. The clinical application of TCM compound, such as Qingwenbaidu decoction (Leng et al., 2012), Dachengqi decoction, and Xuebijing injection (Ma and Song, 2009), is prevalent to therapy spesis; however, there is limited research on the TCM monomer. However, the mechanisms of action and target sites of TCM extracts are still unclear, and issues such as the lack of standardized dosages for effective ingredients from these components remain unresolved. The chemical structure of medicine, either similar to or belonging to the genus of TCM itself, exhibits a certain correlation. By means of animal and cell experimental research, it is possible to expedite the identification of TCM monomer compositions and leverage modern high-tech advancements for better understanding important targets and advantages in sepsis treatment. Therefore, the researchers can further facilitate the discovery or synthesis of analogs or derivatives, expanding the scope of the new drug research and development of

TCM. The potential targets of TCM extracts and active components in the treatment of sepsis and its complications have been identified through a network pharmacological prediction, molecular docking analysis, and visual analysis in the field of molecular biology research. The experimental study on the mechanism of TCM treatment for sepsis, combined with the aforementioned methods, offers a novel direction in the quest for more efficacious intervention strategies. The research field requires the introduction of new systematic, normative, and operable guidelines to gain international recognition for the therapeutic advantages of TCM in treating acute and critical diseases. Most traditional Chinese medicines are administered orally, thereby entering the digestive tract. Consequently, the gut microbiota plays a pivotal role in the metabolism of these medications. Moreover, numerous traditional Chinese medicines exert an influence on the equilibrium of gut microbiota. Henceforth, future research endeavors should consider exploring the correlation between sepsis and gut microbiota status, as well as investigating the regulatory effects of traditional Chinese medicines on gut microbiota.

Author contributions

C-FF: Writing–original draft, Conceptualization. J-LL: Writing–original draft, Conceptualization. J-WC: Writing–original draft, Project administration. HL: Writing–original draft, Visualization. W-RZ: Writing–original draft, Visualization. S-YH: Writing–original draft, Project administration. X-WM: Writing—review and editing, Funding acquisition. X-FY: Writing–review and editing. H-LW: Writing–review and editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was supported by Heilongjiang Province Provincial Higher Education Institutions' Basic Research Operating Expenses Scientific Research Programs (No. 2023-KYYWFMY-0011).

Acknowledgments

The authors would like to thank the reviewers and authors of all references.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Glossary

TCM	Traditional Chinese medicine
DIC	disseminated intravascular coagulation
DAMP	damage-associated molecular pattern
PAMP	pathogen-associated molecular pattern
LPS	lipopolysaccharide
MTT	methyl thiazolyl tetrazolium
ELISA	enzyme-linked immunosorbent assay
TNF	tumor necrosis factor
IL	interleukin
CLP	cecal ligation and puncture
TLR-4	Toll-like receptor 4
SphK1	sphingosine kinase 1
ERK1/2	extracellular signal-regulated kinase 1/2
NF	nuclear factor
HMGB1	high mobility group protein B1
APS	*Astragalus* Polysaccharides
iNOS	inducible nitric-oxide synthase
COX-2	cyclooxygenase-2
PGE2	prostaglandin E2
ICAM-1	intercellular adhesion molecule-1
VCAM-1	vascular cell adhesion molecule-1
IRF4	interferon regulatory factor 4
MUM1	multiple myeloma oncogene 1
NLRP	nucleotide-binding domain, leucine-rich repeat, and pyrin domain-containing protein
TGF-β	transforming growth factor-β
Th	T lymphocyte subgroup
Treg	regulatory T cell
JAK2	Protein tyrosine kinase 2
STAT3	Signal transducers and activator 3
IFN	interferon
MAPK	mitogen activated protein kinase
ERK	extracellular signal regulated kinase
TF	tissue factor
PT	prothrombin time
APTT	activated partial thromboplastin time
THR	threonine
EPCR	endothelial cell protein c receptor
TACE	tumor necrosis factor-a converting enzyme
FIB	fibrinogen
CAP	cholinergic anti-inflammatory pathway
ACh	acetylcholine
α7nAChR	α7 nicotinic ACh receptors
ChAT	choline acetyltransferase
AChE	acetylcholinesterase
LRP1	lipoprotein receptor-related protein 1
CHOP	C/EBP homologous protein
PERK	pkr-like endoplasmic reticulum kinase
IRE1	inositol-requiring enzyme 1
HLA-DR	human leukocyte antigen-DR
ATF6	transcriptional activator 6
RNS	reactive nitrogen species
Nrf2	nuclear related factor 2
ROS	reactive oxygen species
ETC.	electron transport chain
MDA	malondialdehyde
HO-1	Hemeoxygenase-1
NQO-1	ubiquinone oxidoreductase
LC	light chain
PINK 1	PTEN induced putative kinase 1.

frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 18 July 2024
DOI 10.3389/fphar.2024.1412245

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OPEN ACCESS

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RECEIVED 04 April 2024
ACCEPTED 24 June 2024
PUBLISHED 18 July 2024

CITATION
Sadek KM, Shib NA, Taher ES, Rashed F, Shukry M, Atia GA, Taymour N, El-Nablaway M, Ibrahim AM, Ramadan MM, Abdelkader A, Abdo M, Imbrea I, Pet E, Ali LS and Abdeen A (2024), Harnessing the power of bee venom for therapeutic and regenerative medical applications: an updated review. *Front. Pharmacol.* 15:1412245. doi: 10.3389/fphar.2024.1412245

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Harnessing the power of bee venom for therapeutic and regenerative medical applications: an updated review

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Honeybees have been helpful insects since ancient centuries, and this benefit is not limited to being a honey producer only. After the bee stings a person, pain, and swelling occur in this place, due to the effects of bee venom (BV). This is not a poison in the total sense of the word because it has many benefits, and this is due to its composition being rich in proteins, peptides, enzymes, and other types of molecules in low concentrations that show promise in the treatment of numerous diseases and conditions. BV has also demonstrated positive effects against various cancers, antimicrobial activity, and wound healing versus the human immunodeficiency virus (HIV). Even though topical BV therapy is used to varying degrees among countries, localized swelling or itching are common side effects that may occur in some patients. This review provides an in-depth analysis of the complex chemical composition of BV, highlighting the diverse range of bioactive compounds and their therapeutic applications, which extend beyond the well-known anti-inflammatory and pain-relieving effects, showcasing the versatility of BV in modern medicine. A specific search strategy was followed across various databases; Web of sciences, Scopus, Medline, and Google Scholar including *in vitro* and *in vivo* clinical studies.to outline an overview of BV composition, methods to use, preparation requirements, and Individual

consumption contraindications. Furthermore, this review addresses safety concerns and emerging approaches, such as the use of nanoparticles, to mitigate adverse effects, demonstrating a balanced and holistic perspective. Importantly, the review also incorporates historical context and traditional uses, as well as a unique focus on veterinary applications, setting it apart from previous works and providing a valuable resource for researchers and practitioners in the field.

KEYWORDS

apitherapy, anti-inflammation, anticancer, antimicrobial, neuroprotection, wound healing

1 Introduction

Bees are commercially beneficial insects that have been around since the Cretaceous age of the Mesozoic Era. They also help fertilize many different crops. Bees are helpful, but their capacity to administer excruciating and poisonous stings constitutes a risk. Thankfully, most honeybees are not hostile to people and only resort to violence if they perceive danger (Pucca et al., 2019). *Apis mellifera* is the most often used honeybee species for agricultural pollination globally. All bee products, particularly venom, and honey, have been used for centuries, and their medicinal properties have been described in holy writings such as the Bible and the Quran (Ali, 2024; Dinu et al., 2024). Bee venom (BV) treatment involves injecting honeybee venom into the human body to cure various ailments. For over 5,000 years, this technique has been used in complementary therapies. It consists of either an indirect implementation, like isolating BV via stimulating electricity and then administering it into the body, or an immediate administration, such as bee stings (Wehbe et al., 2019). Applying BV in medicine stemmed from discovering that beekeepers rarely experience rheumatism or joint discomfort (Ullah et al., 2023). Across different cultures, BV has been employed in ethnomedicine as a natural remedy for a wide range of health conditions. Traditional practices have incorporated BV for its anti-inflammatory effects, pain relief properties, and immune system stimulation. In ethnomedicinal practices, BV has been used to alleviate symptoms of arthritis, rheumatism, and inflammatory conditions. Additionally, BV has been applied for its potential in wound healing, skin conditions, and even as an antimicrobial agent (Bava et al., 2023). BV is a colorless, scentless liquid of numerous molecules with a slightly acidic pH (4.5–5.5), which bees utilize as protection from enemies. A single drop of BV comprises 88% water and only 0.1 g of dry venom (Bellik, 2015). BV consists of several compounds and a significant quantity of water (Farook et al.; Essam Elenany, 2024). BV content has previously been identified via omics and fractionation techniques (Pucca et al., 2019). BV is a biotoxin or api-toxin produced by a gland in the bee's abdomen cavity. It comprises a complicated blend of many physiologically active peptides-incredibly intricate peptide combinations. Melittin, adolapin, apamin, and MCD-peptide are only a few examples. It also has powerful enzymes, like phospholipase A2 (PLA2), hyaluronidase, acid phosphodiesterase, -D-glucosidase, and lysophospholipase are enzymes, and in addition to low-molecular-weight molecules naturally occurring amines (e.g., histamine and epinephrine) and micronutrients (Moreno and Giralt, 2015). Apitoxin formulation is influenced by bee age, where they live, seasonal shifts, and social standing. Melittin, apamin, hyaluronidase, and PLA2 levels are particularly sensitive to change.

Furthermore, the procedures for collecting BV influence the volatile component composition. Electricity that is used to stimulate histamine can vanish during BV extraction (Schmidt, 2018). Many investigations have examined the curative effects of these substances in managing numerous illnesses and conditions (Wehbe et al., 2019). BV possesses anticancer, antimicrobial, and antiviral activities (Gajski et al., 2024).

This article offers an updated and in-depth review of the intricate chemical profile of BV, emphasizing the wide variety of bioactive compounds and their therapeutic potential and clinical applications against various illness. This highlights the adaptability of BV in contemporary medicine.

2 Sources of bee venom

Female honeybees generate venom from a gland situated in their abdomen cavity (Ali, 2024). The gland is attached to a container capsule. Apis social insects rely heavily on their seminiferous system for protection. Bees sting near apiaries to defend their colonies (Piek, 2013). The queen uses stings to eliminate competitors (Portelli, 2020). When many queens are born simultaneously, they escape with a particular number of bees, murder the unborn queens in their cell, or participate in a dying battle. Each hive may only have one queen. BV has the maximum protein concentration in the initial 1–3 days of life, then declines following 7 days. This is critical for killing competitors in the fight for beehive leadership. Honeybee venom's protein concentration decreases when the gland degenerates in subsequent days. Female honeybees do not produce detectable venom at the moment of emergence (Bava et al., 2023). Instead, it swiftly climbs during the next 2 days, stays steady for 14 days, and then decreases. Older honeybees generate less venom than younger bees. The venom's makeup varies with age. Melittin is secreted as a dormant component becomes active during growth and passes into the guardian stage around day 20 of age (Bava et al., 2023). Honeybees withdraw their venom sac and pointed stinger from their abdomen when stinging.

In contrast to similar insects, they are limited to stinging once before dying (Elieh Ali Komi et al., 2018). When a bee strikes a human or an animal, the stinger remains attached under the outer layer of skin, and the honey bee passes away by pulling out its guts, muscles, and nerve center to remove itself. Many of the bees' bodies are lost, leading to death. The stinger's sharp tip has microscopic

hooks that prevent it from being eliminated without causing damage. Once lodged, the venom is pushed into the wound using a separate piston mechanism (Lee et al., 2020). The stinger integrates into the tissue, discharging the components of the venom chamber within a few minutes (Elieh Ali Komi et al., 2018). BV sends an alarm pheromone that stimulates other honeybees to protect the beehive (Elieh Ali Komi et al., 2018). BV can induce localized inflammatory reactions, such as discomfort, warmth, and irritation, as well as systemically triggered allergic responses that can lead to anaphylactic shock and even death (Annila et al., 1996).

BV can be collected using various techniques, with the most common method being the electric shock extraction process. This method involves exposing bees to a low-voltage electric current (20–30 V), which induces them to release venom that is then collected on a glass plate. The bees are not harmed during this process, making it safe for them. Another traditional method involves surgically removing the venom gland from bees or squeezing individual bees until a drop of venom is extracted from the stinger tip. However, this method is less common compared to the electric shock technique. The venom collected through the electric shock process is considered effective and is widely used in clinical applications. The final product has varying properties depending on the extraction process used, with the most effective venom being that which is collected under water to prevent the evaporation of the highly volatile components (Otręba et al., 2021).

After collection, BV undergoes processing to extract its active components for medical use. Techniques such as chromatographic separation and molecular genetic methods are employed to isolate individual BV active ingredients for specialized medical purposes. These processes allow for the extraction of specific compounds like melittin and apamin, which possess unique therapeutic properties (Pucca et al., 2019; Bava et al., 2023).

To ensure the reproducibility and safety of BV-based therapies, it is crucial to accurately identify and measure the components of BV. Standardization of BV is necessary to guarantee the consistency of the final product and its therapeutic efficacy. Factors such as the bee species, season, and geographical location can influence the composition of BV. Therefore, quality control measures, including analysis of moisture content, protein profile, and cytotoxicity, are essential during the processing and extraction stages (El Mehdi et al., 2022).

3 Composition of bee venom

BV is a complex mixture composed primarily of water (88%) and various peptides, enzymes, and other compounds. The main components of BV including (1) peptides; melittin (50%–60% of dry venom), a small peptide with 26 amino acids that causes pore formation in membranes; apamin (2%–3% of dry venom), a neurotoxic peptide mast cell degranulating (MCD) peptide; and adolapin. (2) Enzymes; phospholipase A2 (10%–12% of dry venom) destroys phospholipids in cell membranes, hyaluronidase, phosphatase, α-glucosidase. (3) Biogenic amines; histamine, epinephrine (adrenaline). (4) Other compounds; lipids, carbohydrates, free amino acids, and minerals.

The composition of BV can vary depending on factors like bee species, season, and geographical location. However, melittin and phospholipase A2 are consistently the most abundant components, making up over 80% of dry BV. The complex chemical nature of BV contributes to its wide range of biological activities and therapeutic potential (Sharaf et al., 2024a). Hymenoptera venom includes several active elements, small-molecular-weight compounds, and aliphatic contents (Table 1) (Silva et al., 2015). The makeup is complex, with proteins accounting for 80% of the total. Proteins generally have large molecular weights, while peptides have lower molecular weights. Biogenic amines, such as dopamine, histamine, and serotonin, are crucial low-molecular-weight compounds found in BV. BV peptides, like adolapine, melittin, apamin, and peptide, have been extensively studied for their bioactivities and potential therapeutic applications (Isidorov et al., 2023).

The appropriate collection method is crucial to ensure the highest quality BV. It is essential to collect the venom without impurities from pollen, honey, or other colony byproducts to maintain its purity and efficacy (Essam Elenany, 2024). While there are no formal quality criteria for BV due to its classification outside the regulatory frameworks for medications and food products, researchers have suggested using quantitative analysis of stable components and comprehensive characterization as indirect means of assessing the purity and quality of BV. Additionally, efforts have been made to standardize and manage the quality control of Hymenoptera venom, including BV, to ensure its therapeutic efficacy (Oliveira Orsi et al., 2024).

3.1 Melittin

MEL is BV's primary potent physiological ingredient, representing 40%–50% of the dry mass. It can dissolve in linear, cationic, and hemorrhaging and weighs 2,840 Da. It is made up of 26 amino acids. The molecular structure $C_{131}H_{229}N_{39}O_{31}$, with the N-terminal part predominantly hydrophobic due to +4 charges and little lytic activity. The area located at the C-terminus is hydrophilic due to the +2 protons, responsible for the lytic activity, for a total of +6 charges at physiological Ph (Bava et al., 2023). Due to its amphipathic structure, Melittin is water soluble in monomeric and tetrameric forms. The previous study found that MEL possesses antimicrobial and anti-cancer benefits. It can mechanically and chemically damage many cellular membranes (Shi et al., 2016). MEL binds to the negatively charged membrane surface (Figure 1). As a result, it compromises the functioning of phospholipid bilayers by creating pores, atomic ion, and molecule leaks, and enhanced permeability, ultimately leading to cell lysis (Jamasbi et al., 2016). Melittin connects to membranes as monomers but works across the whole membrane. According to the dosage, this biopeptide might induce transitory or stable holes. Only ions can travel through the membrane when a transient pore is formed. When regular holes emerge, the membrane becomes accessible to big compounds like glucose. Melittin has recently been discovered to activate and sensitize nociceptor cells, resulting in neuronal plastic changes along pain signaling pathways. Similarly, melittin can function as a PLA2 activator. It is also a critical physiologically active component of BV, generating

TABLE 1 The biological significance of bee venom and its constituent ingredients.

Composition	Nature	Effect	Reference
Melittin	Peptide	The peptide has biological action. Melittin inhibits blood coagulation, inhibits pathogens, and protects against radiation. Melittin acts as an anti-inflammatory in tiny doses. It possesses both hemolytic and cytotoxic properties.	Jafari et al. (2024)
Phospholipase A2	Enzyme	Phospholipase is the most allergenic and damaging ingredient in bee venom.	Lee and Bae (2016b)
Hyaluronidase	Enzyme	This enzyme permits venom to enter tissues by widening blood vessels and boosting tissue permeability, increasing blood circulation.	Khan et al. (2018)
Acid phosphatase	Enzyme	Allergen.	Kim and Jin (2016)
Apamin	Peptide	Bioactive polypeptide: a neurotoxin.	Gu et al. (2020)
Mast cell degranulating peptide	Peptide	Causes the release of biogenic amines.	Starkl et al. (2022)
Protease inhibitor	Enzyme	It possesses anti-inflammatory and bleeding-related characteristics and suppresses proteases.	Lee et al. (2023)
Adolapin	Peptide	Anti-inflammatory, antirheumatic, and painkiller.	Bindlish and Sawal (2024)
Histamine	Biologic amine	It expands blood vessels and promotes the permeability of capillaries. It's an allergy.	de Graaf et al. (2021)
Dopamine, noradrenaline	Biologic amine	Neurotransmitters have an impact on sensory perception and function.	Ahmed-Farid et al. (2021)
Alarm pheromone	Peptide	It sets the entire community on heightened alert.	López-Incera et al. (2021)
Secapin	Peptide	-Antibacterial -Anti-elastolytic -Anti-fibrinolytic	Lee et al. (2023)
Procamine A,B	Peptide	Suppresses the action of proteolytic enzymes, hence lowering inflammatory responses.	Răsinar et al. (2023)

numerous biological effects when administered to the patient's acupoint (Wehbe et al., 2019).

3.2 Apamin

Apamin is considered the second most abundant active peptide identified in BV. It represents 1% of all dried BV. This peptide possesses anti-inflammatory and antinociceptive qualities that enhance defensive line capacity. Following crossing across the blood-brain barrier, it enters the brain and affects the neurological system. Apamin suppresses vascular smooth muscle cell migration and growth in the vascular wall by targeting the Akt and Erk signaling pathways. It suppresses Erk1/2 and Akt phosphorylation generated by PDGF-BB. Apamin has been shown to block the G0/G1 cell cycle via the PDGF-BB signaling pathway, making it a possible therapy for atherosclerosis (Kim JungYeon et al., 2015).

Its structure consists of a disulfide bridge between cysteine residues 3 and 15, which is crucial for its biological activity. Apamin is a selective inhibitor of small-conductance calcium-activated potassium (SK) channels, exhibiting nanomolar or even subnanomolar affinity. This specificity allows apamin to modulate SK channel activity without affecting other ion channels or receptors. Apamin has been investigated for its therapeutic potential in various diseases, including ataxia, epilepsy, and inflammatory conditions (Kuzmenkov et al., 2022). The peptide's ability to modulate SK channel activity has been linked to its therapeutic effects, as SK channels play a crucial role in neuronal excitability and synaptic plasticity. Additionally, apamin has been shown to have anti-inflammatory and anti-fibrotic effects, making it a promising candidate for the treatment of chronic diseases. Apamin has been explored for its potential in neuroprotection and neuroregeneration (Kim H. et al., 2021). Studies have demonstrated that apamin can cross the blood-brain barrier and bind to SK channels in the central nervous system, potentially contributing to learning and memory control. Furthermore, apamin has been shown to have neuroprotective effects in models of neurodegenerative diseases, such as Alzheimer's and Parkinson's. Apamin has also been investigated for its potential in cancer treatment (Villa et al., 2020). Studies have suggested that apamin can inhibit the growth of cancer cells by modulating SK channel activity and inducing apoptosis. Additionally, apamin has been shown to have anti-tumor effects in animal models, making it a promising candidate for the development of novel cancer therapies (Gu et al., 2020; Saravanan et al., 2023; Bindlish and Sawal, 2024).

3.3 Mast cell degranulating (MCD) peptide

Mast cell degranulating (MCD) Peptide, or peptide 401, is a BV polypeptide of 22 amino acids equivalent to apamin because it has two disulfide bridges (Răsinar et al., 2023). It makes up around 2%–3% of BV's dry weight. The term MCD alludes to the biological function of mast cells in producing histamine. It is an epileptogenic neurotoxin, a significant antagonist of K+ channels, and may drastically drop rat blood pressure (Gajski et al., 2024). Some MCD biological activity takes different routes and might be an excellent example of the structure-function link (Figure 2). Based on research, MCD is a potent anti-inflammatory drug that might be utilized to investigate the secretion mechanisms of cells associated with inflammation, potentially leading to the creation of medicinal substances (Ullah et al., 2023).

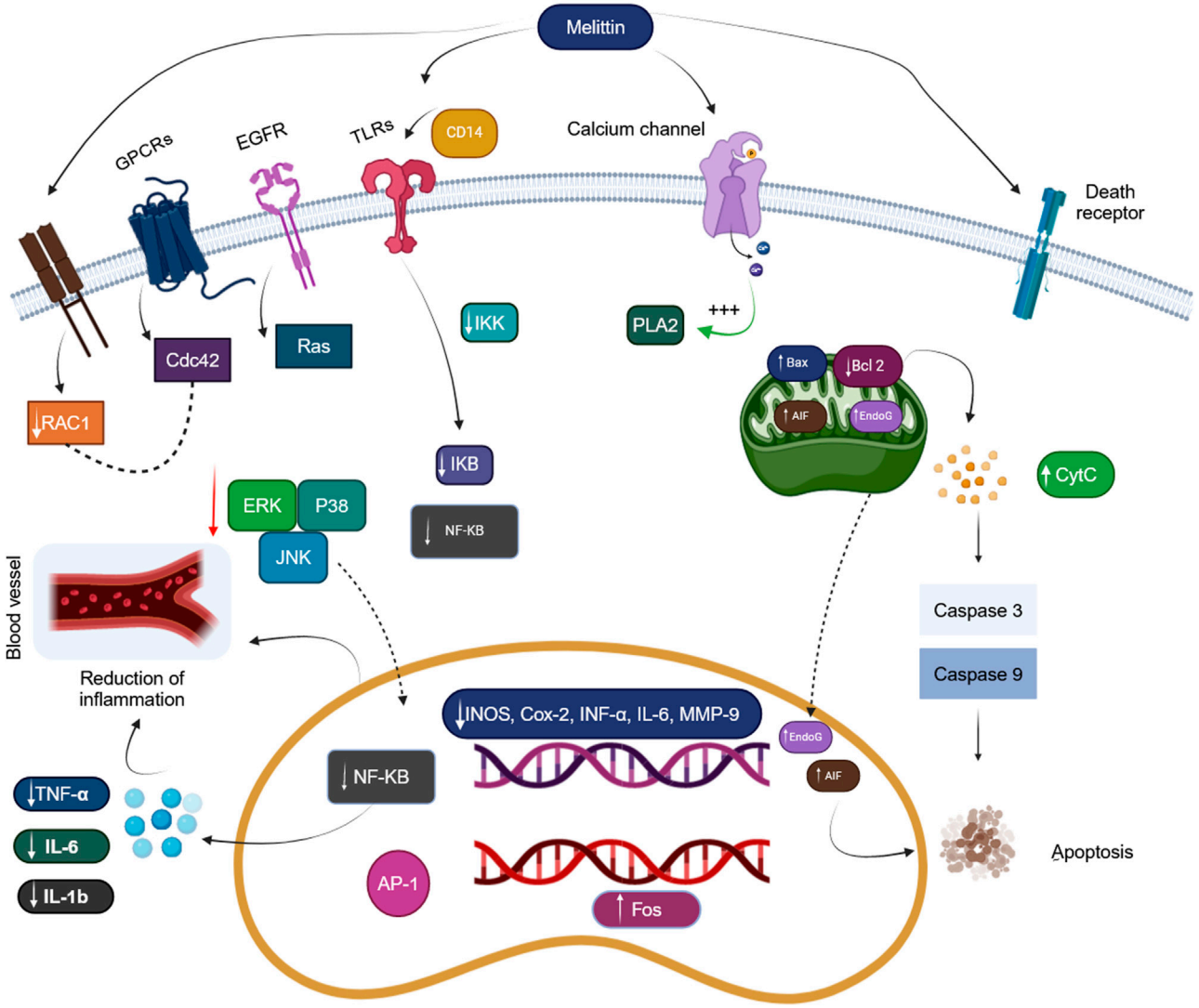

FIGURE 1
Mechanistic actions of bee venom melittin. (Generated by BioRender software).

3.4 Adolapin

Adolapin is a polypeptide with 103 amino acid sequences. It can be identified at 1% dry BV levels. It accounts for 2%–5% of the polypeptides present in BV (Gajski et al., 2024). Adolapin inhibits prostaglandin production and Cyclooxygenase function., providing anti-inflammation, anti-allergic, and fever resolution properties. It suppresses thrombocyte lipoxygenase activity and could possess painkilling characteristics (Sawicka et al., 2024).

3.5 Secapin

Secapin is a 25 amino acid polypeptide with a high proline content and one disulfide bond. It is a non-toxic polypeptide that makes up just 0.5% of dried BV. The Asiatic honey BV contains secapin (AcSecapin-1), which possesses antifibrinolytic, anti-elastolytic, and antibacterial effects (Dinata et al., 2023).

3.6 Procamine and tertiapin

Procamine, a polypeptide isolated from BV, suppresses protease activities, lowering inflammatory responses (Dinata et al., 2023). Tertiapin is a 21 amino acid peptide produced from BV. It has two disulfide bridges and an amidated C-terminal residue. It is a neurotoxic peptide similar to apamin. Tertiapin is a small part of the BV, representing 0.1% of its net content. Tertiapin inhibits the action of potassium gates throughout our bodies. It regulates potassium channels (Świątły-Błaszkiewicz et al., 2020).

3.7 Phospholipase A2

Phospholipase A2 (PLA2), BV's considerably more dangerous enzyme, is a polypeptide chain of 128 amino acids with four disulfide bridges. Its fundamental pH accounts for 12%–15% of BV's dry mass

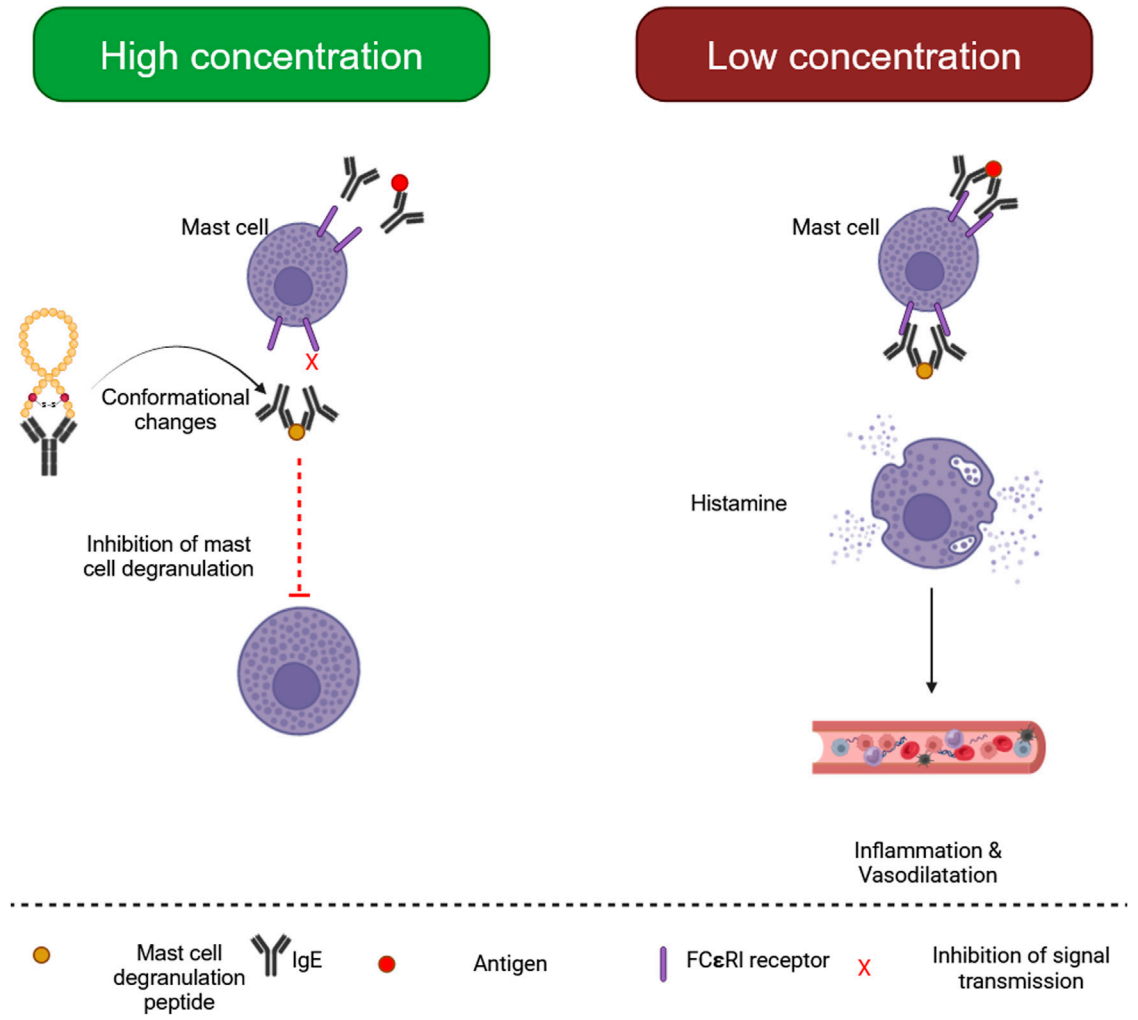

FIGURE 2
Biological processes of mast cell degranulating (MCD) peptide. (Generated by BioRender software).

(Gajski et al., 2024). Melittin, interestingly, can boost its action. This has been proven to occur throughout the red blood cell lysis process, showing synergistic activity between bvPLA2 and melittin (Frangieh et al., 2019). In addition, fresh, experimental results reveal that bvPLA2 has protective immunological reactions against several illnesses (Kim KH. et al., 2019). BvPLA2 causes microglial inactivation and decreases CD4+ T cell recruitment (Figure 3) (Jafarzadeh et al., 2023).

3.8 Hyaluronidase

Hyaluronidase amounts to about 1.5%–2% of BV dry weight and has been shown to break down hyaluronic acid. BV hyaluronidase enables the biologically functioning elements in BV to reach the targeted tissues with greater effectiveness by modifying its architectural durability and improving blood supply in the region. These two activities promote the spread of venom (Bava et al., 2023).

Aside from peptides, BV includes carbohydrates, free amino acids, and minerals. BV contains carbohydrates such as glucose and fructose. The primary components of amino acids are -aminobutyric acid and B-aminoisobutyric acid. BV also includes minerals such as magnesium, calcium, and phosphorus. It also includes volatile chemicals termed pheromones, symbolized by complicated ethers, which warn bees of hazards and trigger stinging responses (Salem and Marzouk, 2024). The RHGXRSP domain distinguishes these acid phosphatases from others. It releases histamine and creates particular IgE, which can be utilized in immunotherapy (Hossen et al., 2016). Dipeptidyl peptidase IV (DPIV) or Api m5 has a molecular mass of 102 kDa (Rzetecka et al., 2024). It is connected to transforming pro-toxins into activated metabolites (Blank et al., 2010; dos Santos-Pinto et al., 2018). Vitellogenin or Api m12 is BV's compound with the most significant molecular weight (200 kDa) (dos Santos-Pinto et al., 2018). Furthermore, it has antibacterial and antioxidant properties (Park HeeGeun et al., 2018).

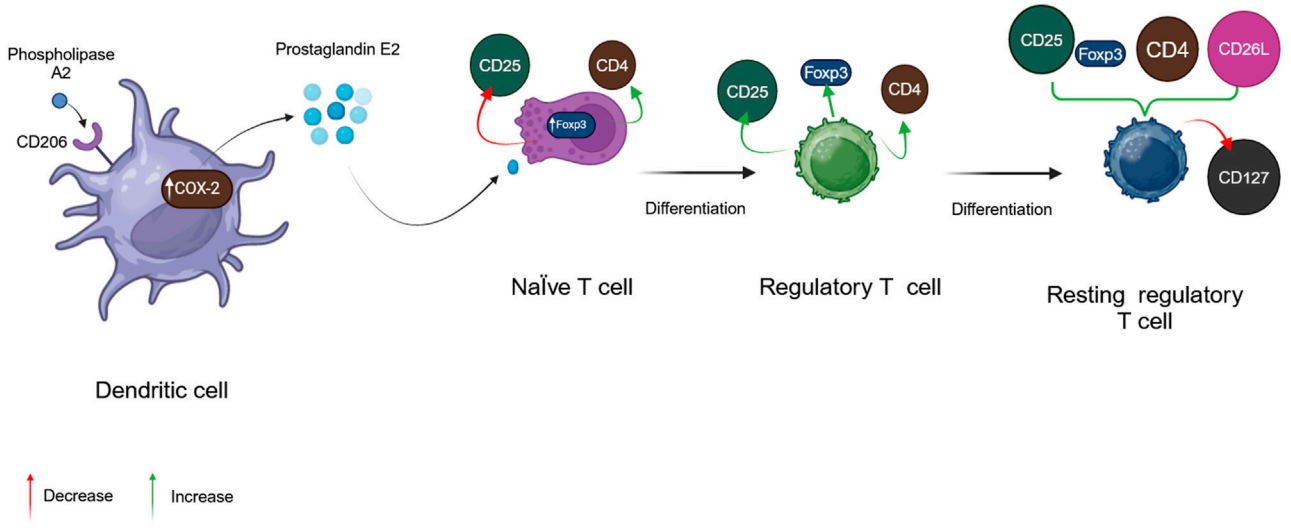

FIGURE 3
Physiological activity of phospholipase A2. (Generated by BioRender software).

4 Delivery approaches of bee venom

Oral delivery is complicated because the protein composition of BV can be degraded by gastrointestinal enzymes (Carpena et al., 2020). However, a study conducted by Meligi and his group, who studied the protective potential of oral administration of BV against liver damage caused by lipopolysaccharide and carbon tetrachloride in rats contradicts other reports (Meligi et al., 2020). BV therapy is usually applied in different ways including bee stings at specified places, injections of pure and sterile Apis homeopathic preparations, BV ointments, lotions, capsules, drops, and phonophoresis. Honeybee sting therapy is considered a classic therapeutic approach. It has several disadvantages, including discomfort. Since melittin's short half-life and the annoyance it gives patients, continuous administration of multiple rounds of stings or injections in the blood requires sting-induced inflammation and trouble maintaining average concentrations (Izhar et al., 2023). Due to BV's short duration of action and the challenge of estimating its dosages, scientists and professionals have promoted and created alternate options, such as mixing polymers or nanoparticles (NPs) (Ojha et al., 2023; Sharma et al., 2024a).

Traditional methods of administering BV therapy have typically involved topical applications, such as intravaginal suppositories or gels containing BV extracts. These methods have been used to target specific areas affected by conditions like bacterial vaginosis (BV). However, advancements in drug delivery have introduced more sophisticated approaches to BV therapy. Controlled drug release systems now allow for precise delivery of the active ingredients, ensuring the right amount reaches the intended site of action. The shift towards advanced drug delivery methods has the potential to enhance the efficacy of BV therapy by improving targeted delivery, increasing bioavailability, and reducing side effects associated with traditional administration routes. For instance, nanotechnology-based delivery systems offer high sensitivity and specificity, allowing for rapid diagnosis and treatment of BV. These modern approaches not only improve the precision of drug delivery but also optimize therapeutic outcomes by ensuring that the active components of BV reach the target tissues more effectively.

5 Therapeutic mechanisms

According to its biological ingredients, BV has a wide variety of medicinal effects. Several studies have discovered the pharmaceutical advancement in one or several features of BV, primarily focusing on pain-relieving (Lee et al., 2005), neuroprotective (Ye et al., 2016b), anti-inflammation (Jeong et al., 2017), enhancing wound recovery (Hozzein et al., 2018), anti-microbial impacts (El-Seedi et al., 2020), and cancer-fighting (Małek et al., 2023). These show that BV has a wide variety of therapeutic uses, which might be due to its multi-target and multi-pathway features (Figure 4). The complex composition of BV, with its mixture of both inflammatory and anti-inflammatory compounds, contributes to its dual nature. While the venom can cause immediate pain and swelling upon a sting, it also has the potential to exert beneficial effects, such as reducing inflammation and providing pain relief, when used in controlled therapeutic settings (Sadek, 2023). The precise mechanisms of action for the various components of BV and their interactions are still being actively investigated. Ongoing research aims to investigate the specific pathways and targets through which BV and its constituents exert their pharmacological effects, both beneficial and detrimental, to optimize its therapeutic potential while minimizing adverse reactions (Pandey et al., 2023a).

5.1 Antioxidant activity

BV includes compounds with high antioxidant activity (AOA) (Martinello and Mutinelli, 2021). The phenomenon is caused by phospholipases A2, apamin, and melittin. The antioxidant action is based in many ways, such as free radical elimination, proton

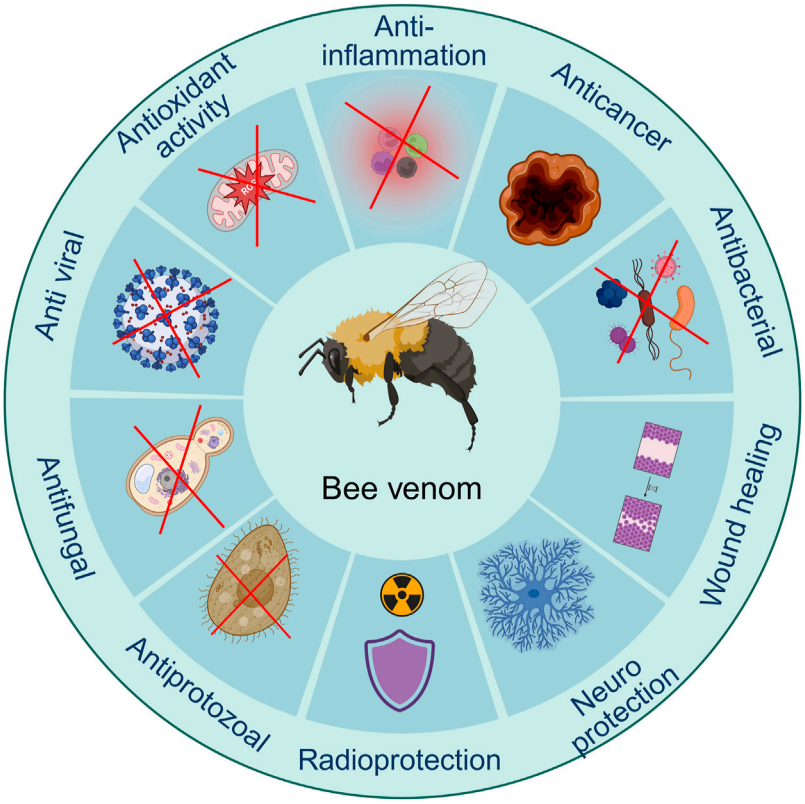

FIGURE 4
Biological activities of bee venom. (Generated by BioRender software).

transfer, metallic ions binding, solitary oxygen suppressing, and serving as a trigger for removing superoxide and hydroxyl radicals (Yaacoub et al., 2023). The antioxidant benefits may be due to the substances' capacity to impede lipid peroxidation and increase superoxide dismutase functionality. Superoxide dismutase is an enzyme that decreases tissue destruction by eliminating the superoxide radical in most cells when subjected to oxygen (Pinmanee et al., 2023).

BV includes extra antioxidants. Vitellogenin is a protective agent in mammalian cells, shielding them from oxidative stress and defending against free radicals. Few studies have employed traditional methods to determine the AOA of BV (Sobral et al., 2016; Somwongin et al., 2018). All samples included antioxidants, unconnected to the elements found and quantified in them. Evidence suggests that melittin alone has lower AOA than BV extracts, perhaps due to other metabolites (Pavel et al., 2014). Other tiny molecules may have a role in BV biological properties, with combinatorial or antagonistic effects at different concentrations resulting in varying consequences.

Previous studies found that honey BV significantly inhibited nonenzymatic lipid peroxidation. It competes with dimethyl sulfoxide for hydroxyl radicals, indicating its high detoxification properties. The findings suggest that honey BV's antioxidant activity contributes to its anti-inflammatory characteristics, particularly its capacity to reduce interleukin-1 synthesis *in vitro* (Değer et al., 2023; Abd El-Aziz et al., 2024; Virk et al., 2024). Additional investigations assessed antioxidant capacity in combination with other criteria. For example, rabbits received 0.1, 0.2, and 0.3 mg Under the skin twice weekly for 20 weeks (El-Hanoun et al., 2020). The study found that treating rabbits boosted glutathione S-transferase (GST) and glutathione (GSH) levels. The readings of malondialdehyde (MDA) and thiobarbituric acid–reactive substances (TBARS) were also lower. These studies confirmed BV's antioxidant capabilities. BV improved reproductive performance and semen antioxidant activity. Elkomy et al. (2021) found delivering BV via subcutaneous injection to rabbits improved total antioxidant capacity (TAC). Kim et al. introduced doses ranging from 10 to 500 μg per kilogram to the standard diet (Kim D-H. et al., 2019). The scientists found that ingesting BV improved the antioxidant properties and altered fatty acid metabolism in broilers.

5.2 Anti-inflammatory activity

Melittin may induce itching, inflammation, and localized discomfort in large dosages, although lower doses of melittin offer widespread anti-inflammatory benefits (Sawicka et al., 2024). Melittin inhibits the Toll-like receptors (TLRs) 2,4, CD14, nuclear factor-κB essential modulator (NEMO), and Platelet-derived growth factor receptor (PDGFR) signaling pathways. This decreases inflammatory reactions in numerous tissues (Lee and Bae, 2016a). BV has anti-nociceptive properties for inflammatory pain. BV has been utilized for decades to treat numerous discomfort reactions, including visceral, inflammatory, and heat (Ullah et al.,

2023). Hypodermic apipuncture with BV decreases thermally and mechanically related hyperplasia Arthritis discomfort is caused by collagen (Elieh Ali Komi et al., 2018). Furthermore, a single ingredient of BV, adolapin, has analgesic effectiveness. Pre-treatment with BV has anti-nociceptive properties against the spinal cord. Fos activity correlates with formalin-generated discomfort in rats (Galante et al., 2023). Apamin is a pain reliever that reduces inflammation and muscle discomfort (Wehbe et al., 2019). Oxaliplatin can cause mechanical allodynia and coldness; however, phospholipase A2 can mitigate these effects (Hossen et al., 2017a). Subcutaneous injection of BV has been demonstrated to offer anti-nociceptive effects in mouse models for both visceral and somatic pain. Additionally, it prevents nociceptive responses in mice caused by acetic acid (Qi et al., 2023). BV is more effective than adjuvants for treating arthritic pain because of its anti-nociceptive properties (Ullah et al., 2023). BV acupuncture effectively alleviated neuritis pain caused by paclitaxel in mouse models (Shen et al., 2020).

5.3 Anticancer activity

Cancer is one of the most severe diseases harming humans and is one of the leading causes of death around the globe. According to current data, around 10 million freshly diagnosed patients with the illness each year, and over 6 million people die from it, accounting for around 12% of all fatalities globally. In 2020, 15 million new cancer cases will be diagnosed (Morgan et al., 2023). Cancer growth and advancement are complex activities. Smoking, pathogenic organisms, environmental contaminants, a lousy diet, and internal variables that include transmitted down anomalies in genes, hormonal changes, and immunological conditions might all contribute to the start of this illness (Golubnitschaja et al., 2016). BV has been frequently used to treat several malignancies (Varol et al., 2022). BV metabolites have received a lot of interest for their possible utility in oncotherapy. Melittin, a powerful antitumor polypeptide, maybe a more practical alternative than the entire BV (Rady et al., 2017).

Melittin has high membrane-perturbing action, which accounts for its anti-inflammatory, anti-microbial, and cancer-fighting abilities (Roversi et al., 2023). When numerous melittin particles enter the cell membrane, they degrade the phospholipids, resulting in cellular fragmentation. It breaks down both phospholipid and synthesized bilayers. It has been found to cause apoptosis and have anticancer properties. Melittin targets several tumor cell types, including kidney, hepatic, pulmonary, prostate, and urinary malignancies, in addition to malignancies of the breast and leukemia (Zarrinnahad et al., 2018).

5.4 Anti-microbial activity

Microbial infections are a challenge, especially with the growth of medication resistance; thus, scientists are seeking Recent discoveries of bioactive potential. Products from nature are considered sources of sustainability with fewer problems that can provide various active substances (Thawabteh et al., 2019).

5.4.1 Anti-bacterial activity

Blending BV with regular medications is effective, helpful, and safe. Against specific microorganisms, but its uses are being carefully considered in both preclinical and clinical studies. BV and melittin have wide-ranging antibacterial properties against Gram-positive and Gram-negative bacteria, with MIC values ranging from 10 to 100 μg/mL and 30 to >500 μg/mL, respectively. BV, melittin, and oxacillin effectively killed Methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 33591. BV and melittin treatment led to modifications in the bacterial cell membrane due to membrane integrity loss and cellular shape changes involving cellular deformation. And cytoplasm loss (Pereira et al., 2020). Melittin's antibacterial capacity was tested against numerous bacteria (Roversi et al., 2023). Gram-positive bacteria are more sensitive to melittin compared to Gram-negative bacteria due to variations in their cell membrane structures (Gong et al., 2023). Melittin may pass through the peptidoglycan layer of Gram-positive cell membranes more effectively than Gram-negative cell membranes, which are protected by a lipopolysaccharide layer. The proline residue at position 14 has been proven to play a crucial role in melittin's antibacterial action (Sharma et al., 2024b). Melittin can dissolve biofilms generated by *S. aureus* and *Escherichia coli* (Rouhi et al., 2024).

5.4.2 Anti-viral activity

Viral infections have appeared in the previous 10 years. These have jeopardized the lives of millions of people throughout the world, particularly immunocompromised individuals. BV and its constituents have significant antiviral activity against viral infections (Table 2) (Mohanty and Murhekar, 2023). An enhanced understanding of Melittin's processes will help us enhance antiviral approaches. Several antimicrobial peptides (AMPs) primarily disrupt membranes to achieve their effects. Melittin's direct interaction with viral capsid proteins or envelopes in this scenario hinders the binding or absorption of cells capable of producing viruses (Memariani et al., 2020). Furthermore, additional possible antiviral modes of action include inhibiting viral multiplication, reducing viral mRNA expression levels, producing changes in viral genome conformation (Memariani et al., 2020), inhibiting the virus's cytotoxic properties, and inhibiting virally mediated cell fusion (Mohanty and Murhekar, 2023), and inhibit viral bundling (Peng et al., 2023). The possible use of melittin-loaded nanoparticles to eliminate the human immunodeficiency virus while leaving uninfected cells alone suggests a preventative method in which these nanoparticles are employed to make a vaginal lotion that stops HIV transmission. The theoretical underpinning is that Melittin molecules may be discovered on the viral envelope. Pore-like attack complexes develop when nanoparticles fuse (Peng et al., 2023). Another research discovered that bvPLA2 can similarly inhibit viral replication. The same team found the bvPLA2 peptide sequence, suppressing HIV replication (Wehbe et al., 2019).

5.4.3 Anti-fungal activity

Fungal infections harm over a billion people globally and cause about 1.5 million deaths each year (Silva et al., 2019). Antifungal drugs have specific restrictions concerning biocompatibility, spectrum of action, and pharmacological characteristics. These

TABLE 2 Application of bee venom in controlling the viral diseases.

Bee venom/isolated compounds	Model	Technique	Outcome	Reference
Phospholipase A2 (sPLA2)	Hepatitis C virus (HCV)	Plaque assay	PLA2 and its related compounds are promising prospects for the establishment of wide-spectrum antiviral medications targeting viral outer lipid bilayers produced from the endoplasmic reticulum membrane.	Chen et al. (2017)
Melittin	Human immunodeficiency virus (HIV)	Transient transfection Assays	Blocks viral replication. Blocks transcription of genes. Reduces intracellular protein and RNA production. Reduces the long terminal repeat (LTR) activity with an ID50 value. 0.9–1.4 μM after 24 h.	Wachinger et al. (1998)
Bee venom	Herpes simplex virus (HSV)	Plaque assay	Limits viral propagation EC50 1.52 ± 0.11 μg/mL	Uddin et al. (2016)
Bee venom	Papillomaviruses (HPV16 E6)	Reverse transcription assay	Blocks mRNA transcription. Blocks cell proliferation. Reduces protein levels. At 10 μg/mL, it decreases 0.35 ± 0.06-fold after 24 h.	Kim et al. (2015c)

TABLE 3 Applications of bee venom in controlling the fungal infections.

Bee venom/ isolated compounds	Microbe	Technique	Outcome	Reference
Apamin	*Alternaria alternate*	Not reported	Melittin and apamin could prevent the fungal-related synthesis of inflammatory messengers and extracellular matrix (ECM) by nasal fibroblasts.	Shin et al. (2017)
Melittin	Unicellular fungal (UCF) pathogens (*Cryptococcus neoformans*, *Kodamaea ohmeri*, and *Candida albicans*)	automated Vitek2 system	BV/chitosan nanoparticles (CHNPs) inhibited biofilm development and the yeast-to-hyphal transformation of the investigated (UCF) pathogens. These findings demonstrated that BV-chitosan nanoparticles are a potential natural chemical for treating fungal diseases.	El-Didamony et al. (2022b)
Bee venom/melittin	*C. krusei*	Broth microdilution	Bee venom and melittin had intense antifungal action (MIC measures ranging from 30 to 100 μg/mL).	Issam et al. (2015)
Bee venom	*C. albicans*	Disc diffusion	Suppresses fungal dimorphism	Ali (2014)
Bee venom	*Trichophyton rubrum*	Disk diffusion method	The bee venom in its entirety provided a high amount of suppression, and the venom in isolated forms was ineffective. Furthermore, BV-based compounds may serve as promising antifungal treatments.	Park et al. (2018)

concerns are worsened when these medicines can be utilized extensively and inappropriately, hastening the evolution of drug-resistant mutations over time (Smith et al., 2019).

The shortage of novel antifungal drugs under development needs the creation of potent antifungal agents, particularly those derived from natural ingredients. BV is effective against various fungi at micromolar doses (Table 3). Furthermore, emerging evidence suggests that melittin inhibits fungal growth via several processes, such as membrane permeability and apoptotic activation via the ROS-induced mitochondrial/caspase-dependent route, suppression of (1,3)-D-glucan synthase, and alterations in fungus genetic transcription. Animal models should be employed to evaluate melittin's safety and effectiveness in the future. Melittin will likely offer new paths for numerous biological uses, from medicine to agriculture (Memariani et al., 2019).

5.4.4 Anti-protozoal activity

Investigations have shown that BV category III sPLA2 possesses anti-trypanosomiasis—activity BV group III sPLA2 transcription in a genetically engineered mosquito's midgut inhibits Plasmodium ookinetes Cecropin, a melittin hybrid, effectively inhibits Leishmania Donavan promastigote by damaging its plasma membrane (Ullah et al., 2023). Melittin affects membrane integrity in prokaryotic and eukaryotic species, leading to membrane lysis and increased permeability. Melittin exhibits antibacterial, anti-fungal, and antileishmanial properties due to this interaction (Berhe et al., 2024). Previous studies suggest that in controlled circumstances, PLA2 has antiprotozoal activity against *Trypanosoma brucei*. Lasioglossins, a peptide found in BV, have potent antibacterial properties due to their membrane contact and DNA binding (Ullah et al., 2023). The peptide melittin has been shown to have antiprotozoal efficacy versus *Toxoplasma gondii*,

TABLE 4 Application of bee venom in wound healing.

Active component	Action	References
Tertiapin	Anti-inflammatory properties action via inhibiting potassium gates.	Dadar et al. (2019)
Secapin	Anti-fibrinolytic, anti-elastolytic, and antimicrobial properties	Lee et al. (2016a)
Peptide 401 The mast cell degranulation peptide	Benefits include relief from pain, as well as anti-inflammatory and nociceptive effects.	Bellik (2015)
Melittin	Antioxidant, anti-inflammatory, and pro-angiogenic.	Shaik et al. (2023)
Hyaluronidase	Breaks down hyaluronic acid, allowing the venom to penetrate tissue. It causes dilatation and greater blood artery permeation, increasing blood circulation.	Son et al. (2007)
Apamin	Inhibits cyclooxygenase-2 and phospholipase A2.	Kim et al. (2020b)
Adolapin	Antioxidative, anti-apoptotic, and anti-inflammatory.	Han et al. (2011)
Acid phosphatase	Human-sensitized basophils produce a potent histamine releaser. It can be used in anti-BV immunotherapy.	Grunwald et al. (2006)

Trypanosoma cruzi, *Plasmodium*, and *Leishmania*. Melittin has been used in vaccine production to boost protection against leishmaniasis. Melittin can kill *T. cruzi* (Memariani and Memariani, 2021). Although BV has antiprotozoal properties, its specific impact, route of action, and potential for therapeutic use remain unclear.

5.5 Wound healing

The reconstruction of wounds is an intricate mechanism that consists of four concurrent stages: hemostasis, clot formation, inflammation, and division (Duda et al., 2023). The research provided demonstrates that BV treatments have an impact on all stages of the healing process. BV can be used topically or subcutaneously on wounds (Table 4). An animal model study suggests BV might be a viable damage-healing therapy for diabetes patients. BV promotes the initial stage of wound healing (hemostasis) in diabetic mice by increasing the production of Transforming growth factor beta (TGF-β) and Vascular endothelial growth factor (VEGF).

Activating Transcription Factor 3 (ATF3, and inducible nitric oxide synthase (iNOS) has been shown to impact on extracellular matrix biosynthesis through increased collagen synthesis (Wang et al., 2021b). In the Reepithelization stage, BV triggers human epidermal keratinocyte division and migration while decreasing interleukin-8 (IL-8) and Tumor necrosis factor-α (TNF-α). BV affects angiogenesis and neovascularization in diabetic mice by inhibiting caspase-3, -8, and -9 activity while enhancing TGF-β and VEGF levels. TGF-1, fibronectin, and VEGF levels are lowered during remodeling, whereas raising collagen-I mRNA levels reduces the expression of ATF-3 and Inos (Kurek-Górecka et al., 2020).

5.6 Neuroprotection

Neuroinflammation induced by prolonged stimulation of glial cells and microglia is linked to neurodegenerative disorders. Various BV components, such as PLA2 and apamin, have been investigated as anti-neuroinflammation medicines to boost the efficiency of multiple treatments against neurodegenerative illnesses (Mohammadi-Rad et al., 2019). It has been proposed that melittin might help address neurodegenerative disorders linked to stimulation of microglial cells since it substantially inhibits BV2 microglia's pro-inflammatory activities (Zhang et al., 2024). The progressive neurodegenerative illness amyotrophic lateral sclerosis (ALS) damages motor neurons in the brain and spinal cord, resulting in muscle weakening and atrophy (Zarei et al., 2015). Yang et al. found that melittin therapy enhances the anti-inflammatory ability of the proteasome in the central nervous system (CNS) of Amyotrophic Lateral Sclerosis (ALS) model mice (Yang et al., 2011). Melittin-treated mice demonstrate less neuronal loss in the spinal cord and better motor control, which enhances motor performance. In addition, melittin therapy lowers p38 phosphorylation and the microglial cells count in the brainstem and spinal cord. Furthermore, melittin inhibits-synuclein post-transcriptional modification, a critical ALS pathogenic pathway, and activates chaperones to prevent protein misfolding. The findings support melittin's anti-neuroinflammatory properties. Although the CNS is the major issue with ALS, the liver, spleen, and lungs are all impacted. In an animal model of ALS, melittin therapy lowers inflammation while increasing, suggesting cell viability in the spleen and lung (Lee and Bae, 2016a). AD, the most common neurodegenerative disease, comprises several pathogenic mechanisms that contribute to its development (Vickers, 2017). Although the etiology of Alzheimer's disease is still unknown, research suggests that inflammatory reactions may play an important part. In its progression (Cummings et al., 2018). Current therapies for cognitive loss caused by Alzheimer's disease include acetylcholinesterase (AChE) inhibitors and muscarinic or nicotinic receptor ligands. As an alternative strategy, bvPLA2 was shown to be effective in slowing the course of Alzheimer's disease in transgenic mice. This is attributed to. bvPLA2's capacity to minimize accumulation while improving cognitive function in mice brains. It also reveals that bvPLA2 can enhance brain glucose metabolism and lower the hippocampus's neuroinflammatory reactions, potentially inhibiting AD progression (Ye et al., 2016b). Recent research has also shown that bvPLA2 therapy in a 3xTg-AD mouse model might affect regulatory T-cell populations. To prevent AD's unfavorable inflammatory response, scientists developed a novel therapeutic

method that combines bvPLA2 medication with AB vaccine therapy (Baek et al., 2018). In addition to creating protective benefits against inflammatory disorders, bPLA2 possesses anti-inflammatory actions, as previously stated (Palm et al., 2013). A recent study investigated PD in mice using a combination of 78% bPLA2 and 15% melittin (Carpena et al., 2020). bPLA2 reduced inflammation by activating Treg cells. Additionally, bPLA2 activation reduced the loss of dopaminergic cells (Kim KH. et al., 2019).

5.7 Radioprotective activity

Research indicates that BV can protect against the detrimental effects of ionizing radiation. In many evaluation systems, BV protects against gamma and X-ray radiation (Djahonkulovna, 2023). Additionally, BV contains antioxidant properties that neutralize free radicals while shielding the body from harmful radiation ()Shaaban et al., 2019. BV protects bone marrow cells from chromosomal mutations (aberrations) in the Wistar mouse model (Yu et al., 2023). It promotes hematopoiesis, induces the production of histamine from MCD, and lowers blood oxygen pressure through phospholipase A2 (Gajski et al., 2024). PLA2, a BV component, contains a forkhead box P3 (Foxp3), + CD4, + CD25, + Treg cell that protects against radiotherapy-induced extensive pulmonary inflammation (Hossen et al., 2017b). BV therapy significantly lowers levels of IL-6 and TNF-a following gamma radiation therapy (Hasan et al., 2023). BV decreases serum Aspartate transaminase (AST), Lactate Dehydrogenase (LDH), Creatine Kinase MB (CK-MB), Cardiac Troponin I (cTnI), and Alanine aminotransferase (ALT) concentrations in Wistar mice via decreasing hepatic NF-kB transcription. However, the amounts rose following gamma radiation (Salimo et al., 2023). According to investigations, BV exhibits radioprotective properties against oxidative and basal DNA damage (Montoro et al., 2023). Administering melittin a day before being subjected to the radiation (8.5 Gy) dramatically improves survival rates in mice (Duché and Sanderson, 2024). BV shields human peripheral blood lymphocytes against damaging gamma radiation (Yu et al., 2023). In an analogous study, BV was shown to protect blood cells against radiation (3–4 Gy) when administered 24 h beforehand (Khateeb and Taha, 2024). Contemporary technologies and non-ionizing radiation have become more prevalent daily, leading to detrimental consequences on the body. BV has demonstrated radioprotection, making it a potential non-toxic and efficient radioprotector agent for the future.

6 Medical applications

BV is a well-known pharmacologically active product of the hive synthesized by the venom glands associated with the sting apparatus of workers and queens, stored in the venom reservoir, and injected through the sting apparatus during the stinging process (Aparna, 2020). Due to its anti-oxidants, anti-coagulants, anti-inflammatory properties, and bioactive substances like melittin and phospholipase BV is mainly used to treat many inflammatory disorders such as arthritis, cancer, diseases of the nervous system, heart and blood system abnormalities, skin diseases, and others (Khalil et al., 2021). Furthermore, therapeutic application of BV includes their use in the management of bursitis, tendonitis, dissolving scar tissue, in the management of post herpetic neuralgia, Lyme disease, rheumatoid arthritis, osteoarthritis, multiple sclerosis, TMDs, and more (Table 5) (Kurek-Górecka et al., 2020).

6.1 Liver diseases

Chronic liver disease (CLD) is a significant source of death and disability globally, with 2 million people dying from it every year (Younossi et al., 2023). In this sense, CLD is the 10th reason for mortality globally (Younossi and Henry, 2016; Heron, 2021). Besides death, CLD causes severe damage to patients' quality of life, as well as substantial medical and economic costs (Stepanova et al., 2017). Nevertheless, various socioeconomic and medical shifts have happened worldwide during the previous 2 decades, potentially altering the impact of each CLD. BV has been demonstrated to help minimize hepatic damage, inflammation, and fibrosis (Table 6). Melittin reduces TNF release and IL-1 and IL-6 transcription in TNF-treated hepatic stellate cells (HSCs). Melittin suppresses inflammation and fibrosis in thioacetamide-induced liver fibrosis by blocking the NF-κB signaling pathway. Furthermore, they postulated that the controlled inflammatory response may impact melittin's anti-fibrotic action in activated HSCs. They then demonstrate that melittin prevents liver failure in the D-galactosamine/LPS-induced mice liver failure model via suppressing NF-B signaling and apoptosis (Li et al., 2023). Melittin lowers the high mortality rate, alleviates hepatic pathological damage, suppresses hepatocyte apoptosis, and suppresses hepatic inflammatory responses (Jafari et al., 2024). They also observed that melittin inhibits NF-B transcription in TNF-induced liver injury. Melittin suppresses NF-κB DNA binding and promoter activity in TNF-treated hepatocytes, demonstrating that melittin prevents hepatocyte death by decreasing NF-κB activation. Recently, researchers observed that giving melittin decreased bile duct inflammation and fibrosis (Kim et al., 2015a).

6.2 Cancer treatment

At the beginning of the 1950s, apitoxin's influence on the colchicine-induced carcinogenic pathway was documented (Gajski et al., 2024). In turn, the study's findings of reasons for mortality among qualified beekeepers compared with most of humanity revealed that BV had oncoprotective potential, particularly in the case of lung malignancy (Małek et al., 2023; Ali, 2024). Starting in the early 1980s, papers detailing the findings of studies on the cancer-fighting potential of BV began to appear (Table 7).

MEL from *A. mellifera* is the most often used pharmacological peptide in cancer investigations. MEL may impact signaling and regulatory routes, resulting in numerous cancer-related death processes, including a decrease in the division, stimulation of the death of cells, limitation of vascular development, interruption of the cell cycle, and restriction of malignant tumor circulation, movement, the spread of cancer cells and invading forces according to available literature (Rady et al., 2017). How melittin antitumor activity has

TABLE 5 Physiochemical properties, doses, and benefits of bee venom, and its components in numerous medical applications.

Bee venom/ Metabolites	Physiochemical properties	Disease	Model	Method of application	Dosage of use	Outcomes	References
Bee venom	Anti-inflammation and damaging effects on cells on several kinds of tumour cells.	Liver carcinoma Breast cancer Cervical cancer	Liver (Hep-G2), breast (MCF-7), and cervical (HPV-18 infected HeLa cells)] and two normal cells (splenocytes and macrophages (MQ		NA	Having an effect on IL-10, TNF-α, and IFN-γ. Increased Cas3 levels.	Salama et al. (2021)
	Inhibiting cervical-cancer tumorigenesis	Cervical cancer	HPV-positive cervical-cancer cell lines, such as Caski and HeLa cells, and not to HPV-negative cervical-cancer cells (C33A)		0, 0.625, 1.25, 2.5, 5, and 10 μg/mL of BV for 24 h	Cyclin A, cyclin B, Bcl-2, and Bcl-XL levels decreased. Effects on caspases and intercellular signaling pathways.	Kim et al. (2020a)
	Small conductance K+ (SK) channel inhibition may be effective in managing Parkinson's disease in both short and long term.	Parkinson's disease	Human	Injection	s.c. bee venom injections (100 μg)	Delayed disease progression.	Hartmann et al. (2016)
	Significant decrease in muscle tonus.The VAS scale decrease was statistically significant.	Temporoma ndibular disorder (RDC/TMD Ia and RDC/TMD Ib)	Human	Local dermal massage to the area of masseter muscle	0.0005% bee venom ointment	Relived masticatory muscles myalgia	Nitecka-Buchta et al. (2014)
	Increase locomotor velocity. ↑ Parkinson's Disease Quality of Life Questionnaire. ↑ Motor manifestations	Parkinson's disease (PD)	Human trial	Acupuncture	0.1 mL, diluted to 0.005% twice a week for 12 weeks.	significant improvements in gait speed	Doo et al. (2015)
	(RLPP) ↓ TNF-α	Recalcitrant localized plaque psoriasis (RLPP)	Human trial	Intradermal injection	Inject 0.05–0.1 mL of commercial BV (Epivac®) once a week for 3 months.	↓ Recalcitrant localized plaque psoriasis	Eltaher et al. (2015)
	(i) Lower blood glucose levels (ii). Reduced blood cholesterol concentrations, reduced low density lipoproteins, and boosted high density lipoproteins.	Diabetes	Rabbits	A bee sting	Includes 0.2–0.5mL of BV for 14 days.	Management of hyperglycemia and hyperlipidemia	Khulan et al. (2015)
	Remarkable improvement in blood levels of TNFα (−14%), IL1β (−52%), IL-6 (−53%), NFKB (−32.6%), and ESR (−39.8%).	Chronic Low Back Pain	Human trial	Bee stinging at GV14 acupuncture sites.		Effectively managed low back pain.	El Gendy et al. (2017)
	Destructive and apoptosis outcomes.	Breast cancer	MCF-7		2.5, 5, 7.5, 10, 12.5 (μg/mL)	CBV (in a dose-related fashion). NO generation, caspase-3 stimulation, MCF-7 survival, functioning of catalase, and glutathione level. CBV caused destruction of DNA in MCF-7 cells in a dosage-dependent way.	Kamran et al. (2020)
Melittin	Reduced amino acids in the proline/ glutamine/arginine system. Reduced amounts of carnitines, polyamines, ATP, and NAD+.	Ovarian cancer	Human ovarian cancer cell lines A2780 (cisplatin-sensitive) and A2780CR (cisplatin-resistant)		4.5, and 6.8 μg/mL	Anti-cancer activity	Alonezi et al. (2016)
	Increased apoptosis. Ca2+ is released from the endoplasmic reticulum and accumulates in the mitochondria.	Leukemia	Acute lymphoblastic leukaemia (CCRF-CEM) and chronic myelogenous leukaemia (K-562)		0.001–100 μM	Disruption in mitochondrial membrane potential, annexin V binding, and caspase 3/7 activation.	Ceremuga et al. (2020)
	Anti-angiogenic, and anti-inflammatory.	Lung carcinoma	Mouse		0.1–5 mg/kg	Reduced the number of macrophages in the tumor environment and reduce VEGF and CD206 transcription in bone marrow-derived M2 macrophages.	Lee et al. (2017)

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TABLE 5 (*Continued*) Physiochemical properties, doses, and benefits of bee venom, and its components in numerous medical applications.

Bee venom/ Metabolites	Physiochemical properties	Disease	Model	Method of application	Dosage of use	Outcomes	References
	Anti-inflammatory.	Liver inflammation	Mouse	i.p. injection, twice a week for 12 weeks	0.1 mg/kg	Reduced inflammation, fibrosis, and the production of VCAM-1, IL-6, and TNF-α in the liver.	Park et al. (2011)
	Anti-inflammatory	Neuro inflammation	*In vitro*		0.5–2 μg/mL	Reduced NO and iNOS levels Repressed NF-κB activity by inhibiting IκBα degradation and JNK and Akt phosphorylation.	Moon et al. (2007)
	Antitumor activity, and anti-angiogenic ations.	Non-small cell lung cancer	Human non-small cell lung cancer cell line, A549	subcutaneous	1 and 10 mg/kg	Lowering of HIF-1α and VEGF levels.	Zhang and Chen (2017)
	Anticancer	Malignant melanoma	Human malignant melanoma (A375) cells		3, 4 and 6 μg/mL	Increased expression of cytochrome c leads to its translocation to the cytosol, as well as Cas3 and Cas9. Decline in EGFR transcription.	Sangboonruang et al. (2020)
	Anti-cancer effects	Gastric cancer Colon cancer	AGS, COLO205 and HCT-15 cell lines		10–20 μg/mL	Membrane damage.	Soliman et al. (2019)
	Anti-inflammatory properties 100 times more potent than hydrocortisone Antimicrobial properties antinociceptive effect Antitumor activities Radioprotection	Atopic dermatitis	Mouse	TNF-α/IFN-γ stimulated human keratinocytes	(100, 200, and 500 g mixed with normal saline) was applied 5 times/week for 4 weeks	Reduced ad skin lesions. BV and melittin inhibited the generation of chemokines (e.g., CCL17 and CCL22) and cytokines associated with inflammation (e.g., IL-1β, IL-6, and IFN-γ) generated by 2,4-dinitrochlorobenzene.	An et al. (2018)
		Arthritis	raw 264.7 and synoviocytes obtained from patients with rheumatoid arthritis, *in vitro*		5–10 μg/mL	Blocked the transcription of LPS-induced COX-2, PGE2, cPLA2, NO, and iNOS Reduced JUK and NF-κB activation, IκB release, and nuclear translocation of the p50 subunit.	Park et al. (2004)
		Amyotrophic lateral sclerosis	Mouse, s.c. injection at ST36 acupoint three times a week		0.1 μg/g	Microglia and phospo-p38 levels have decreased in the spinal cord and brainstem. Improves motor abilities and prevents neuronal death in the spinal cord. Inhibited a-synuclein misfolding. Reduced transcription of Iba-1 and CD14 in the lung Obliterated expressed CD14 and COX-2 in the spleen. Elevated pERK and Bcl-2 in the spleen	Lee et al. (2014b)
	Anticancer activity	Osteosarcoma	Human MG63 osteosarcoma cells		1 μM	Stimulation of phospholipase A2 leads to cell death and Ca^{2+} influx, whereas inhibition of cell proliferation occurs via the stimulation of inositol-requiring protein-1a and Xbox-binding protein 1.	Chu et al. (2007)
Apamin	Anti- apoptosis, and anti-inflammatory actions	Atherosclerosis	THP-1 cell treated with oxLDL - LPS injection with high fat diet		0.5, 1 and 2 μg/mL	Reduced NF-κB signaling pathway.	Kim et al. (2012)
	SK channel blockage. Increased the action potential duration	Heart failure	Pacing induced heart failure	Rabbit	(100 nmol/L) infusion	Reduce reduced ventricular fibrillation (VF) vulnerability.	Yin et al. (2017)

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TABLE 5 (*Continued*) Physiochemical properties, doses, and benefits of bee venom, and its components in numerous medical applications.

Bee venom/ Metabolites	Physiochemical properties	Disease	Model	Method of application	Dosage of use	Outcomes	References
	Blocked MAPK, Smad, and TGF-_1 signalling pathways.	Liver fibrosis	HSC-T6 cells, an immortalized rat hepatic stellate cell line		0.5, 1 and 2 μg/mL	Suppressed hepatic fibrosis	Kim et al. (2017)
	Improved memory acquisition	Alzheimer's disease	Transgenic mouse			Enhanced efficiency of nicotinic signaling	Proulx et al. (2020)
	Increased membrane potential in postsynaptic cell	Neurofibromatosis	Heterozygous Nf1+/mouse model			SK channel blockage.	Kallarackal et al. (2013)
Mast-Cell Degranulating (MCD) Peptide (Peptide 401)	Anti-inflammatoryactivity	Edema	Carrageenin-induced swelling of the Rathin paw		10 mg/kg	Suppressed edema	Banks et al. (1990)
Adolapin	Analgesic, and anti-inflammatory actions	Brain, and spleen inflammations	Rat spleen and brain		40 μg/kg	Inhibited cyclooxygenase. Elevation of c-GMP level. Decrease of c-AMP.	Koburova et al. (1985)
Phospholipase A_2	Neural protective effect	Multiple sclerosis (Experimental autoimmune encephalomyelitis)	C57BL/6 mouse	Injection	(0.2 mg/kg, i.p., daily for a period of 10 days)	bvPLA2 reduces limb paralysis and CD4+ cell invasion, but loses its benefits when Tregs are reduced.	Lee et al. (2019b)
	Anti-inflammatory and anti-arthritic activities	Rheumatoid arthritis	Male DBA/1 mouse	Intraperitoneal injections	(0.1, 0.5, 1.0 mg/kg, i.p., for 5 weeks)	Prevent bodily weight reduction. Reduce squeaking score. Paw thickness and arthritis index.	Choi et al. (2021)
	Alleviated atopic skin inflammation	Atopic dermatitis (AD)	Male C57BL/6 mouse	Alleviates atopic skin inflammation	(80 ng/ear, topical application, four times a week for 3 weeks)	Reduce atopic dermatitis-related skin edema, optimize ear thickness, reduce Th1 and Th2 cytokine expression, promote Treg, reduce epidermal and dermal thickness and macrophage invasion, and inhibit mast cell penetration.	Shin et al. (2018)
Hyaluronidase	Sperm like structure	Male Infertility	*In vitro*			Substantial homology with PH-20, a membrane protein of guinea pig sperm that regulates sperm-egg attachment.	Gmachl and Kreil (1993)
Acid phosphatase	Densensitization	Allergy	*Trichoplusia ni* cells			Optimized component-resolved strategy for honeybee venom allergic reactions, with a focus on developing improved diagnostic methods and improving allergenic immunotherapy.	(Stahelin, 2016; Murakami et al., 2017)

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TABLE 6 Application of bee venom in controlling the liver diseases.

Active component	Model	Outcome	Reference
Apamin	Cholestatic liver fibrosis in mice	Apamin therapy has reduced liver damage and pro-inflammatory cytokines concentrations.	Kim et al. (2017)
Bee venom	High-fat diet (HFD)-induced nonalcoholic fatty liver (NAFL) in rats	BV-treated rats had considerably decreased levels of all hepatic enzymes. Treatment resulted in the correction of adiponectin levels and substantial reductions in hepatic triglyceride and cholesterol levels.	Hanafi et al. (2018)
PLA2	Liver injury induced by acetaminophen in mice	PLA2 protects against hepatic impairment and stimulates anti-inflammatory cytokines to be generated in acetaminophen-injected rats.	Kim et al. (2014)
MEL	Acute liver failure (ALF) in mice	MEL lowered total bilirubin, ALT, AST, TNF-α, and IL-1β levels in acute liver failure mice, improving survival rates, reducing symptoms, and relieving hepatic inflammation. MEL demonstrated antioxidant and anti-inflammatory properties in LPS-stimulated RAW264.7 macrophages.	Fan et al. (2021)
phospholipase A2 (bvPLA2)	Cholestatic liver injury and fibrosis in a murine model	BvPLA2 treatment prevented hepatocyte death. Furthermore, bvPLA2 lowered cytokine production and inhibited the nuclear factor kappa-B pathway.	Kim et al. (2021b)

TABLE 7 Application of bee venom in cancer therapy.

Active component	Model	Action	Outcome	Reference
Bee venom	Human Lung cancer	Regulation of genetic transcription: Poly (ADP-ribose) polymerase (PARP), caspase-9, p53, BCL2, Box.	Apoptosis and cellular suppression.	Hwang et al. (2022)
Bee venom	Human Glioblastoma	Reduction in inflammation-promoting cytokines concentrations.	Insufficient toxic effects.	Sevin et al. (2023)
Bee venom	Pancreatic Cancer Human	Cyclin and Cyclin-dependent kinase (CDK) transcription and p53-p21 system activity can be modified.	Cell death and cellular arrest.	Zhao et al. (2022)
Melittin	Leukemia Human	Melittin-triggered decrease in mitochondria energy metabolism	Cytotoxic	Gasanoff et al. (2021)
Melittin	Gastric cancer Human	Inhibiting MMP2 and MMP9 activity leads to decreased activity in the Wnt/BMP and MMP-2 signaling pathways. Suppression of molecular adhesion.	Cytotoxicity, attachment, and infiltration suppression.	Huang et al. (2021)
Bee venom	Head and neck squamous cell carcinoma Human	Increased expression of Bax leads to reduced levels of Bcl2 and Epidermal growth factor receptor (EGFR), affecting the cell cycle.	Cisplatin has cytotoxic properties, arrests the cell cycle, and increases activity.	Grawish et al. (2020)
Bee venom, melittin	Malignant melanoma Human	Inhibits Phosphoinositide 3-kinases (PI3Ks)/AKT/mammalian target of rapamycin (mTOR) and mitogen-activated protein kinase (MAPK) processes. Increased transcription of Casp3 and Casp9	Cell death inhibits motility and colonization.	Lim et al. (2019)
Melittin	Ovarian cancer Human	Reduced amino acids in the proline/glutamine/arginine pathway. Decreasing amounts of carnitines, polyamines, ATP, and NAD+.	Cytotoxic	Alonezi et al. (2016)

been studied, and it has been found that melittin may increase the death of hepatocellular carcinoma cells (HCC) through the CAMKII-TAK1-JNK/p38 system. In addition, melittin has been found to encourage TRAIL-resistant HCC cells to TRAIL-induced cell death, probably through stimulating the CAMKII-TAK1-JNK/p38 route and blocking the IKK-NFB signaling. The outcomes are consistent with melittin activating calcium channels, increasing Ca^{2+} concentrations inside the cells, and calcium-responsive CaMKII (Balasubramani et al., 2023). It has also been reported that BV and melittin inhibit the malignant expansion of cells by triggering caspases (3 and 9) and suppressing NF-B signaling (Ghadiri et al., 2024). It has been proven that BV inhibits proliferation and causes

apoptotic cell death by activating death receptors (DR4 and DR5) (Khaliq et al., 2022). The primary reasons for cancer progression are metastasis and the infiltration of malignant cells. As a result, cancer researchers emphasize identifying the biological processes that drive cancerous cells to migrate and potential strategies to inhibit metastasis (Zuazo-Gaztelu and Casanovas, 2018).

In this context, melittin has been shown to impede *in vitro* and *in vivo* HCC cell movement via lowering Rac1-dependent processes (Haque et al., 2023). In contrast, a recent study demonstrated that combining melittin with anticancer drugs, such as temozolomide (TMZ), dramatically lowers melanoma cell growth and invasion compared to circumstances where TMZ or melittin was provided alone (Lim et al., 2019). Although MEL's promise as a carcinoma treatment modality has long been identified, its fast breakdown in circulation and unexpected cellular lysis activities provide substantial obstacles. To address these concerns, current optimization strategies rely on nanoparticle-based melittin delivery. Nanotechnology has enabled the creation and practical testing of melittin conjugates against a variety of tumors of human kinds in preliminary studies (Singh et al., 2023; Jafari et al., 2024). To develop an effective and safe melittin delivery method that can lower hemolytic activity while maintaining cytotoxic properties. Consequently, a dual-secured nano-sting (DSNS) was created by combining zwitterionic glycol chitosan and disulfide links. At low concentrations, melittin-loaded DSNS displayed a virtually complete cytotoxic impact on numerous cancer cell types, including undamaged red blood cells (Giribaldi et al., 2021).

Only one study on cell cultivation found no statistically noteworthy cytotoxic impact on cancer cells (human glioblastoma). BV and melittin influence several intracellular pathways, and their blockage triggers apoptosis., melittin inhibits the PI3K/Akt/mTOR pathway in breast cancer cells (Pandey et al., 2023b), or liver cancer cells (Lv et al., 2023). Lim et al. found that BV and melittin suppress the PI3K/Akt/mTOR and MAPK pathways in cancerous melanoma cells (Lim et al., 2019). In two investigations on ovarian carcinoma cells, Alonezi et al. reveal a new explanation of melittin activity where compounds in the tricarboxylic acid cycle, oxidative phosphorylation, purine, and pyrimidine metabolic rate, and the arginine/proline pathways are decreased. In addition, the investigators discovered that melittin reduced carnitines, polyamines, adenosine triphosphate (ATP), and nicotinamide adenine dinucleotide (NAD+) (Alonezi et al., 2017). Erkoc et al. found that melittin induces calcium signaling apoptosis and inhibits Cyclic adenosine monophosphate (Camp) in breast cancer cells, resulting in cell death and an anti-division effect (Erkoc et al., 2022). BV also alters cell shape, resulting in DNA and protein disintegration (Jung et al., 2018). Li et al.'s study on lung cancer cells indicates that melittin boosts the generation of reactive oxygen species (ROS) and the formation of intracellular iron while affecting the activity of glutathione peroxidase 4 (GPX4); it causes mitochondrial dysfunction and death, a condition called ferroptosis (Li et al., 2022). In the past few years, some research has concentrated on the influence of BV. on cancer cell migration. The tumor mass grows complexly, with proteolytic enzymes gradually destroying the stromal tissue. Matrix metalloproteinases (MMPs) play a significant part in this process. BV inhibits the expression of MMP-2 and MMP-9 in glioblastoma cells in a dose-responsive way, but not in healthy hippocampus cells (Małek et al., 2022). It is important to note that, unlike several other pharmaceutical chemicals, BV active ingredients possess the ability to cross the blood-brain barrier, making them a potentially feasible approach for the management of CNS disorders in coming years. Additionally, research on gastric cancer cells show that melittin inhibits MMP-2 and MMP-9 function. BV and melittin have comparable effects on hepatocarcinoma cells (Mansour et al., 2021). Inhibiting angiogenesis is also linked to reduced tumor invasion. Shin et al. revealed that melittin suppresses hypoxia-inducible factor 1-alpha (HIF-1α), an element that controls the production of (VEGF), a strong amplifier of vascular development. Zhang et al. found similar outcomes in non-small cell lung cancer (Zhang et al., 2015).

In another investigation on the U87 human glioblastoma line, significant cytotoxic properties began at a 5 μg/mL dose. The investigation skipped over the influence of venom on native cells. Gajski et al. found that BV had a cytotoxic impact on glioblastoma A1235 cells at 20 μg/mL (Gajski et al., 2016). However, 2 μg/mL of the crude BV caused a considerable reduction in cell survival (above 60%) in human colon cancer HCT116 cells. Another research employing the same colon cancer cell line confirmed the cytotoxic impact of BV at 1 μg/mL. Furthermore, BV had no harmful effects on FHC colon epithelial normal cells, even at a dosage of 10 μg/mL (Małek et al., 2023). The discovered BV levels that inhibit cancer cells may be inadequate to be considered in systemic-therapy without substantial adverse effects. Melittin's great tendency to trigger hemolysis and quick metabolism might represent a hurdle to employing BV. BV has a cytotoxic impact on tumor cells at concentrations higher than 1 μg/mL (Based on the cellular lineage). To get such a BV concentration in the bodily fluids of a 75 kg individual, around 75 mg of venom would need to be administered. Nonetheless, in animal studies, a venom dosage between 0.5 and 1.0 mg/kg bw had been effectively implemented (Małek et al., 2023). Other disadvantages of systemic therapy might be eliminated by administering BV directly into the tumor. This approach has little toxicity and an excellent therapeutic index (El Bakary et al., 2020). Other disadvantages of systemic therapy might be eliminated by administering BV directly into the tumor. This approach has little toxicity and an excellent therapeutic index (Xing et al., 2003). Cell culture investigations provide for the evaluation of the molecular processes of a drug's activity. Still, they do not answer how cancer behaves in the host's natural environment. As a result, animal models can examine tumor growth in the body's circumstances and the propensity to produce distant metastases. There has been significantly less animal research than *in vitro* studies on the anticancer properties of BV. In the Ehrlich ascites carcinoma mouse model, BV has been found to lower MMP-2 and MMP-9, as well as VEGF, TNF-α, and nitric oxide (NO) levels, hence decreasing tumor growth and preventing angiogenesis (El Bakary et al., 2020). Lee et al. discovered that melittin reduced VEGF levels in animals with lung cancer (Lee et al., 2017). Rocha et al. reported that melittin inhibited the formation of colorectal cancer metastases in a mouse model (Rocha et al., 2022). El-Beltagy et al. found that restoring histological abnormalities in ovarian and breast cancer rat models decreased serum MMP-1, NF-κB, and TNF-α levels (El-Beltagy et al., 2021).

Both *in vitro* and animal investigations show that BV and melittin have anticancer properties. The findings thus far put us

closer to conducting clinical studies on using BV in cancer in the future (Jafari et al., 2024). Maybe additional proof of BV's favorable benefits will lead to the initiation of human studies. As previously stated, the influence of BV on the body is multidirectional, and determining the effect of its different components on the complete human body takes extensive investigation. It is unclear if BV may achieve therapeutic quantities in tumor tissue without eliciting unpleasant consequences. It should be emphasized that in addition to the anti-cancer action, the venom has additional systemic properties that may contribute to the anticancer activity of BV.

6.3 In periodontal therapy

Periodontitis is a bacterial illness that damages tooth-supporting tissue, destroying ligaments and bones. Periodontitis can lead to loss of teeth, cardiovascular problems, and rheumatoid arthritis (Alkhursani et al., 2022; Atia et al., 2022; Nasser Atia et al., 2022; Atia GaN. et al., 2023). In individuals with periodontitis, osteoclasts stimulate bone resorption (Atia GaN. et al., 2023). Periodontitis is commonly treated with mechanical techniques and antibiotics. Nevertheless, there is a shortage of therapies addressing the disease's immunological elements (Nasser Atia et al., 2022). *Porphyromonas gingivalis* is a crucial bacterium linked to chronic periodontitis commonly found in periodontal disease locations (How et al., 2016). *P. gingivalis* produces virulence-related substances to survive in the oral environment (Jia et al., 2019). These contaminants stimulate host cells, including gingival macrophages and fibroblasts, leading to inflammation in periodontal tissue and alveolar bone resorption (Xu et al., 2020; Atia GA. et al., 2023). Periodontal infection triggers inflammation that increases host immune cells and promotes the breakdown of bones through cytokines, chemokines, and attachment molecules (Cekici et al., 2014). BV inhibits *P. gingivalis*-triggered proinflammatory alveolar bone destruction *in vivo* and RANKL-associated osteoclasts development, stimulation, and metabolism *in vitro* (Gu et al., 2019).

6.4 Management of autoimmune diseases

Autoimmune illnesses that include rheumatoid arthritis, systemic lupus erythematosus, and multiple sclerosis have long been thought to be T helper cells (Th cells) 1-dominant; nevertheless, the importance of Th17 cells and Tregs in autoimmune diseases has only recently been discovered (Rosenblum et al., 2015). Rheumatoid arthritis is a prevalent autoimmune condition, although current treatment options are not always practical (Guo et al., 2018). BV has been utilized for decades for the management of chronic inflammatory conditions, such as rheumatoid arthritis (Wang et al., 2021a). BV's anti-rheumatic and anti-inflammatory properties have been known for almost a century (Nainu et al., 2021).

6.4.1 Rheumatoid arthritis

Rheumatoid arthritis is an autoimmune condition characterized by synovial growth and cellular penetration. It can cause joint damage, malformation, incapacity, swelling, and pain in numerous joints (Sharif et al., 2018). Low-dose methotrexate is the most frequent therapy for rheumatoid arthritis. Methotrexate can cause hepatotoxicity, leading to insufficient drug tolerance (Wang et al., 2018). As a result, patients are more prone to pursue alternative therapies. Previous research suggests that BV injection may reduce inflammation and pain in animals with rheumatoid arthritis (Figure 5) (El-Tedawy et al., 2020). Li et al. employed Freund's adjuvant-induced animal model to study arthritis. They discovered that treatment with BV at ST-36 inhibited Fos transcription in the superficial layer of the lumbar spinal cord, resulting in reduced paw edema and nociceptive behaviors on the injected side (Li et al., 2010). In a type II collagen-induced arthritis (CIA) model, Lee et al. discovered that BV injection treatment inhibits immunological responses. TNF-α production decreased significantly in the BV group compared to the control group, whereas IL-1β levels remained constant (Lee JaeDong et al., 2004). Another study found that rats with Cytosolic Iron-Sulfur Cluster Assembly (CIA) had much higher amounts of free radicals and protease activity than normal rats. BV injection (0.25 mg/kg) effectively modulated rheumatoid arthritis by reducing protease activity and eliminating ROS (Petronek et al., 2021). In conjunction with methotrexate, BV could boost effectiveness and have antiarthritic properties (Darwish et al., 2013).

BV injection has been shown to provide antiarthritic and antinociceptive effects in rats with arthritis, making it a potential alternative treatment for rheumatoid arthritis (RA) (El-Tedawy et al., 2020). Melittin treatment in arthritis decreases LPS-induced p50 redistribution into the nucleus, lowering inflammatory gene expression (Zhang et al., 2024). Melittin binds to p50 with high affinity (Kd = 1.2 108 M). They also looked at melittin's anti-inflammatory properties through its associations with IKKs. Melittin suppresses TNF/LPS-induced NO and PGE2 generation in a mouse macrophage cell line and synoviocytes of rheumatoid arthritis patients (Yang et al., 2023). Additionally, they found that melittin suppresses inflammatory target gene expression and NF-κB activation through the JNK pathway (Xing et al., 2024).

6.4.2 Multiple sclerosis

Multiple sclerosis is an inflammatory illness that can cause brain and spinal cord damage through inflammation of the central nervous system. The cause of multiple sclerosis is uncertain. It might be caused by a viral infection, environmental causes, or hereditary factors (Ghasemi et al., 2017). After 2000, numerous alternatives have been employed for the management of multiple sclerosis, including BV and cannabis-derived substances. The preliminary therapy with decreased nerve degeneration and glial stimulation, and cytokine production, including IFN-γ, IL-17, IL-17A, TNF-α, IL-1β, and chemokines (MCP-1 and MIP-1α). BV treatment can decrease CD4+, CD4+/Interferon-gamma (IFN-γ+), and CD4+/IL-17 + T cells while raising CD4+/Foxp3+ T cells in the nervous system and lymphatic systems of rats with acute experimental autoimmune encephalomyelitis (EAE) (Lee MJ. et al., 2016).

6.5 Management of neurological conditions

Among these neurological problems, neurodegenerative ailments have a substantial influence on not only people but also

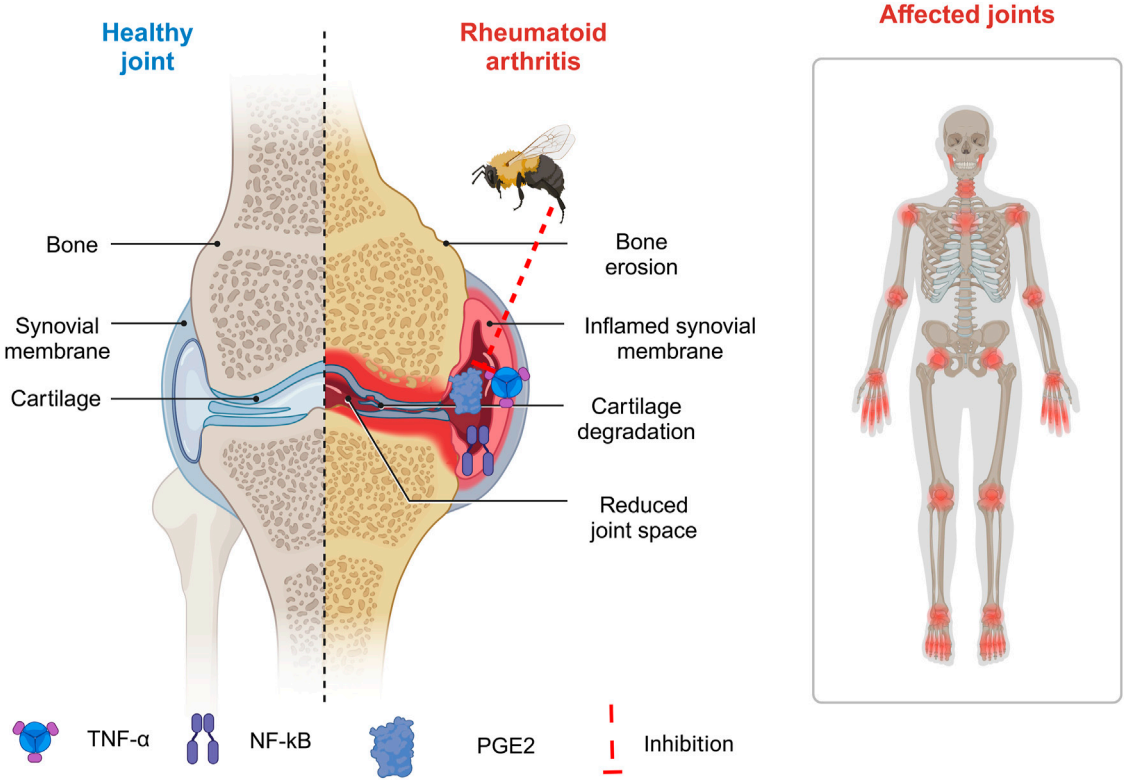

FIGURE 5
Implementation of bee venom in management of rheumatoid arthritis. (Generated by BioRender software).

carers and society. The most common neurological illness is Alzheimer's, followed by Parkinson's and epilepsy (Nassan and Videnovic, 2022). They are a varied group with unrelenting progression, and aging is a crucial risk factor in their development. Regardless of their differences, all of these disorders are associated with intellectual disability, motor abnormalities, and behavioral abnormalities. The particular cause of neuronal degeneration and protein accumulation in many illnesses is unclear (Lamptey et al., 2022). BV products prove to be helpful in the management of neurological conditions (Table 8).

6.5.1 Parkinson's disease

Parkinson's disease (PD) is a degenerative movement illness that causes gradual impairment in sufferers. The illness is characterized by gradual loss of dopaminergic neurons in the substantia nigra and the appearance of Lewy bodies, which include aggregation of alpha-synuclein, extensively dispersed proteins in the cerebral tissues. Improper stimulation of microglial cells is a pathogenic marker in several neurodegenerative disorders, such as Parkinson's (Cai et al., 2021). Several preclinical experiments examined how BV affects leukocyte migration and microglial activation in animal and cellular models (Avalo et al., 2022; Gao et al., 2023). In experimental mice models of Parkinson's disease, BV acupuncture therapy (BVA) was shown to protect from rotenone-triggered oxidative damage, neurological inflammation, and cytotoxicity (Mi et al., 2024). Rotenone, a pesticide, may alter the pathophysiology of Parkinson's disease (Tanner et al., 2011). BV effectively prevented dopamine depletion following rotenone injection.

Furthermore, BV treatment in PD mouse models restored locomotor activity (Morin Jr and Boudreau, 2024). The therapy reduced DNA damage and suppressed the transcription of cell death genetic factors Bax, Bcl-2, and caspase-3 in the brains of PD mice (Eleiwa et al., 2023). BV has been shown to safeguard dopamine neuronal cells against neurodegeneration in preclinical PD studies (Hassani and Esmaeili, 2024).

6.5.2 Alzheimer's disease

Alzheimer's disease is the most prevalent neurodegenerative illness, with several pathogenic mechanisms contributing to its development (Aksoz et al., 2019). Although the cause of Alzheimer's disease is uncertain, research suggests that inflammation could have a significant role in its progression (Twarowski and Herbet, 2023). Ye et al. discovered that bvPLA2 may successfully suppress the development of Alzheimer's disease in transgenic mice (Ye et al., 2016b). BvPLA2 reduces Aβ buildup and improves cognitive performance in mouse brains. The study found that bvPLA2 can improve glucose metabolism and minimize neuroinflammatory reactions in the hippocampus, perhaps limiting AD development (Ye et al., 2016b). Recent research found that bvPLA2 therapy can change regulatory T-cell populations in a 3xTg-AD animal model. The investigators proposed pairing bvPLA2 therapy with Aβ vaccine therapy to slow the onset of Alzheimer's disease while preventing harmful inflammatory responses (Baek et al., 2018).

TABLE 8 Application of bee venom in the treatment of neurological diseases and disorders.

Active component	Disease	Model	Outcomes	Reference
Wistar rats	Spinal Cord Injury (SCI)	3–4 animals/group	BVA raised IL-10 expression at six h and decreased IL-6 production at 24 h following SCI compared to controls.	de Souza et al. (2017)
Bee venom	Intervertebral Disk Disease (IVDD)	Canines	BV injection effectively treated dogs with moderate to severe IVDD, reducing clinical rehabilitation time.	Tsai et al. (2015)
Melittin	Peripheral Neuropathy	Human	Following BVA, Patient Health Questionnaire (PHQ) scores and WHO Chemotherapy-induced peripheral neuropathy (CIPN) grades fell. VAS has also decreased.	Yoon et al. (2012)
Bee venom	Peripheral Neuropathy	Sprague–Dawley rats	BVA decreases oxaliplatin-induced acute cold allodynia by activating the serotonergic system and spinal 5-HT3 receptors.	Lee et al. (2014a)
Bee venom	Neuropathic Pain	ICR mice	BVA inhibits nociception through alpha2-adrenoceptors but not naloxone-sensitive opioid receptors.	Kwon et al. (2001)
bvPLA2	Parkinson's disease		Injecting bvPLA2 has been demonstrated to improve motor function, reduce α-sympathy, increase microglial deactivation in the spinal cord, and normalize the M1/M2 microglial phenotypic ratio.	Ye et al. (2016a)
Bee venom/apamin	Parkinson's disease	C57BL/6 mice	Apamin can somewhat mimic the neuroprotective properties of dopaminergic neurons.	Alvarez-Fischer et al. (2013)
Bee venom	Parkinson's disease	C57BL/6 mice	Avoided the breakdown of tyrosine hydroxylase and reduced phospho-Jun immunoreactivity.	Doo et al. (2010)

6.5.3 Amyotrophic lateral sclerosis (ALS)

ALS is a CNS illness that leads to the loss of nerve cells that control movement. ALS is characterized by aberrant buildup of mutant superoxide dismutase (SOD) 1 protein complexes (Quatorze et al.). Soares et al. characterized a mouse model of ALS with a mutant mtSOD1 gene with a Glycine to Alanine substitution (SOD1G93A) to clarify the etiology of the disease (Soares et al., 2023). Previous investigations on mutant SOD1 transgenic mice revealed pathogenic processes in motor neurons, including protein misfolding, mitochondrial malfunction, and neurofilament buildup (Zhou et al., 2023). Additionally, BV showed some potential for treating this illness. Unlike age-matched control mice, BV treatment at a particular stage of ALS development increases motor function and longevity in SOD1G93A mutant mice. This might be due to blocking active microglia, as shown in mouse models of ALS (Barreto-Núñez et al., 2024). The research found that BVA at ST36 reduces inflammation in neurons in the spinal cord of ALS animals, including TLR4, CD14, and TNF-α (Cai et al., 2015).

6.5.4 Neuropathic pain

BV also helps to alleviate neuropsychiatric problems. Cold allodynia is a crucial indicator of neuropathic pain. Research suggests that BV injections may alleviate cold allodynia in mice with sciatic nerve CCIs (Er-Rouassi et al., 2023). BV, combined with peripheral β-adrenoceptor limitation, may help reduce inflammation-related pain. BVA may impact other receptors, including α4β2 nicotinic acetylcholine receptors, and relieve cold allodynia in oxaliplatin-injected mice (Yoon HeeRa et al., 2015). Numerous studies show that BV boosts Fos transcription and decreases nociceptive behavior. Coupled with BV, intrathecal clonidine-induced painkilling was much more effective (Ullah et al., 2023).

6.5.5 Peripheral neuropathy

Chemotherapeutic drugs can produce peripheral neuropathy by damaging sensory and motor nerves in the peripheral nervous system. Oxaliplatin, an anticancer medication, causes neuropathic cold allodynia. A few studies have explored whether BVA can reduce mechanical allodynia (Liu F. et al., 2023; Peralta et al., 2023). Yoon et al. found that injecting DBV (0.1 mg/kg) at ST36 of the right hind leg for 2 weeks and then administering oxaliplatin (10 mg/kg) resulted in significantly decreased ipsilateral mechanical allodynia (Yoon et al., 2013). Another research study found that methysergide can inhibit BVA's antiallodynic action linked to the serotonin system. BV has been shown to boost serotonin levels in the spinal cord and relieve cold allodynia in rats treated with oxaliplatin via activating spinal 5-HT3 receptors (Kim WooJin et al., 2016). Paclitaxel, a chemotherapy medication used to treat tumors, also caused agonizing peripheral neuropathy. In this research, BV reduced the negative consequences of paclitaxel. Additionally, BVA therapy had a strong antihyperalgesic impact at acupoint ST36 (Choi et al., 2017). Yoon et al. found that BV pharmacopuncture can lower the recommended toxicity guidelines for peripheral neuropathy (Yoon et al., 2012).

6.6 Male reproductive impairment

Recent research suggests that bee products may have preventative and therapeutic benefits for health, particularly infertility in men (Table 9). According to World Health Organization recommendations, 15%–25% of couples struggle to conceive. Infertility in men is triggered by changes in sperm quantity, movement, and/or shape that are found in collected samples. Recent research suggests that bee products may have preventative and therapeutic benefits for health, particularly infertility in men (Regeai et al., 2021; Suleiman et al., 2021).

TABLE 9 Application of bee venom in controlling the male reproductive impairment.

Active component	Model	Outcomes	Reference
Bee venom	Rabbit	Lower progesterone levels, with concomitant increase in had a boost in conception (17%) and fertility (10%).	Elkomy et al. (2021)
Bee venom	Mouse	- Restoration of normal levels of reproductive hormones and increased sperm quantity and quality.	Al-Shaeli et al. (2022)
Mellitin	Rat	Mellitin binds with proteins in tight connections between Sertoli cells.	El-Hanoun et al. (2020)
Bee venom	Rabbit	BV demonstrated a considerable favorable effect on several semen quality features, sexual behavior, antioxidant activity, lipid peroxidation indicators, and immunological response	(Al-Sayigh et al.)

TABLE 10 Application of bee venom in skin diseases.

Skin diseases	Model	Mechanism	References
hyperpigmentation	Hartley guinea pigs	Photoprotective, anti-melanogenic	Han et al. (2017)
Atopic dermatitis (AD)	BALB/c mouse	This medicine inhibits T cell proliferation and access and the production of Th1 and Th2 cytokines, IL-4, and IgE, which are common allergic Th2 responses in the bloodstream.	Sur et al. (2015)
Acne Vulgaris	HaCaT and THP-1 cells	Includes anti-inflammatory effects for Propionibacterium acnes. P. acnes secreted less IFN-γ, IL-1β, IL-8, and TNF-α after TLR2 expression was blocked.	Kim et al. (2015b)
Alopecia	C57BL/6 mouse	Increase the concentrations of the growth factors FGF-2, IGF-1R, and VEG.	Park et al. (2016)
Tissue regeneration	Mouse	Preventing inflammatory reactions may promote collagen synthesis and wound healing.	Sarhan and Azzazy (2017)
Aging marks	Human trial	Clinical investigations revealed decreases in total wrinkle area, count, and average depth, although the exact mechanism is uncertain.	Han et al. (2015)
Vitiligo	Normal human epidermal melanocyte	Triggers melanocytes to generate cAMP. The number of melanocytes increased.	Jeon et al. (2007)
Psoriasis	Twenty-five patients	TNF-α concentrations were significantly reduced than in the control group.	Eltaher et al. (2015)

According to WHO recommendations, 15%–25% of couples have difficulty conceiving. Infertility in men is triggered by changes in sperm quantity, movement, and/or shape that are found in collected samples. Infertility can be caused by various mechanisms, including steroidogenic pathway defects, pro and antioxidant activity imbalances, apoptotic pathway irregularities, pro and anti-inflammatory markers, and reactive oxygen species production (El-Hanoun et al., 2020). A study found that Iraqi bee stings protect and maintain sexual performance in mice by inhibiting Sertoli cell discharge of activin-B, which promotes spermatogonia and induces replication to create spermatocytes (Al-Sayigh et al.).

6.7 Skin care

BV has been utilized in medicine for therapy and as a beauty component. It has antimicrobial and anti-inflammatory properties, making it suitable for use in dermatology (Table 10). BV has a suppressive impact on *Cutibacterium acnes*. *C. acnes* is the primary cause of inflammation in acne.

An et al. found that applying BV to mice's skin after injecting *C. acnes* into their ears decreased inflammation and levels of TNF-α and IL-1β. BV decreased the levels of TLR2 and CD14 in *C. acnes*-injected tissue (An et al., 2014). The results imply that BV can successfully cure acne. Research suggests that BV-based cosmetics can effectively treat acne vulgaris. At a dosage of 0.5 mg, concentrated BV effectively decreased C. acnes numbers (Han et al., 2013). Melittin in BV has antibacterial properties (Elieh Ali Komi et al., 2018). It effectively eliminates *S. aureus*, *Staphylococcus* epidermidis, and *Staphylococcus* pyrogens (Kim H. et al., 2019). Melittin is a poisonous peptide that destroys the bacterial cell wall (Abd El-Wahed et al., 2019). BV can treat fungal and viral skin problems. BV has been proven to have antifungal properties (Kim H. et al., 2019). The antiviral impact of BV on herpes simplex virus has been investigated. BV reduced the growth of this virus (Uddin et al., 2016). BV can block five α-reductase, which converts testosterone to dihydrotestosterone and promotes hair development, as demonstrated by studies on alopecia. Various percentages of BV (0.001%, 0.005%, and 0.01%) were compared to 2% minoxidil. BV enhanced hair development and prevented shifting from the anagen to the catagen phase. BV suppressed the levels of SRD5A2, a 5-α-reductase (Park et al., 2016).

BV may serve as a novel therapeutic for localized plaque psoriasis. Localized plaque psoriasis can be effectively treated with intradermal BV alone or oral propolis. BV includes melittin, which inhibits the production of inflammatory genes. BV suppresses COX-2 transcription, reducing the generation of inflammatory prostaglandins (Hegazi et al., 2013). BV components can have varying and sometimes contradictory immunological responses. Polypeptide adolapin suppresses prostaglandin production and inhibits the action of PLA2 and human lipoxygenase (Tusiimire

et al., 2016). BV's anti-inflammatory properties make it effective for treating atopic dermatitis. Emollients containing BV resulted in decreased eczema areas, severity indexes, and visual analog scale values compared to those without (You et al., 2016). BV's biological activity has been utilized to cure wounds. Wound healing involves the production of TGF-β1, fibronectin, VEGF, and collagen-I.

The study on mice found that lowering wound size led to increased epithelial division. Local application of BV reduces wound size in animal models (Zhao et al., 2024). BV is used in wound dressing alongside polyvinyl alcohol and chitosan. Using 4% BV as a wound treatment in diabetic rats led to faster healing and less inflammation (Ivanov et al., 2023). Research found that combining 6% BV with chitosan improved wound healing (ÖZgenÇ and Sevin, 2023). BV was shown to enhance the growth and movement of human epidermal keratinocytes. Combining BV with hydrogel led to enhanced collagen synthesis. BV promotes wound healing through anti-inflammatory, antimicrobial, and antioxidant properties. BV is extremely successful in treating human melanoma A2058 cells. It was found that BV causes cell death by generating hydroxyl radicals (Gajski et al., 2024). In recent years, BV has been employed as an antiwrinkle treatment. Twenty-two women from South Korea used 0.006% BV serum as a cosmetic component, using 4 mL twice a day for 12 weeks. It reduced overall wrinkle area, measurement, and thickness. BV inhibits tyrosinase-related proteins, resulting in antimelanogenic action (Han et al., 2015). Han et al. found that BV reduces matrix metalloproteinase protein levels, indicating photoprotective action.

BV efficiently suppresses photoaging mechanisms and can treat photodamaged skin (Han et al., 2017). A gel with 0.06% BV did not cause photosensitive dermatitis, as proven in animals (Han et al., 2017). Melittin treats skin inflammation and protects against P. acnes-induced inflammatory reactions *in vitro* and *in vivo*. They studied the anti-inflammatory effects of melittin therapy in heat-killed P. acnes-treated HaCaT cells, which inhibited NF-B and p38 MAPK signaling. Furthermore, the anti-inflammatory efficacy of melittin was studied in a live P. acnes-induced inflammatory skin disease animal model. In the animal model, melittin-treated ears showed considerably lower swelling and granulomatous responses to P. acnes injection. Another study found that melittin reduced heat-killed P. acnes-induced apoptosis and inflammation in human THP-1 monocytic cells (Zhang et al., 2024).

6.8 Prevention and treatment of common cardiovascular diseases

BV can enter the body via direct stings or manual injection (Ali, 2012; Moreno and Giralt, 2015). Wehbe et al. thoroughly examine BV's chemical makeup, biological characteristics, and mechanisms of action (Wehbe et al., 2019). Yook et al. discovered that sweet BV may impact fluctuations in heart rate, although nothing is known about its effect on CVDs (Yook et al., 2008). Wang et al. discovered that melittin, a polypeptide present in BV, can attenuate CVB3-induced myocarditis. Melittin treatment (0.1 mg/kg) lowered myocardial cell death, lowered Bax and caspase-3 expression, and increased Bcl-2. It also improved cardiac activity, as seen by echocardiography (Wang et al., 2016).

6.9 Management of asthma

Asthma is a potentially fatal inflammatory lung disorder with elevated CD4+ T cell counts. Research discovered that injecting BV (0.1 and 1 micro g/mL) increased regulatory T cells, inhibited cytokine production, and reduced peribronchial and perivascular inflammatory cell infiltrates in Balb/c mice with an ovalbumin-induced allergic asthma model (Reuter et al., 2024). Natural regulatory cells expressed more CD4+, $CD25^{+}$, and FOXP3, whereas IgE levels in test animals' blood dropped considerably (Haque et al., 2024).

6.10 Anti-hyperglycemic activity

Diabetes mellitus (DM) is a life-changing metabolic condition caused by abnormal insulin production, receptor activation, or both (Kharroubi and Darwish, 2015). Hyperglycemia causes excessive glycation of sugars and unbound amino groups in proteins, which alters their physical and chemical characteristics (Feng et al., 2023). BV doses (10, 20, and 40 μg/mL) were investigated *in vitro* for their effect on hemoglobin glycation when incubated with glucose. The amount of heme was evaluated by separating free amino groups with fluorescamine. The researchers discovered that BV prevents glycation-driven heme breakdown in hemoglobin. Because it has a solid antiglycation activity. It has been proposed as a natural therapy for glycation difficulties in DM (Jafari et al., 2024).

6.11 Protective impact against renal damage

Sepsis-induced acute kidney injury is a major worldwide health problem. Sepsis-induced acute kidney injury is a worldwide health problem. During septicemia, the endotoxin Lipopolysaccharide (LPS) causes systemic inflammation. BV, MEL, and apamin alleviated LPS-triggered severe renal damage in mice by lowering cellular oxidation damage, inflammatory processes, and cell death. MEL lowered TNF-α and IL-6 levels, decreased immune cell aggregation in the kidney, and blocked the NF-κB system. MEL decreased MDA levels, inhibited NOX4 expression, boosted Nrf2-mediated antioxidant defenses, and blocked apoptotic and necrotic processes following LPS administration. Furthermore, MEL treatment significantly prolonged the survival of mice treated with LPS (Son et al., 2007). MEL may help prevent and treat sepsis-related renal issues (Tiwari et al., 2022). In addition, MEL decreased cisplatin-induced acute kidney injury in mice by regulating M2 macrophage expression (Kim J-Y. et al., 2020).

Chronic accumulation of developing renal fibrosis produces irreversible harm to the kidneys, resulting in end-stage conditions such as renal failure. Apamin decreased renal fibrosis from unilateral ureteral obstruction *in vivo* and inhibited TGF-β1-induced activation of renal fibroblasts *in vitro*. This peptide inhibited TGF-β1/Smad2/3 and STAT3 pathways, leading to decreased inflammation, tubular atrophy, myofibroblast activation, and fibrotic gene expression (Gwon et al., 2021).

Dietary BV improved breast meat's efficiency, yield, and nutritional value in grilled chickens. It also lowered the height of internal organs, caecal short-chain fatty acid, and ileal villus. This

shows that BV might be an organic alternative to antibiotics in feed for improving animal health (Kim et al., 2018).

7 Clinical studies on the effect and safety of bee venom

BV has shown promising clinical effectiveness in treating a range of conditions, including asthma, allergic rhinitis, insomnia, headache, pain relief, and inflammation (Jang and Kim, 2020; Lin and Hsieh, 2020). However, its use is not without risk, as it can cause life-threatening adverse reactions (Jang and Kim, 2020). Despite this, BV is effective in treating skin varicosities veins, with potential benefits in dermatology. Further research is needed to fully understand the clinical applications and safety of BV therapy.

The safety of BV has been extensively studied, with mixed results (Bae et al., 2015; Cherniack and Govorushko, 2018; Sung et al., 2022). Cha, et al. found no significant adverse effects in mice, suggesting its potential as a safe natural antibiotic (Cha et al., 2012). However, Jang, et al. and Park, et al. both highlighted the need for caution, as BV therapy can lead to adverse events, including skin reactions and anaphylaxis (Park et al., 2019; Jang and Kim, 2020). Lee also emphasized the importance of practitioner education and qualifications in ensuring the safety of BV therapy (Lee CH. et al., 2019). Therefore, while BV may have potential as a therapeutic compound, its safety in clinical trials is still a matter of concern.

8 Contraindications and provisions

Many situations limit the use of BV in human therapeutics, including children under five, pregnancy, nursing, infections, post-vaccination, and medically compromised patients (Khalil et al., 2021). Systemic responses to bee stings have been seen in up to 3.4% of children and 7.5% of adults. Hence, allergy testing should be undertaken before employing BV treatment. These allergic responses can be modest, affecting only the cutaneous tissues, resulting in life-threatening anaphylaxis. Regarding venom-related allergies, venom immunotherapy is the sole medicine to prevent additional systemic sting responses (Sturm et al., 2018). To test for allergies, 0.05 mL (1.0 mg of unprocessed dried venom dissolved in 1.0 mL of normal saline is administered into the patient's forearm flexor surface. It is infected slowly, resulting in a tiny hemispherical bleb. The absence of systemic manifestations 15–30 min after a subcutaneous administration of BV means the individual is judged not allergic to the examination. In the case of an allergic reaction, the patient should receive rapid medical assistance, which may involve the injection of adrenaline and/or dexamethasone. Since BV tolerance differs across individuals, there is no universal technique for everyone getting BV treatments. To get the finest benefits from BV treatments. All BV treatment courses should incorporate the following recommendations: (a) 2–3 mg of vitamin C daily; (b) 650 mg of acetaminophen can be administered in the event of fever and chills; (c) alcohol intake is forbidden during BV therapies; (d) an ice pack is helpful at the injection site if local reactions occur like edema; and (e) in anaphylactic shock, the patient should receive epinephrine (adrenaline) instantly and then be moved to the hospital (Bilò et al., 2019).

Advancements in controlled drug release systems and nanotechnology-based delivery methods can improve the targeted delivery of BV, increase bioavailability, and reduce side effects associated with traditional administration routes. These modern approaches allow for precise delivery of BV's active ingredients, ensuring the right amount reaches the intended site of action. Nanotechnology-based delivery systems offer high sensitivity and specificity, allowing for rapid diagnosis and treatment of conditions like bacterial vaginosis while minimizing side effects. By optimizing therapeutic outcomes through more efficient drug delivery, these advancements have the potential to enhance the efficacy and appeal of BV therapy.

9 Toxicity of bee venom

Toxicology reports on BV have highlighted both its benefits and potential risks. Studies have documented adverse effects related to BV therapy, including hyperventilation, fatigue, appetite loss, extreme pain, increased bleeding risk, and vomiting (Jang and Kim, 2020; Lee et al., 2021). Furthermore, research has focused on the cellular toxicity assessment of BV microspheres in prostate cancer treatment, emphasizing the need for a thorough understanding of its impact on cellular health (El-Didamony et al., 2022a). Toxicological studies have delved into the hemolytic activity of BV in various species, such as horses, humans, sheep, and rabbits, shedding light on its cytotoxic effects. Additionally, investigations have explored the accidental ingestion of honeybee venom, emphasizing the importance of understanding the potential risks associated with exposure to BV components (de Roodt et al., 2020; Lee et al., 2021; Tanuwidjaja et al., 2021). The presence of compounds like melittin, phospholipase A2, and biogenic amines in BV has been a subject of interest, with melittin, in particular, being a major toxin known for its cytotoxic effects (Shi et al., 2022). While, BV therapy has shown promise in treating various health conditions, including inflammation and chronic illnesses, caution is warranted due to the potential for serious side effects. The use of BV products or undergoing BV therapy should be supervised by trained medical professionals to mitigate risks. Research has highlighted the dual nature of BV, with compounds like melittin, and apamin exhibiting anti-inflammatory and pain-relieving properties, but also carrying the risk of irritation and allergic reactions in sensitized individuals (Jang and Kim, 2020; Ullah et al., 2023).

10 Scaffolds based delivery for bee venom delivery

Biological preparations comprise substances from vegetation and animals; some of these products possess an extensive record of use, while others have been found in recent years (Giannenas et al., 2020). For instance, the therapeutic efficacy of natural

extracts and components has been hampered by a variety of issues, notably an absence of targeting capability and low bioavailability. Scientists recommended employing scaffolds infused with BV to address its disadvantages and boost its physiological and medicinal benefits (Atia GA. et al., 2023; Liu H. et al., 2023; Atia et al., 2024; El-Nablaway et al., 2024). Despite advances in cancer treatment, the illness remains the leading cause of death globally. BV has a variety of biological characteristics and has been shown *in vitro* to be cytotoxic against many cancer cell lines. However, its use in humans remains challenging because to allergic responses, discomfort at administration sites, and other serious toxic events including hemolysis, nonspecific cytotoxic effects, itching feeling, and hypersensitivity during therapeutic therapy. BV-loaded nanoliposomes (BV-NLs) were synthesized and characterized, and their anticancer efficacy was tested *in vitro* against the HepG-2, MCF-7, and HCT-116 cell lines. They outperformed raw BV in terms of anticancer efficacy against the 3 cell lines evaluated. It demonstrated preferential cytotoxicity and was more potent against the HCT-116 cell line, with an IC50 of 4.16 μg/mL. BV-NLs also regulate the mRNA transcription of apoptosis genes (Abd El-Gawad et al., 2023). Another study used the freeze-thawing process to create various formulations of polyvinyl alcohol (PVA) and chitosan (Ch) hydrogel wound dressings incorporating BV. It was more swollen, flexible, and elastic than the other formulations. Furthermore, it demonstrated anti-inflammatory properties equivalent to diclofenac gel, commonly used anti-inflammation medicine. Meanwhile, wound tissues coated with this mixture demonstrated greater hydroxyproline and glutathione amounts and decreased IL-6 levels relative to the control (Amin and Abdel-Raheem, 2014). The BV or its contents (e.g., melittin) were intensively explored as prospective therapy and antagonists of tumor types; they shown numerous prospective biological anticancer processes (Borojeni et al., 2020; Lebel et al., 2021; Zein et al., 2024). Nanotechnology has become widely used in most medical disciplines, with nanomaterials being used as antimicrobial, cancer-fighting, or loaded with biologically active drugs/compounds that improve their solubility, stability, performance, and transport to the human body (Alkhursani et al., 2022; El-Nablaway et al., 2024). In this line, nano chitosan produced from Fusarium oxysporum-grown mycelia had been utilized to transport BV. The *in vitro* antitumor capacity evaluation against HeLa cervical carcinoma indicated that they all displayed significant dose-related cancer-fighting properties, with BV/NFC nanoconjugates being the most efficient. The fluorescent labeling of these cells demonstrated the presence of initial apoptosis, subsequent apoptosis, and additional necrosis indicators, and their increase with contact extension (Alalawy et al., 2020). Due to the increasing prevalence of adaptive human microorganisms across the world, antimicrobial resistance has become one of the most pressing medical concerns. Chitosan synthesized from dead bee exoskeletons laden with BV has previously been developed and evaluated against eight prevalent human infections (two fungal and six bacterial strains) as well as two cancer cell lines. It has increased antibacterial action against six prevalent human diseases, including Gram-positive and Gram-negative bacterial and fungal strains. Although hazardous at high doses, the nanoparticles demonstrated exceptional activity against the human colon cancer cell line (Caco2 ATCC ATP-37) and human liver cancer cell line (HepG2 ATCC HB-8065), eliminating around 72% of cancer cells (Sharaf et al., 2024b). In another work, BV-loaded chitosan nanoparticles (ChNPs) had weak anti-MERS-COV activity (SI = 4.6), whereas ChNPs had considerable anti-MERS-COV activity (SI = 8.6). Meanwhile. It showed significant anti-MERS-COV activity (SI = 12.1). Furthermore, the synthesized platform demonstrated better antibacterial action against both Gram-positive and Gram-negative bacteria when contrasted with ChNPs, BV, or the used experimental medication (Elnosary et al., 2023).

11 Conclusion

BV is a complex combination of compounds widely researched because of their biological effects. Proteins and peptides comprise most of its content, with additional molecules present in small amounts. Between its constituents, melittin is the most prevalent and researched constituent of BV, after which comes PLA2, an enzyme that is regarded (along with histamine) as the primary allergen of BV. Furthermore, additional investigation is needed, particularly on BV's less significant elements. Numerous physiological characteristics have been documented. However, a study has focused chiefly on anti-inflammation and immune-modulating benefits. However, further *in vitro* and *in vivo* research is required to understand the BV action mechanisms better. Due to its beneficial effects on certain conditions, including musculoskeletal and neurological ailments, BV's primary applications are therapeutic. Nowadays, attempts are being made to develop safer dosages and methods and new trends like novel delivery technologies to reduce undesirable effects. In addition, the study advances the comprehension of the pathways of BV ingredients and evaluates their medicinal applicability.

Author contributions

KS: Conceptualization, Software, Visualization, Writing–original draft, Writing–review and editing. NS: Conceptualization, Software, Visualization, Writing–original draft, Writing–review and editing. ET: Writing–original draft, Writing–review and editing. FR: Writing–original draft, Writing–review and editing. MS: Conceptualization, Software, Visualization, Writing–original draft, Writing–review and editing. GA: Software, Visualization, Writing–original draft, Writing–review and editing. NT: Writing–original draft, Writing–review and editing, Software, Visualization. ME-N: Writing–original draft, Writing–review and editing. AI: Writing–original draft, Writing–review and editing. MR: Writing–original draft, Writing–review and editing. AfA: Writing–original draft, Writing–review and editing. MA: Writing–original draft, Writing–review and editing. II: Writing–original draft, Writing–review and editing, Software. EP: Writing–original draft, Writing–review and editing. LA: Data

curation, Visualization, Software, Writing–review and editing. AhA: Conceptualization, Writing–original draft, Writing–review and editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This work is supported via Prince Sattam bin Abdulaziz University project number (PSAU/2023/R/1445), Al-Kharj, Saudi Arabia. This research is also funded partially by Zarqa University-Jordan and Mutah University, Jordan. The project 6PFE of the University of Life Sciences "King Mihai I" from Timisoara and Research Institute for Biosecurity and Bioengineering from Timisoara is also appreciated. The authors would also like to thank AlMaarefa University, Riydah, Saudi Arabia for supporting this research.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Glossary

ACHE	Acetylcholinesterase
AD	Alzheimer's disease
ALF	Acute liver failure
ALS	Amyotrophic lateral sclerosis
ALS	Amyotrophic lateral sclerosis
ALT	Alanine aminotransferase
Amps	Antimicrobial peptides
AOA	Antioxidant activity
AST	Aspartate transaminase
ATF3	Activating transcription factor 3
BV	Bee venom
BV-nls	BV-loaded nanoliposomes
Bvpla2	Bee venom phosphao lipase A2
Camp	Cyclic adenosine monophosphate
C-AMP	Cyclic adenosine monophosphate
CDK	Cyclin-dependent kinase
C-GMP	Cyclic guanosine monophosphate
Ch	Chitosan
ChNPs	Chitosan nanoparticles
CIA	Collagen-induced arthritis
CK-MB	Creatine Kinase MB
CLD	Chronic liver disease
COX-2	Cyclooxygenase-2
CTNI	Cardiac Troponin I
DM	Diabetes mellitus
DPIV	Dipeptidyl peptidase IV
DSNS	Dual-secured nano-sting
EAE	Experimental autoimmune encephalomyelitis
ECM	Extracellular matrix
Foxp3	Forkhead box P3
GSH	Glutathione
GST	Glutathione S-transferase
HCC	Hepatocellular carcinoma cells
HCV	Hepatitis C virus
HFD	High-fat diet
HIF-1α	Hypoxia-inducible factor 1-alpha
HIV	Human immunodeficiency virus
HPV	Papillomaviruse
Hscs	Hepatic stellate cells
HSV	Herpes simplex virus
IFN-γ	Interferon-gamma
IL-8	Interleukin-8
iNOS	Inducible nitric oxide synthase
LDH	Lactate Dehydrogenase
LPS	Lipopolysaccharides
MAPK	Mitogen-activated protein kinase
MCD	Mast cell degranulating
MDA	Malondialdehyde
MEL	Melittin
MIC	Minimum inhibitory concentration
MMPS	Matrix metalloproteinases
NAD^+	Nicotinamide adenine dinucleotide
NAFL	Nonalcoholic fatty liver
NF-κB	Nuclear factor kappa B
NO	Nitric oxide
PDGF-BB	Platelet derived growth factor-BB
PI3Ks	Phosphoinositide 3-kinases
PLA2	Phospholipase A2
PVA	Polyvinyl alcohol
RA	Rheumatoid arthritis
RANKL	Receptor activator of nuclear factor kappa-B ligand
RLPP	Recalcitrant localized plaque psoriasis
RNA	Ribonucleic acid
SK	Small-conductance calcium-activated potassium
SOD	Superoxide dismutase
STAT3	Signal transducer and activator of transcription 3
TAC	Total antioxidant capacity
TBARS	Thiobarbituric acid–reactive substances
TGF-β	Transforming growth factor beta
Th cells	T helper cells
TLR4	Toll-like receptor 4
TMDs	Tempormandibular disorders
TMZ	Temozolomide
TNF-α	Tumor necrosis factor-α
Tregs	Regulatory T-cells
UCF	Unicellular fungal
VEGF	Vascular endothelial growth factor

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TYPE Review
PUBLISHED 22 July 2024
DOI 10.3389/fphar.2024.1435524

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RECEIVED 20 May 2024
ACCEPTED 02 July 2024
PUBLISHED 22 July 2024

CITATION
Qian Q, Pan J, Yang J, Wang R, Luo K, Wu Z, Ma S, Wang Y, Li M and Gao Y (2024), Syringin: a naturally occurring compound with medicinal properties.
Front. Pharmacol. 15:1435524.
doi: 10.3389/fphar.2024.1435524

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Syringin: a naturally occurring compound with medicinal properties

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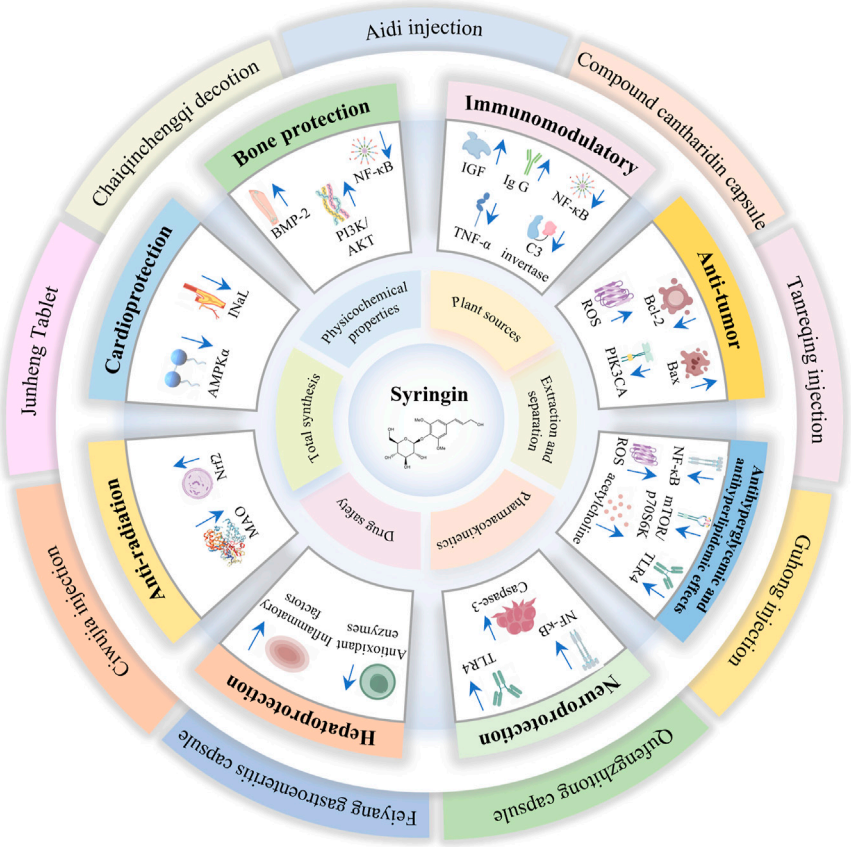
Syringin, a phenylpropanoid glycoside, is widely distributed in various plants, such as *Acanthopanax senticosus* (Rupr. et Maxim.) Harms, *Syringa reticulata* (BL) Hara var. mandshurica (Maxim.) Hara, and *Ilex rotunda* Thumb. It serves as the main ingredient in numerous listed medicines, health products, and foods with immunomodulatory, anti-tumor, antihyperglycemic, and antihyperlipidemic effects. This review aims to systematically summarize syringin, including its physicochemical properties, plant sources, extraction and separation methods, total synthesis approaches, pharmacological activities, drug safety profiles, and preparations and applications. It will also cover the pharmacokinetics of syringin, followed by suggestions for future application prospects. The information on syringin was obtained from internationally recognized scientific databases through the Internet (PubMed, CNKI, Google Scholar, Baidu Scholar, Web of Science, Medline Plus, ACS Elsevier, and Flora of China) and libraries. Syringin, extraction and separation, pharmacological activities, preparations and applications, and pharmacokinetics were chosen as the keywords. According to statistics, syringin can be found in 23 families more than 60 genera, and over 100 species of plants. As a key component in many Chinese herbal medicines, syringin holds significant research value due to its unique sinapyl alcohol structure. Its diverse pharmacological effects include immunomodulatory activity, tumor suppression, hypoglycemic action, and hypolipidemic effects. Additionally, it has been shown to provide neuroprotection, liver protection, radiation protection, cardioprotection, and bone protection. Related preparations such as Aidi injection, compound cantharidin capsule, and Tanreqing injection have been widely used in clinical settings. Other studies on syringin such as extraction and isolation, total synthesis, safety profile assessment, and pharmacokinetics have also made progress. It is crucial for medical research to deeply explore its mechanism of action, especially regarding immunity and tumor therapy. Meanwhile, more robust support is needed to improve the utilization of plant resources and to develop extraction means adapted to the needs of industrial biochemistry to further promote economic development while protecting people's health.

KEYWORDS

syringin, extraction and separation, pharmacological activities, preparations and applications, pharmacokinetics



GRAPHICAL ABSTRACT

1 Introduction

Syringin (E−4-3-Hydroxy-1-propenyl-2, 6-dimethoxyphenyl-*β*-D-glucopyranoside; also known as Eleutheroside B) is a phenylpropanoid glycoside extracted from the roots of *A. senticosus* (Rupr. et Maxim.) Harms, a medicinal plant species traditionally used as folk medicine in Russia, China, Korea, and Japan (Davydov and Krikorian, 2000). Previous research has demonstrated that syringin possesses broad pharmacological effects, including immunomodulatory properties (Singh et al., 2023), anti-tumor activity (Wang et al., 2008), antihyperglycemic and antihyperlipidemic effects (Niu et al., 2007), neuroprotection (Tan et al., 2021), and hepatoprotection (Jiang et al., 2019) et al. Additionally, it meets all the criteria according to *Lipinski's rule of five*. As all the values were within the acceptable range, it is evident that syringin can be considered a potential drug-like molecule (Ahmed et al., 2021). Furthermore, it serves as the main ingredient in various marketed drugs, health products, and foods such as Aidi injection (Yang et al., 2022), Compound cantharidin capsule (Sun et al., 2017), and Tanreqing injection (Wang L. et al., 2021).

Despite its incredibly high and comprehensive utilization values, syringin has not yet been fully explored. Lack of clarity regarding its action mechanism impedes further development and application of syringin-related formulations. It is essential to explore plant resources for syringin, enhance its resource utilization, and elucidate its pharmacological mechanism to promote the development and clinical application of products containing syringin. Thus, there is a need for comprehensive analysis to develop its potential further.

This article systematically reviews the physicochemical properties, plant sources, extraction and separation, total synthesis, pharmacological activities, drug safety, preparations and applications, and pharmacokinetics of syringin. The information on syringin was obtained from internationally recognized scientific databases through the Internet (PubMed, CNKI, Google Scholar, Baidu Scholar, Web of Science, Medline Plus, ACS Elsevier, and Flora of China) and libraries. Syringin, extraction and separation, pharmacological activities, preparations and applications, and pharmacokinetics were chosen as the keywords. The purpose is to provide valuable references for further expanding the research and application of this component.

2 Physicochemical properties

Syringin is a phenylpropanoid glycoside with a molecular formula of $C_{17}H_{24}O_9$, a relative molecular weight of 372.37, a density of 1.415 g cm^{-3}, and a melting point of 192°C. Its chemical structural formula is shown in Figure 1. At room temperature, syringin exists as colorless needle crystals and is soluble in hot water, ethanol, and methanol. It is slightly soluble

10.3389/fphar.2024.1435524

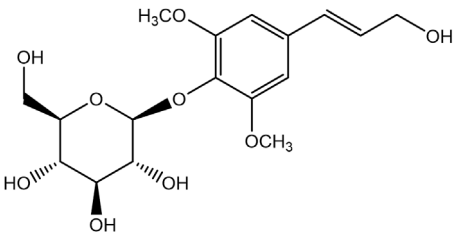


FIGURE 1
Structural formula of syringin.

TABLE 1 IR spectrum data of syringin.

NO.	Frequency (cm^{-1})	Vibration type, functional group
1	3,570	ms, —OH
2	3,385.3	vs., —OH
3	3,300–3,000	vw, —C=C—
4	2921.8	—OCH_3, —CH_2—, —C—
5	1584.9, 1508.5	ms,
6	1464.7	ms, —OCH_3, —CH_2
7	1418.3	ms, —CH_2OH, —CHOH
8	1241.7	ms, —OCH_3, —CH_2—, —CHOH
9	1133.2	ms, Ar—OCH_3, —CHOH,
10	964.9	ms, —C=C—
11	844.2	m, —glu
12	784.4	m, —glu—CH_2
13	630.3	m, —OH, —glu

TABLE 2 ^{13}C and ^{1}H NMR data of syringin.

Position	Li et al. (2018)		Noshita et al. (2021)	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1	6.78, s	135.9	6.74, s	135.8
2		154.4		154.4
3		105.5		105.5
4		135.3		135.3
5	6.78, s	105.5	6.74, s	105.5
6		154.4		154.4
7	6.58, dt (15.9,1.6)	131.3	6.54, d (15.9)	131.3
8	6.35, dt (15.9,5.6)	130.0	6.32, dt (15.8,5.6)	130.0
9	4.25, dd (5.6,1.6)	63.6	4.21, dd (5.5,1.1)	63.6
-OCH3	3.89, s	57.0	3.85, s	57.0
1′	4.90, d (7.5)	105.4	4.85, d (7.6)	105.4
2′	3.41–3.54 (overlap)	75.7	3.51–3.37, m	75.7
3′	3.41–3.54 (overlap)	78.4	3.51–3.37, m	78.4
4′	3.41–3.54 (overlap)	71.4	3.51–3.37, m	71.3
5′	3.24, m	77.8	3.24–3.15, m	77.8
6′a	3.81, dd (11.9, 2.4)	62.6	3.77, dd (12.0, 2.3)	62.6
6′b	3.69, dd (11.9, 5.2)		3.65, dd (12.0, 5.1)	

in cold water and acetone but insoluble in benzene, chloroform, and ether. When colored with 10% ethanol sulfate upon thin-layer inspection, syringin appears as purple spots (Mahadeva et al., 2015). The ultraviolet (UV) spectrum (MeOH) shows λ max (log ϵ) at 221 (4.50) nm and 266 (4.19) nm (Min et al., 2004). The infrared ray (IR) spectrum data for syringin can be found in Table 1. However, it should be noted that the data is outdated and may require updating by relevant researchers. Furthermore, Table 2 presents the mass spectrometry data for syringin for reference purposes.

3 Plant sources

Syringin is a bioactive compound isolated from numerous pharmacologically essential plant species. According to statistics (Wang F. et al., 2021), syringin is found in 23 families, over 60 genera, and over 100 plant species, some of which are listed in Table 3. The data indicates that the content of syringin is relatively high in the phloem of *S. reticulata* (BL) Hara var. mandshurica (Maxim.) Hara and *I. rotunda* Thumb (Figure 2).

Among these plants, *A. senticosus* (Rupr. et Maxim.) Harms, also known as Siberian ginseng, is a medicinal and edible plant with multiple biological activities. This popular medicinal plant is widely used in China and Russia and is one of the top ten herbal dietary supplements in the United States (Davydov and Krikorian, 2000). Its functions and indications have been recorded in the *Compendium of Materia Medica* and the *Records of Famous Doctors* throughout various dynasties. *Acanthopanax senticosus* (Rupr. et Maxim.) Harms is warm, spicy, slightly bitter, non-toxic, and belongs to the spleen and kidney meridians. It is commonly used in traditional Chinese medicine for symptoms such as heart and body deficiency, spleen and kidney yang deficiency, insomnia, and excessive dreaming (Chen et al., 2024). Due to its significant health benefits, *A. senticosus* (Rupr. et Maxim.) Harms have been processed into herbal tea and capsules for daily use (Hu et al., 2024). According to the United States Pharmacopoeia (USP) and the European Pharmacopoeia standards (Guo et al., 2014), one of the quality requirements is that the content of syringin should be more than 0.8%.

4 Extraction and separation

The extraction, separation, and purification processes of syringin are complex and costly, resulting in low yields that limit its application in medicine and clinics.

TABLE 3 Some plant resources with syringin.

NO.	Family	Genera	Species	Part and content
1	Oleaceae Hoffmanns. and Link	S. Linn	*S. reticulata* (BL) Hara var. mandshurica (Maxim.) Hara	Stem bark (3.59%), stem xylem (0.10%), root (0.42%) (Zhang et al., 2010a)
2			*S. dilatata*	Stem bark (1.53%), stem xylem (0.19%) (Mun and Mun, 2015)
3			*S. Pubescens* Turcz	Stem bark (1.15%) (Xu, 2021)
4	Aquifoliaceae	I. L	*I. rotunda* Thumb	Stem bark (3.53%), leaves (0.42%), fruits (0.40%) (Wang et al., 2016)
5	Crassulaceae J. St.-Hil	Rhodiola L	*Rhodiola crenulate* (Hook.f.et Thoms.) H. Ohba	Root and rhizome (3.02%) (Yang, 2016)
6	Cornaceae Bercht. and J. Presl	Toricellia DC.	*Toricellia Angulata* Oliv. var. intermedie (Harms) Hu	Root phloem (0.56%) (Han et al., 2017)
7	Araliaceae Juss	Eleutherococcus Maxim	*A. senticosus* (Rupr.et Maxim.) Harms	Stem (0.32%) (Zhang et al., 2010b)
8		Aralia L	*Eleutherococcus sessiliflorus* (Rupr. and Maxim.) S.Y. Hu	Stem (0.08%) (Zhang et al., 2010b)
9		Lysionotus D. Don	*Aralia decaisneana* Hance	Root phloem (0.17%) (Zhou et al., 2013)
10			*Acanthopanar gracilistylus* W. W. Smith	Root phloem (0.09%) (Wang et al., 2011)
11	Thymelaeaceae Juss	Daphne Linn	*Daphne tangutica* Maxim	Whole-plant (0.25%) (Wang and Li, 2018)
12		Edgeworthia Meisn	*Edgworthia chrysantha*	Whole-plant (0.18%) (Tang and Tang, 2014)
13	Buxaceae Dumort	Buxus L	*Buxus microphylla Sieb.*et Zucc. var. sinica Rehd.et Wils	Thick stem (0.03%), twig (0.05%) (Yang et al., 2017)
14	Asteraceae Bercht. and J. Presl	Saussurea DC.	*Saussurea involucrate* (Kar.et Kir.) Sch.Bip	Aboveground (0.06%) (Zhai et al., 2008)
15	Eucommiaceae	Eucommia Oliv	*Eucommia ulmoides* Oliv	leaves (0.05%) (Zhang et al., 2019)
16	Magnoliaceae Juss	Magnolia L	*Magnolia officinalis* Rehd.et Wils	leaves (0.04%) (Wu et al., 2018)
17	Campanulaceae Juss	Codonopsis Wall	*Codonopsis tangshen* Oliv	Root (0.04%) (Hu, 2017)
18	Loranthaceae Juss	Viscum L	*Vhcum coloratum* (Komar.) Nakai	Leafy stem branch (0.02%) (Gao et al., 2018)

He (2022) utilized ethanol reflux extraction to extract syringin from *A. senticosus* (Rupr. et Maxim.) Harms. The optimal extraction conditions included a 75% ethanol concentration, an extraction time of 1.5 h, and petroleum ether, chloroform, and ethyl acetate for extraction, followed by silica gel column chromatography for purification and isolation. The purity of the extracted syringin was determined to be 95.19%, providing a solid research foundation for potential industrial applications.

Duan et al. (2014) developed a method for preparing syringin from the *I. rotunda* Thumb. The process involves the following steps: 1) Crushing *I. rotunda* Thumb to 20–50 mesh, extracting it with water, and concentrating it by centrifugation to obtain the upper column solution; 2) Passing the upper column solution through a resin column and eluting it with water and 10%–40% ethanol (v/v). The eluates were collected in sections; 3) Concentrating the eluted portion of 10%–40% ethanol (v/v) by membrane separation and obtaining the product by spray drying. The method is simple, feasible, cost-effective, and suitable for industrial production.

Yan et al. (2004) successfully isolated syringin from the n-butanol extract of the stems and barks of *Edgeworthia chrysantha* Lindl for the first time. The extraction process involved a two-phase solvent system consisting of ethyl acetate-ethanol-water at a volume ratio of 15:1:15 (v/v/v), which was optimized for this study. Preparative high-speed counter-current chromatography isolated 28 mg of syringin with over 96% purity from 110 mg of the partially purified extract, as confirmed by high-performance liquid chromatography (HPLC) analysis.

Ethanol reflux extraction is commonly utilized in laboratory research because it effectively extracts various chemical components. However, the properties of different compounds vary, leading to a generally low extraction rate as the optimal conditions for each compound cannot be precisely defined. The primary goal of researching the extraction of Chinese medicinal materials or monomer compounds is to facilitate their clinical use. The extraction and separation technology for the syringin monomer still needs further development, emphasizing the necessity to enhance extraction methods to improve its pharmacological effects.

5 Total synthesis

Due to the significant pharmacological effects of syringin and its potential for clinical application, as well as the need for further

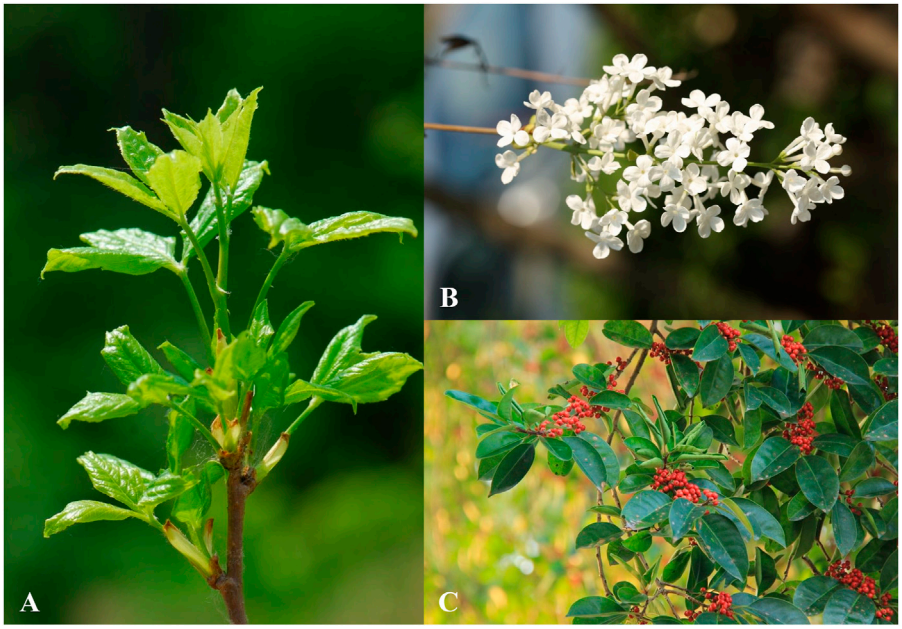


FIGURE 2
(A) *A. senticosus* (Rupr. et Maxim.) Harms; **(B)** *S. reticulata* (BL) Hara var. mandshurica (Maxim.) Hara; **(C)** *I. rotunda* Thumb. (The copyright of these pictures was obtained from https://www.iplant.cn/frps/vol).

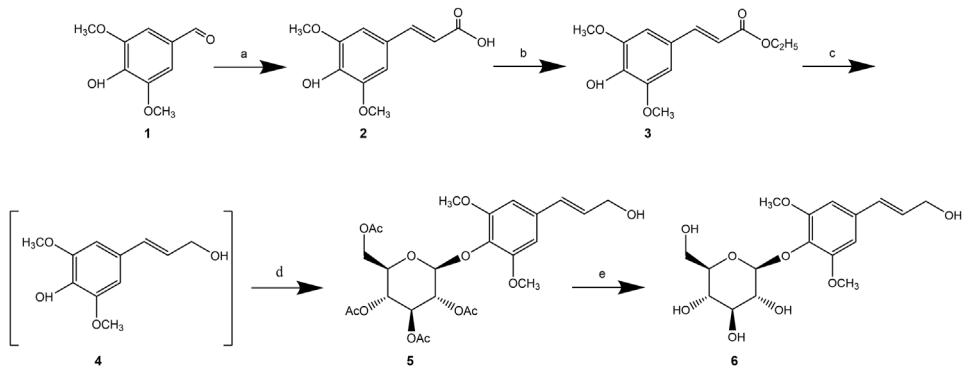


FIGURE 3
Synthetic route of syringin (Dong et al., 2021).

development in extraction and isolation methods, the synthesis process holds crucial research value in medicinal chemistry.

For instance, Dong et al. (Dong et al., 2021) described a 5-step synthesis of syringin using Doebner-Knoevenagel condensation of syringaldehyde with malonic acid as the key step to form the α, β-unsaturated carboxylic acid. The final yield of syringin was reported to be 54% (Figure 3).

Additionally, Wang et al. (2023) synthesized natural product syringin from commercially available starting materials in five steps with an overall yield of 58%. The palladium-catalyzed C(O)-C bond activation of aryl ketone was identified as a crucial step in the total synthesis process. Furthermore, various syringin analogues bearing alkynyl, alkenyl, aryl, alkyl, siliconyl, and boronyl groups were also constructed via the aryl palladium intermediate (Figure 4).

6 Pharmacological activities

Syringin exhibits significant pharmacological effects, including immunomodulatory, anti-tumor, antihyperglycemic, and antihyperlipidemic effects. It also provides neuroprotection, hepatoprotection, anti-radiation, cardioprotection, and bone protection. These properties suggest the potential for syringin to be developed into drugs and health products (Table 4). Its mechanism of action is primarily associated with the elimination of oxidative free radicals, enhancement of antioxidant enzyme activity, inhibition of inflammatory signaling pathways activation, and suppression of inflammatory factor production (Shen et al., 2020; Dai et al., 2021; Tan et al., 2021).

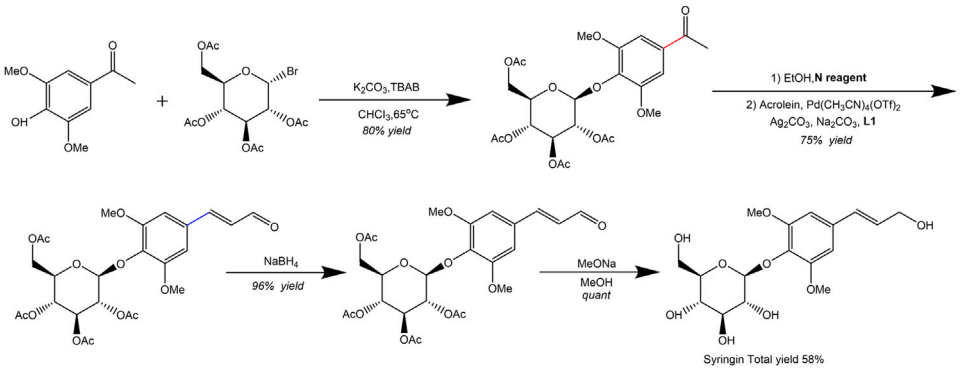


FIGURE 4
Synthetic route of syringin (Wang et al., 2023).

6.1 Immunomodulatory

The primary function of the immune system is to recognize and eliminate microorganisms, foreign cells, or macromolecules that invade the body, as well as to remove cells with altered surface antigens to protect the body from harm. When immune cells identify a foreign body and migrate to the damaged area, they initiate an inflammatory response to eliminate the foreign body and promote tissue repair. Developing immune-enhancing agents with fewer adverse effects and lower costs holds excellent promise. Syringin has been identified as an effective natural immunostimulant for ameliorating immune-related diseases (Singh et al., 2023).

Research has indicated that syringin can inhibit the classical activation pathway of complement C3 convertase and *in vitro* immune hemolysis of sheep red blood cells coated with guinea pig serum antibodies. It also demonstrates a dose-dependent effect. Additionally, syringin can significantly increase the content of immunoglobulin G in serum and activate macrophages, thereby exerting further immune regulatory effects (Kapil and Sharma, 1997). In a study by Dai et al. (2021), a mouse asthma model was established, revealing that syringin can inhibit airway inflammation and the onset of asthma, suggesting its potential anti-inflammatory effects. Furthermore, it can reduce levels of peroxidation products such as malondialdehyde (MDA) and myeloperoxidase, thus preventing additional damage to inflammatory tissues.

Zhang et al. (2020) discovered that syringin has the potential to exert anti-inflammatory effects by inhibiting the overproduction of interleukin-1β (IL-1β), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and cyclooxygenase-2 (COX-2) induced by dextran sulfate sodium (DSS) or lipopolysaccharide (LPS). Additionally, it was found to hinder the nuclear factor kappa-B (NF-κB) p65 pathway by reducing the phosphorylation of the NF-κB inhibitor alpha (IκBα) Ser 32 site and promoting the activation of antioxidant nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway, thereby exerting protective effects against intestinal inflammation. Syringin also dose-dependently inhibited the proliferation of mouse leukemia cells of monocyte macrophage (RAW264.7), TNF-a production, and mouse T cell CTLL-2 stimulated by LPS, demonstrating an antiallergic effect. However, high concentrations of syringin did not inhibit either NO or CD4 T cell proliferation (Cho et al., 2001). Studies have shown that syringin may attenuate LPS/D-galactosamine (D-GalN)-induced fulminant liver failure (FHF) by inhibiting NF-κB activation and reducing TNF-α production (Gong et al., 2014). It was claimed that syringin increased intestinal barrier function, tight junction protein expression, and anti-inflammatory cytokines while decreasing pro-inflammatory cytokine synthesis in porcine intestinal epithelial (IPEC-J2) cells (Che et al., 2019). Furthermore, it has been suggested that c-Jun N-terminal kinases 3 may be a key target for the anti-inflammatory properties of syringin (Geng et al., 2022). Syringin is expected to be developed as a COX-2 selective inhibitor for treating rheumatoid arthritis (RA) and osteoarthritis (OA) (Chen Q. et al., 2021).

These studies have demonstrated that syringin exhibits immunomodulatory effects primarily through the activation of cytokines, downregulation of the NF-κB signaling pathway, and upregulation of the Nrf2 and insulin-like growth factor signaling pathways (Figure 5). These findings suggest that syringin holds excellent potential for treating inflammation-related disorders and enhancing macrophage phagocytosis.

6.2 Anti-tumor

Currently, chemotherapeutic drugs used for treating tumors often lead to severe adverse reactions. This has led to a new trend in extracting effective anti-tumor natural ingredients from traditional Chinese medicine. Syringin, in particular, has shown promising anti-tumor effects (Figure 6). Further investigation into the related molecular mechanism can provide a solid theoretical foundation for the development of clinically targeted eugenol drugs (Wang F. et al., 2021).

Syringin exhibited significant inhibitory effects on human cervical cancer (HeLa) cells, human breast cancer (MCF-7) cells, human non-small cell lung cancer (A549) cells, and human prostate cancer (PC-3) cells, with an apparent quantity-efficacy relationship observed in all cases. Furthermore, experiments conducted on tumor-bearing mice demonstrated that syringin could effectively suppress the growth of solid tumors, with tumor inhibition rates of 61.16% and 25.89% for the high and low-dose groups, respectively

TABLE 4 Pharmacological activities of syringin.

Pharmacological activities	Subjects	Syringin doses	Mechanism of action	Ref.
Immunomodulatory	Croton oil-, arachidonic acid- and fluorescein-isothiocynate -induced mouse ear oedema model; LPS-stimulated RAW264.7 cells	Syringin (0.7, 7, 70, and 700 mg/kg); (62.5, 125, 250, 500, and 1000 μM)	Inhibited TNF-α production and cytotoxic T cell proliferation	Cho et al. (2001)
	Female BALB/c mice	Syringin (25, 50, and 100 mg/kg)	Improved asthma symptoms in ovalbumin-induced mice by modulating NF-κB pathway activation	Dai et al. (2021)
	DSS-induced colitis model; LPS-induced rat small intestine crypt epithelial (IEC6) cells	Syringin (100 mg/kg); Syringin (12.5 and 25 μg/mL)	Inhibited NF-κB and activated the Nrf2 signaling pathway in colitis	Zhang et al. (2020)
	LPS and D-GalN-induced FHF in mice	Syringin (10, 30, and 100 mg/kg)	Inhibited NF-κB activation and reduced TNF-α production	Gong et al. (2014)
	IPEC-J2 cells	Syringin (0, 0.05, 0.10, and 0.20 mg/mL)	Decreased cellular membrane permeability and mRNA expression of proinflammatory cytokines, increased tight junction protein expression and anti-inflammatory cytokines	Che et al. (2019)
	Rat adjuvant-induced arthritis and anterior cruciate ligament transection-induced OA rats	Syringin (50, 100, and 200 mg/kg)	Selectively inhibited COX-2	Chen et al. (2021b)
Anti-tumor	HGC-27 cells	Syringin (90, 180, and 360 μM)	Induced apoptosis by upregulating Bax protein expression and downregulating Bcl-2 protein expression. Inhibited tumor cell proliferation by arresting the cell cycle in the G0/G1 phase	He (2022)
	S180 tumor-bearing mice	Syringin (200 and 400 mg/kg)	Inhibited the growth of solid tumors, and the tumor inhibition rates were 61.16% and 25.89%, respectively	Wang et al. (2010b)
	Human breast cancer (MDA-MB-231 and MCF-7) cells	Syringin (0, 20, 40, 80, 160, and 320 μg/mL)	Against BC via PI3K-AKT-PTGS2 and EGFR-reticular activating system-Recombinant C-Raf Proto Oncogene Serine - mitogen-activated protein-extracellular regulated protein kinases (RAS-RAF-MEK-ERK) pathways	Wang et al. (2022a)
	AuNP modified by bxpc3 pancreatic cancer (BxPC3) and human hepatocellular carcinoma (Huh7) cells	G. diversifolia methanolic extract	BxPC3 (IC50 = 12.5 μg/mL); Huh7 (IC50 = 7.2 ± 2.5 μg/mL)	Aventurado et al. (2020)
	Colon adenocarcinoma (Caco-2) cells	Syringin (10 μM)	Inhibited hCAIX and hXII.	Costa et al. (2020)
	Non-tumorigenic, tumorigenic and metastatic BC cell lines; mouse model of xenograft tumor	Syringin (1, 3, 10, 30, and 100 mM); (5 mg/kg)	Upregulated cyclin-dependent kinase inhibitor 1 A (p21), cleaved cysteinyl aspartate specific proteinase (caspase-3/caspases-9), and PARP but downregulated cyclin-dependent kinase 4 (CDK4) and X-linked inhibitor of apoptosis protein expression concomitant with the suppression of growth in breast carcinoma cells, also induced excessive ROS levels in BC cells	Lee et al. (2019)
Antihyperglycemic and antihyperlipidemic effects	Wistar rats	Syringin (100 g/kg)	Increased the ability of nerve endings to release acetylcholine, stimulated the M3 receptor in pancreatic cells, and increased insulin release, resulting in a hypoglycemic effect	Niu et al. (2007)
	High glucose-induced mouse podocytes	Syringin (2.5, 5, and 10 μM)	Inhibited activation of mTOR/70 kDa Ribosomal Protein S6 Kinase 2 (p70S6K) pathway	Yao et al. (2023)

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TABLE 4 (*Continued*) Pharmacological activities of syringin.

Pharmacological activities	Subjects	Syringin doses	Mechanism of action	Ref.
	Type-2 diabetic SD rats Rat myocardial (H9C2) cells treated with high glucose	Syringin (60 mg/kg) Syringin (15 μM)	Downregulated the TLR4/MyD88/NF-κB pathway and upregulated the peroxisome proliferator-activated receptor γ coactivator-1α (PGC1)/SIRT3 mitochondrial protection pathway	Yao et al. (2021)
	Conscious rats under chemical sympathectomy using an intraperitoneal injection of guanethidine	Syringin (100 μg/kg)	The hypoglycemic effect was related to the sympathetic nerve	Niu et al. (2008a)
	Mice fed on a high-fat diet	Syringin (5 mg/kg)	Through the production of adiponectin, it suppressed chronic inflammation and promoted the activation of skeletal muscle AMPK, thus reducing lipotoxicity and endoplasmic reticulum stress	Kim et al. (2017)
	Mouse embryonic fibroblast (3T3-L1) cells	Syringin (20 μM)	Enhanced the phosphorylation of AMP-activated protein kinase and acetyl-coenzyme A carboxylase, thus inhibiting adipocyte differentiation and adipogenesis and promoting fat metabolism	Hossin et al. (2021)
	Human breast cancer (MDA-MB-231 and MCF-7) cells	Syringin (10 μM); (6.8 μM)	Activated adiponectin receptor	Sun et al. (2013)
Neuroprotection	Human neuroblastoma cell (SK-N-SH and SK-N-BE) cells treated with amyloid β fragment Aβ (25-35)	Syringin (0, 5, 10, and 20 μM)	Enhanced the expression of miR-124-3p and decreased the level of BID.	Zhang et al. (2021a)
	The middle cerebral artery embolism model was established in SD rats	Syringin (5, 10, and 20 mg/kg)	Reduced rat cerebral ischemia damage and improved nerve function damage through the FOXO3α/NFκB axis	Tan et al. (2021)
	Cerebral ischemia/reperfusion injury in rats	Syringin (50 mg/kg)	Attenuated inflammation and brain injury in rats with ischemia-reperfusion injury by inhibiting the TLR4 receptor	Liu et al. (2022)
	Patch clamp-treated HEK-293 cells	Syringin (6.25, 25, and 100 μM)	Suppressed VRAC channel	Xu et al. (2023)
	Cortical neurons of rats injured by Ab (25-35)	Syringin (1 and 10 μM)	Ab-induced axonal and dendritic atrophy of cortical neurons in rats	Bai et al. (2011)
	Rat neuroblastoma cells treated with Aβ(25-35)	Syringin (25 μM)	Reduced the activity and expression of Caspase-3, reduced PARP, and made DNA fragmented	Yang et al. (2010)
	Human neuroblastoma (SH-SY5Y) cells treated with 6-OHDA	Syringin (2 and 4 μM)	Regulated miR-34α/SIRT1/BECN-1 axis to induce autophagy and prevent 6-OHDA-induced apoptosis and α-synuclein accumulation	Fu et al. (2023)
Hepatoprotection	HBV mice	Syringin (10 mg/mL)	Reduced liver oxidative stress, enhanced immunity, and prevented HBV replication	Jiang et al. (2019)
	Acute liver injury induced by LPS/D-GalN in mice	Syringin (25 and 50 mg/kg)	Inhibited the activation of NF- κB p65 protein in the liver, inhibited the production of inflammatory factors IL-6, IL-1β, TNF- α, and promoted the production of antioxidant enzymes in the liver	Hu et al. (2023)
	Acute liver injury model established by human hepatocyte (L-02) cells	Syringin + costunolide (10 + 40 μM)	Decreased the production of alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, MDA, and ROS, increased the activity of superoxide	Mao et al. (2023)

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TABLE 4 (*Continued*) Pharmacological activities of syringin.

Pharmacological activities	Subjects	Syringin doses	Mechanism of action	Ref.
			dismutase and catalase, decreased the expression of Caspase-3, 7, 9, NF- κB, TNF-α, Cyclin B, recombinant cyclin-dependent kinase 1 (CDK1), and protein kinase inhibitors kappa-B (PKI) κB. The anti-acute liver injury effect was induced by ras-related C3 botulinum toxin substrate 1 (Rac1)/Akt/NF- κB signal pathway	
Anti-radiation	Injury model of mice induced by low-dose radiation	Syringin (10 and 20 mg/kg)	Regulated the disorder of cytokines in the testis of mice, improved the level of oxidative stress in the testis, and restored the testosterone level in the testis of mice	Hu et al. (2023)
	Injury model of mice induced by low-dose radiation	Syringin (20 mg/kg)	Protected spleen and thymus damage	Mei (2023)
	A mouse model of brain injury induced by ^{60}Co-γ irradiation	Syringin (240 μg/kg)	Prevented damage to the learning and memory ability of irradiated mice, protected the neurons of irradiated mice, improved the antioxidant activity of irradiated mice, and changed the level of neurotransmitters in irradiated mice	Song et al. (2022)
Cardioprotection	Model of atrial fibrillation induced by sea anemone toxin II in rabbit atrial myocytes	Syringin (200 μM)	Inhibited cell electrophysiological activity	Zhang et al. (2021b)
	Aortic banding-induced cardiac hypertrophy in mice	Syringin (50 and 100 mg/kg)	Decreased the protein expression levels of recombinant autophagy-related protein 7 (ATG7), BECN1, prostacyclin (p62), and autophagy-related protein LC3 A/B (LC3A/B), and decreased the phosphorylation level of AMPKα	Li et al. (2017)
	Rat intestinal microorganism	Syringin (10 mg/mL)	Stimulated intestinal bacteria to produce short-chain fatty acids, thus further promoting the role of myocardial ischemia	Yu et al. (2022)
Bone protection	Fully differentiated human osteoblast (Hob) cells	Syringin (60, 80, and 100 μM)	Increased alkaline phosphatase activity, increased mineral sedimentation, and regulated autophagy and the BMP-2 signal pathway	Imtiyaz et al. (2020)
	Ovariectomized mice	Syringin (10, 20, and 40 mg/kg)	Prevented bone loss by TNF receptor-associated factor 6 (TRAF6)-mediated inhibition of NF-κB and stimulation of PI3K/AKT, subsequently increasing the recombinant osteoprotegerin/receptor activator of nuclear kappa-B (OPG/RANKL) ratio and inhibiting osteoclastogenesis, finally promoting bone formation	Liu et al. (2018)
	Osteoarthritis induced by transection of the anterior cruciate ligament in rats	Syringin (25, 50, and 100 mg/kg)	Decreased the levels of IL-6, TNF- α, and IL-1β in serum and joint lavage fluid; decreased the protein expression levels of matrix metalloproteinase-13 (MMP-13), Wnt1, and β-catenin	Qiao et al. (2022)

(Wang Z. et al., 2010). Additionally, syringin was found to effectively inhibit the proliferation of human gastric cancer (HGC-27) cells through a mechanism involving significant cytotoxicity. It induces apoptosis by up-regulating Bcl-2-associated X protein (Bax) protein expression while down-regulating B-cell lymphoma-2 (Bcl-2) protein expression and inhibiting cell proliferation by blocking the cell cycle at the G0/G1 phase (He, 2022). Studies have also indicated that syringin exerts inhibitory effects on HeLa cells by inducing apoptosis, blocking the G2/M cell cycle, and inhibiting cell migration (Xia, 2016). Wang F. et al. (2022) identified mitogen-

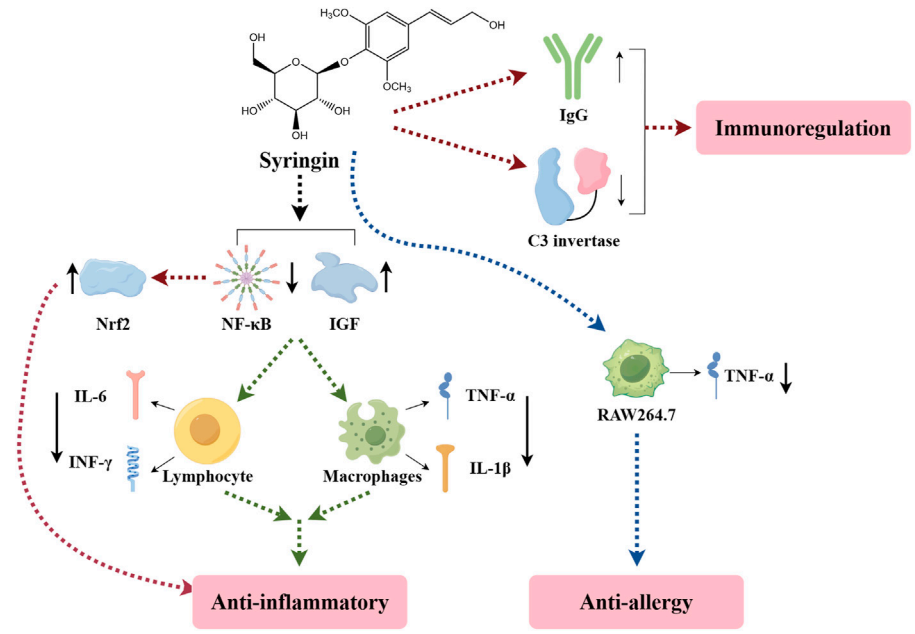

FIGURE 5
Mechanism of immunomodulatory effects of syringin.

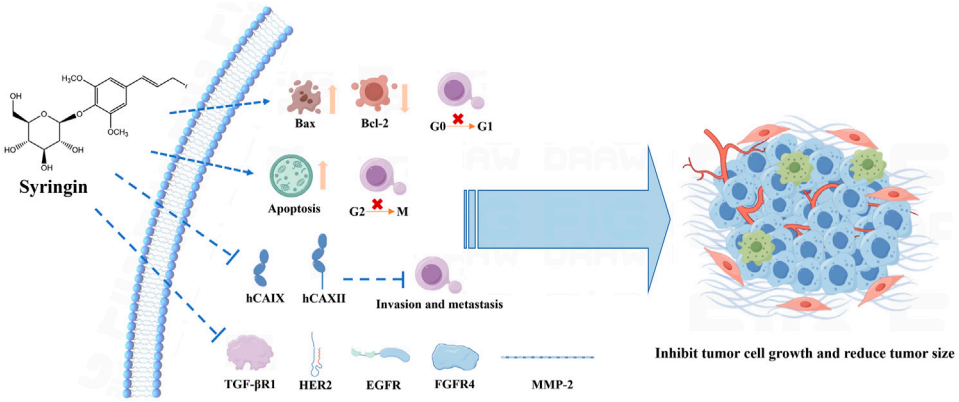

FIGURE 6
Mechanism of anti-tumor effects of syringin.

activated protein kinase 1 (MAP2K1), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA), recombinant V-Ha-Ras harvey rat sarcoma viral oncogene homolog (HRAS), epidermal growth factor receptor (EGFR), cysteinyl aspartate specific proteinase 3 (Caspase3), and prostaglandin-endoperoxide synthase 2 (PTGS2) as pivotal targets of butyroside anti-breast cancer (BC), suggesting that the PIK3CA-hsa-mir-139-5p-linc01278 and PIK3CA-hsa-mir-375 pathways may be closely related to the mechanism of syringin anti-BC. *In vitro* experiments confirmed that syringin inhibits BC cell proliferation and migration while promoting BC cell apoptosis through the above hub targets.

Angiogenesis plays a critical role in tumor growth and metastasis, making the inhibition of angiogenesis a promising approach for cancer therapy. Reverse molecular docking studies have identified syringin as a potential target for angiogenesis inhibition, which was further validated by a chorionic allantoic membrane assay. The study showed that the anti-angiogenic activity of syringin at concentrations of 100 μM and 200 μM was comparable to that of the positive control celecoxib at 200 μM (Aventurado et al., 2020). Furthermore, syringin has been found to inhibit angiogenesis regulatory enzymes such as transforming growth factor beta receptor 1 (TGF-βR1 kinase), human epidermal growth factor receptor 2 (HER2 kinase), EGFR kinase, fibroblast growth factor receptor 4 (FGFR4 kinase), and matrix metallopeptidase-2 (MMP-2) (Aventurado et al., 2023).

The chemical structure of syringin, a component of mustard alcohol, confers antioxidant activity, enabling it to combat free

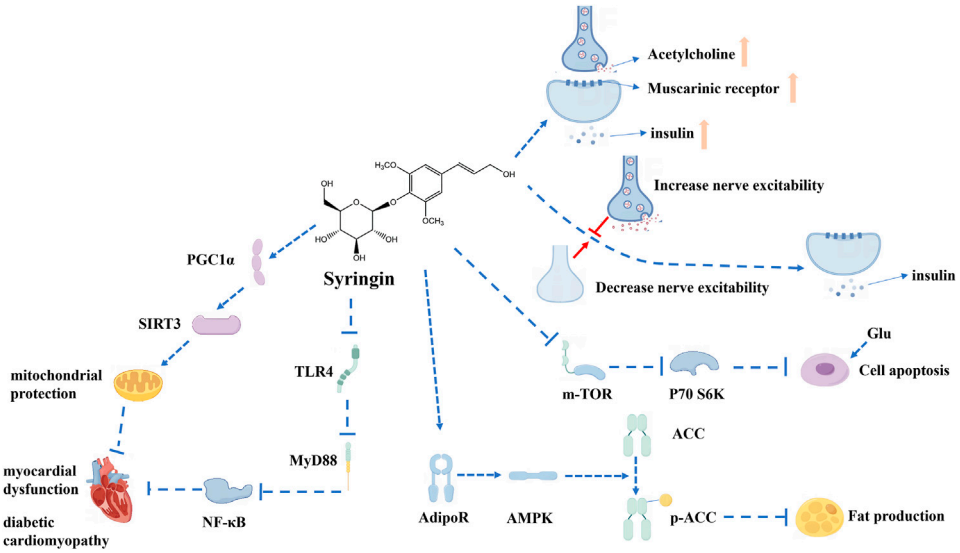

FIGURE 7
Mechanism of antihyperglycemic and antihyperlipidemic effects of syringin.

radical damage and exert antitumor effects. Specifically, carbonic anhydrase 9 (hCAIX) and carbonic anhydrase 2 (hCAXII) are crucial in acidifying the tumor environment and promoting tumor cell invasion and metastasis. Studies have demonstrated that syringin is a potent dual inhibitor of hCAIX and hCAXII, with the most promising outcomes associated with a phenol fraction (Costa et al., 2020). Furthermore, research indicates that syringin may potentially treat BC by increasing reactive oxygen species (ROS), inhibiting cancer cell growth, and significantly reducing tumor size (Lee et al., 2019).

6.3 Antihyperglycemic and antihyperlipidemic effects

In recent years, the antihyperglycemic and antihyperlipidemic effects of syringin have garnered increasing attention from scholars. Syringin has shown particular promise in the treatment of type 1 diabetes mellitus (Niu et al., 2007; Niu et al., 2008b; Liu et al., 2008; Shen et al., 2020) and type 2 diabetes mellitus (Kim et al., 2017; Yao et al., 2021; Deng et al., 2023) as well as in correcting specific haematological parameters (Us et al., 2020) (Figure 7).

Research has indicated that syringin enhances the release of acetylcholine from nerve endings, stimulating muscarinic M3 receptors in pancreatic cells and increasing insulin release (Niu et al., 2007). It has also been suggested that the inhibition of activation of the mammalian target of rapamycin/ribosomal protein S6 kinase (mTOR/p70SK) pathway by syringin in high glucose states may play a role (Yao et al., 2023). Additionally, it has been observed that increased sympathetic nerve activity in awake animals may interfere with the insulin-modulating effects of syringin, suggesting that decreasing sympathetic tone could contribute to its efficacy (Niu et al., 2008a). Furthermore, combination therapy with syringin and tilianin has been found to treat diabetic cardiomyopathy through interactions with toll-like receptor 4/myeloid differentiation primary response gene 88/NF-κB/nucleotide-binding oligomerization domain-like receptor family, pyrin domain-containing 3 (TLR4/MyD88/NF-κB/NLRP3) and human peroxisome proliferator-activated receptor gamma coactivator one alpha/recombinant sirtuin 3/ROS (PGC1α/SIRT3/ROS) signaling pathways (Yao et al., 2021). However, it is insufficient to account for the mechanism of syringin and further study is required.

Studies have demonstrated that syringin is one of the most active lipocalin receptor two agonists (Sun et al., 2013), inhibiting inflammation, lipotoxicity, endoplasmic reticulum stress, and reducing insulin resistance (Kim et al., 2017). Moreover, it effectively promotes phosphorylation of Adenosine 5'-monophosphate (AMP)--activated protein kinase and acetyl coenzyme A carboxylase, inhibiting adipogenesis and promoting lipid metabolism. Therefore, syringin has the potential to be applied as an anti-obesity drug (Hossin et al., 2021).

6.4 Neuroprotection

Neurodegenerative diseases result from the loss of neurons and their myelin sheaths, which deteriorate over time, leading to dysfunction. These diseases can be categorized into acute neurodegenerative diseases (such as cerebral ischemia and brain injury) and chronic neurodegenerative diseases (including Alzheimer's disease and Parkinson's disease). Research has demonstrated that syringin possesses specific neuroprotective effects.

Syringin has been found to exert a protective effect against brain ischemia/reperfusion injury by reducing inflammation associated with cerebral ischemia. This protective mechanism is regulated through the Forkhead box O3 (FOXO3α)/NF-κB pathway. (Tan et al., 2021). In experimental rats, syringin decreased both inflammation reaction and cerebral damage in cases of cerebral

ischemia/reperfusion injury. Furthermore, the neuroprotective properties of syringin may be linked to its inhibition of TLR4 (Liu et al., 2022). Additionally, syringin is considered an inhibitor of volume-regulated anion channel (VRAC). A study revealed that syringin moderately inhibited VRAC currents dose-dependently (Xu et al., 2023).

Bai et al. (2011) demonstrated that the ethyl acetate, n-butanol, and water fractions from the methanol extract of Siberian ginseng could protect against Aβ(25–35)-induced neuritic atrophy. Syringin was identified as one of the main active constituents responsible for this neuroprotective effect. It has been suggested that the mechanism involves reducing apoptosis, as evidenced by a decrease in caspase-3 activity and expression, reduction in cleaved poly ADP-ribose polymerase (PARP), and inhibition of deoxyribonucleic acid (DNA) fragmentation (Yang et al., 2010). Furthermore, syringin shows promise as a candidate agent for preventing and treating Parkinson's disease by inducing autophagy through partially regulating the microRNA-34 Alpha/Sirtuin 1/Beclin-1 (miR-34α/SIRT1/BECN1) axis to prevent 6-oxidopamine hydrobromide (6-OHDA)-induced apoptosis and α-synuclein accumulation (Fu et al., 2023). Additionally, syringin has been found to prevent Aβ25–35 induced neurotoxicity via the microRNA-124-3p/B-cell lymphoma-2 (Bcl-2) homology three interacting domain death agonist (miR-124-3p/BID) pathway, suggesting its potential for broadening the pharmacological treatment options for Alzheimer's disease (Zhang N. et al., 2021).

6.5 Hepatoprotection

The liver is crucial in maintaining physiological processes and is essential for life. It regulates blood volume, metabolizes nutrients, supports the immune system, maintains lipid and cholesterol balance, and breaks down exogenous compounds. Syringin is a potent antihepatotoxic drug that can restore enzyme activity in the microsomal enzyme system and inhibit lipid peroxidation. It promotes liver toxin metabolism and improves overall liver function.

Comparative metabolomics research has revealed that the antiviral effect of syringin in the hepatitis B (HBV) animal model is associated with arachidonic acid, citric acid, ornithine, L-lysine, L-glutamine, uric acid, pyruvic acid, and L-phenylalanine as potential therapeutic targets (Jiang et al., 2019). Studies have shown that syringin (25 and 50 mg/kg) has a protective and dose-dependent effect on LPS/D-GalN-induced liver injury in mice. This mechanism of action is related to its ability to increase antioxidant enzyme activity and inhibit inflammatory factor production (Hu et al., 2023). Additionally, syringin has been reported to provide potent protection against LPS/D-GalN-induced FHF by reducing lethality rates, alleviating hepatic pathological injury, inhibiting hepatocyte apoptosis, and reducing hepatic inflammatory responses. These protective effects are likely mediated by suppressing TNF-α production through NF-κB inhibition (Mao et al., 2023).

6.6 Anti-radiation

Radiation has the potential to cause extensive DNA damage in the human body, leading to severe harm to biological systems through cell death or the induction of mutations in surviving cells, which can ultimately result in cancer (Bugris et al., 2019). Research indicates that syringin exhibits a protective effect against radiation-induced damage.

Specifically, syringin has been shown to ameliorate cytokine regulation disorders induced by low-dose radiation 0.6 Gy (Gy) in mice and effectively reduce reproductive system damage, suggesting its potential as a protective agent against such damage caused by low-dose radiation exposure (Hu et al., 2023). Furthermore, syringin demonstrates a specific protective effect on spleen and thymus damage resulting from low-dose radiation exposure (1 Gy), potentially increasing Nrf2 levels via protein kinase B (AKT) and enhancing Nrf2-mediated antioxidant effects in mouse immune organs (Mei, 2023). In a study by Song et al. (Song et al., 2022), a brain injury model was generated using ^{60}Co-γ ray radiation at 4 Gy in mice. The findings revealed that syringin significantly improved radiation-induced cognitive dysfunction and decreased monoamine oxidase levels to prevent brain damage.

6.7 Cardioprotection

The incidence of heart disease has significantly increased in recent years, leading to high morbidity, mortality, recurrence, disability, and economic burden. Numerous researchers have focused on identifying the molecular mechanism of cardiac injury to develop new drugs for preventing heart failure. Syringin shows potential in the treatment of myocardial ischemia, and further research into its mechanism of action in cardiovascular disease will provide more options for patient care.

Studies have demonstrated that long-term oral administration of syringin attenuates the development of cardiac hypertrophy induced by pressure overload and improves cardiac functions. The protective effects of syringin may be attributed to its inhibition of adenosine-activated protein kinase α (AMPKα) and autophagy-related signaling pathways (Li et al., 2017). In Langendorff-perfused rabbit hearts, the application of syringin (200 μM) or tetrodotoxin (2 μM) substantially decreased incidences of atrial fibrillation (AF), ventricular fibrillation, and heart death induced by anemone toxin II. These results suggest that syringin exerts anti-AF actions mainly via blocking late sodium current, providing a basis for new clinical applications in pharmacology (Zhang P. P. et al., 2021). *In vitro* studies have shown that syringin (10 μg/mL) can significantly stimulate intestinal bacteria to produce short-chain fatty acids, which may contribute to the effect on myocardial ischemia. The impact on isovaleric acid production was particularly significant, with a 27.9% increase at 24 h; propionic acid concentration also increased by 9.5%. Butyric acid showed a lesser extent increase at 16.3% at 24 h, with no significant difference observed (Yu et al., 2022).

6.8 Bone protection

The process of bone metabolism involves the breakdown of old or mature bone (catabolism) and the formation of new bone (anabolism), which is carried out by osteoclasts and osteoblasts, respectively. Any disruption in this process can lead to a loss of bone

mass or osteoporosis. Syringin has been identified as a potent multi-targeted drug for bone formation against osteoporosis.

Research studies have demonstrated that syringin significantly increases alkaline phosphatase activity by 131.01% and enhances mineral deposition by 139.39%. It also regulates autophagy and the bone morphogenetic protein-2 (BMP-2) signaling pathways (Imtiyaz et al., 2020). Furthermore, syringin exhibits strong anti-osteoporosis effects in ovariectomized mice, with its underlying molecular mechanism possibly being the NF-κB and human phosphoinositide-3 kinase/protein kinase B (PI3K/AKT) signaling pathways (Liu et al., 2018). In addition, some scholars have found that syringin improves histopathological damage to articular cartilage in rats with anterior cruciate ligament transection-induced osteoarthritis by inhibiting inflammatory responses, degradation of the extracellular matrix, and activation of the proto-oncogene Wnt-1/recombinant beta-catenin (Wnt/β-catenin) signaling pathway (Qiao et al., 2022).

7 Drug safety

Toxicity studies on Chinese medicines and their main components provide a scientific basis for determining safety limits and taking preventive and control measures. In ancient books, herbs are classified into upper, middle, and lower grades. The upper grade is considered a life-extending, non-toxic medicine. *Acanthopanax senticosus* (Rupr. et Maxim.) Harms were first recorded in *Shennong's Classic of the Materia Medica* and classified as an upper-grade herb (Lee et al., 2019).

In this study, computational tools were used to predict the toxicity of syringin. The blockage of the human Ether-a-go-go-related gene (hERG) K+ channels is closely associated with lethal cardiac arrhythmia. The pred-hERG predicted syringin as non-cardiotoxic with a 50% confidence value. *General Unrestricted Structure-Activity Relationships* predicted the median lethal dose values for rats with four types of administration (oral, intravenous, intraperitoneal, subcutaneous, and inhalation): 0.923, 0.889, 0.503, and 1.208 mmol/kg, respectively. According to the acute rodent toxicity classification of chemicals by the *Organisation for Economic Co-operation Project*, syringin was predicted to be non-toxic for intravenous and subcutaneous routes of administration and categorized in toxicity class 5 for intraperitoneal and oral routes of administration. Regarding environmental toxicity, the predicted bioaccumulation factor is less than five, which indicates low environmental toxicity according to *Annex D of the Stockholm Convention* (Ahmed et al., 2021). Additionally, syringin is not predicted to be an inhibitor of cytochrome P450 (CYP), making it less likely to cause adverse drug reactions. However, it was reported as a weak inhibitor of CYP2C9 and CYP2E1 in rat liver microsomes, and it remains to be investigated whether the weak inhibitory effect on these two CYP isoforms is clinically relevant (Guo et al., 2014).

8 Preparations and applications

Syringin is a key component in numerous pharmaceuticals, nutraceuticals, and food products. It plays a crucial role in the treatment of cancer, cardiovascular and cerebrovascular diseases, inflammation, as well as liver and kidney deficiencies (Table 5) (Figure 8). However, there is currently limited analysis of the active ingredients in these formulations, making it difficult to determine the specific effects of syringin.

Aidi injection is among the Chinese medicinal injections approved by the *China Food and Drug Administration*. It has been assessed for its adjuvant beneficial effects on tumor survival, tumor response, quality of life, and reduction of radiotherapy side effects. This evaluation has focused on lung, liver, and colorectal cancers. Aidi injection has been recognized as one of the most competitive products in cancer therapy in China (Yang et al., 2022). The compound cantharidin capsule is primarily used for treating rectal cancer, lung cancer, malignant lymphoma, primary liver cancer, and gynecological malignancies (Sun et al., 2017). These two formulations utilize *M. phalerata* Pallas as the primary therapeutic agent to exert antidotal effects and dispel pathogenic factors. *Acanthopanax senticosus* (Rupr.et Maxim.) Harms addresses deficiencies while calming the spirit, fortifying the kidneys, and reinforcing essence. Syringin may modulate immunity, combat tumors, protect neurons, and safeguard liver and kidney function (Dai et al., 2024).

Guhong injection is a novel combination of Chinese and Western medicine, approved by the *State Food and Drug Administration of China* in 2003. It has been widely used in China to treat cerebrovascular diseases, especially ischemic stroke. Syringin is considered as one of the chemical compositions (Wang Y. et al., 2022). While syringin has demonstrated cardiovascular and neuroprotective effects, current research does not establish it as the primary active ingredient. Ciwujia injection is a brownish-red sterilized solution of *A. senticosus* (Rupr. et Maxim.) Harms extracted by the method of "hydrotropic alcohol precipitation". It is clinically used for transient ischemic attacks, cerebral arteriosclerosis, cerebral thrombosis, and cerebral embolism caused by liver and kidney insufficiency. It is also used for coronary heart disease, angina pectoris combined with neurasthenia, and menopausal syndrome (Fan et al., 2014). Syringin may be involved in hepatorenal protection, cardiovascular and cerebrovascular health, and immune regulation. However, there is still a lack of relevant studies to confirm these potential effects.

Tanreqing injection is widely utilized in clinical practice to manage respiratory disorders. Research has indicated that syringin is among the active compounds (Wang L. et al., 2021). Feiyang gastroenteritis capsule, a proprietary Chinese medicine, is extensively employed to treat bacterial dysentery and acute and chronic gastroenteritis. Studies have demonstrated that one of its primary components, syringin, possesses anti-inflammatory properties (Li et al., 2019). Chaiqinchengqi decoction has been an efficacious therapy for acute pancreatitis at West China Hospital for many years. The protective impact of syringin on pancreatic acinar cell death was found to be positively associated (Liang et al., 2021).

Junheng Tablet is a health food with syringin as its signature ingredient. According to Wang et al. (2020), syringin can effectively accelerate the removal of metabolic wastes from the body and maintain normal physiological function, thus delaying the onset of fatigue and promoting faster recovery of physical fitness.

TABLE 5 Traditional Chinese medicine preparations with syringin.

Product name	Ingredient	Indication	Execution standards	Ref.
Aidi injection	*Mylabris phalerata* Pallas, *Panax ginseng* C. A. Mey., *Astragalus membranaceus* (Fisch.) Bge. var. mongholicus (Bge.)Hsiao, *A. senticosus* (Rupr.et Maxim.) Harms, et al	Lung cancer, liver cancer, and colorectal cancer	WS3-B-3809-99-2002	Yang et al. (2022)
Compound cantharidin capsule	*M. phalerata* Pallas, *P. ginseng* C. A. Mey., *A. membranaceus* (Fisch.) Bge. var. mongholicus (Bge.) Hsiao, *A. senticosus* (Rupr. et Maxim.) Harms, et al	Rectal cancer, lung cancer, malignant lymphoma, primary liver cancer, gynecological malignant tumors	WS3-B-3272-98	Sun et al. (2017)
Tanreqing injection	*A. membranaceus* (Fisch.) Bge. var. mongholicus (Bge.) Hsiao, *Saiga tatarica* Linnaeus, *Lonicera japonica* Thunb., *Forsythia suspensa* (Thunb.) Vahl, et al	Respiratory system diseases	YBZ00912003-2007Z-2009-2012	Wang et al. (2021b)
Guhong injection	Aceglutamide, *Carthamus tinctorius* L	Cerebrovascular disease	WS-10001(HD-1506)-2004-2012	Wang et al. (2022b)
Qufengzhitong capsule	*Clematis chinensis* Osbeck, *Taxillus chinensis* (DC.) Danser, *Erodium stephanianum* Willd., *Angelica pubescens* Maxim.f. biserrata Shan et Yuan, *C. tinctorius* L	Neurogenic pain, aching lumbus, and knees	WS3-0049 (Z-049)-2001(Z)	Liao et al. (2022)
Feiyang gastroenteritis capsule	*Euphorbia hirta* L., *Polygonum chinense* L., *I. rotunda* Thunb	Bacterial dysentery and gastroenteritis	YBZ09622008	Li et al. (2019)
Ciwujia injection	*A. senticosus* (Rupr. et Maxim.) Harms	Liver and kidney deficiency, cardiovascular and cerebrovascular diseases	WS3-B-3425-98	Fan et al. (2014)
Junheng Tablet	*A. senticosus* (Rupr. et Maxim.) Harms, *Cistanche deserticola* Y. C. Ma, *P. quinquefolium* L., *Salvia miltiorrhiza* Bge	Anti-fatigue	-	Wang et al. (2020)
Chaiqinchengqi decoction	*L. japonica* Thunb. Fl. Jap., *Taraxacum mongolicum* Hand. -Mazz., *Bupleurum chinense* DC., *A. membranaceus* (Fisch.) Bge. var. mongholicus (Bge.) Hsiao, et al	Acute edematous pancreatitis	-	Liang et al. (2021)

The Qufengzhitong capsule is clinically utilized to treat neuropathic, lumbar, and knee pain. Syringin is identified as one of its seven active ingredients (Liao et al., 2022).

9 Pharmacokinetics

Syringin is rapidly eliminated *in vivo* and exhibits low bioavailability. In rats, the area under the curve ($AUC_{0\text{-}T}$) was (3.26 ± 0.60) μg/h/mL, with a plasma half-life time ($t_{1/2}$) of (0.41 ± 0.04) h and elimination rate constant (Ke) of (1.85 ± 0.16) h^{-1} (Li et al., 2007). Tan and Jia (2008) conducted *in vivo* intestinal perfusion experiments on rats and observed variations in drug absorption and corrected intestinal wall permeability of syringin across different intestinal segments, with the following order: duodenum > jejunum > ileum > colon. It was noted that syringin underwent degradation by intestinal enzymes, with the levels of degradation as follows: duodenum > jejunum > ileum > colon. Additionally, syringin was not detected in plasma and bile, suggesting potential metabolism by intestinal enzymes within the intestine, leading to low bioavailability. Yang et al. (2022) discovered that the rat intestinal flora plays a significant role in metabolizing syringin. They analyzed the incubation solution of rat intestinal flora isolated from 0 to 48 h with syringin, revealing that 81% of the syringin was metabolized within 12 h, with erucic alcohol identified as the primary metabolite. By 24 h, syringin was completely metabolized, and the primary metabolite was dextroseyl resinoid phenol; however, erucic alcohol could not be detected after 24 h. Fan et al. (2014) preliminary explored the co-existing component of p-syringin in Ciwujia injection and its pharmacokinetic effect. Male rats were intravenously administered with a syringin monomer and a corresponding dose of Ciwujia injection. The plasma syringin concentration was determined at different time points using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The $AUC_{0\text{-}\infty}$ values of syringin were (429.5 ± 25.6) and (721.0 ± 81.8) μg/h/L, respectively, and the plasma clearance (CL) of syringin was (3.3 ± 0.2) and (2.0 ± 0.2) L/h/kg, respectively, both showing significant differences. It was observed that the coexisting components in Ciwujia injection can increase the accumulation of syringin in rats; however, further confirmation is required to elucidate the specific mechanism.

Lu et al. (2012) analyzed rat plasma using HPLC/quadrupole time-of-flight mass spectrometry (QTF-MS)/MS and automated data analysis. A total of 11 metabolites were detected, with no syringin being found. In rats, syringin (M0) was deglycosylated (Process A) to obtain the glycoside erucinol (M1). Subsequently, M1 was further glucuronidated (Process B) to obtain the glucuronidated erucinol (M2), which was then demethylated (Process C) to produce the demethylated glucuronidated erucinol (M3). The product M4 was obtained from M3 through further demethylation and acetylation (Process D). Additionally, after deglycosylation of M0, it can be further hydroxylated and desaturated to obtain the product

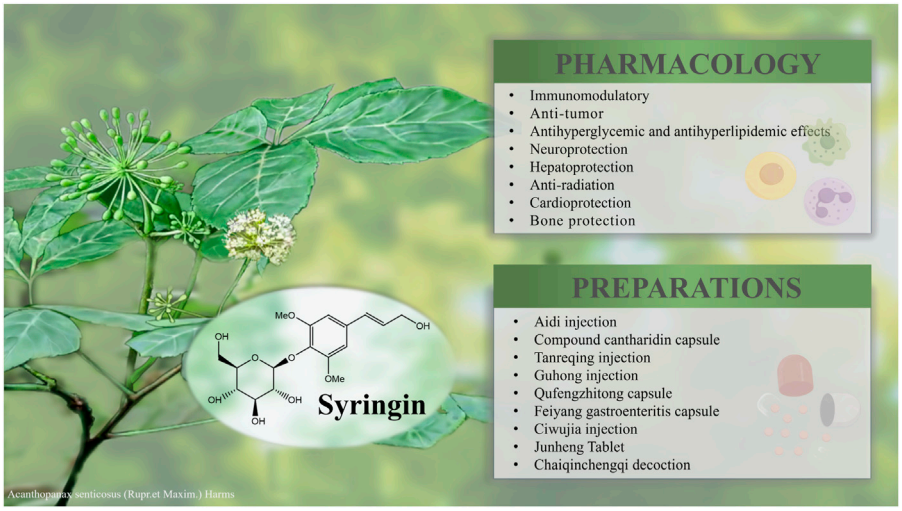

FIGURE 8
Pharmacological effects and clinical applications of syringin.

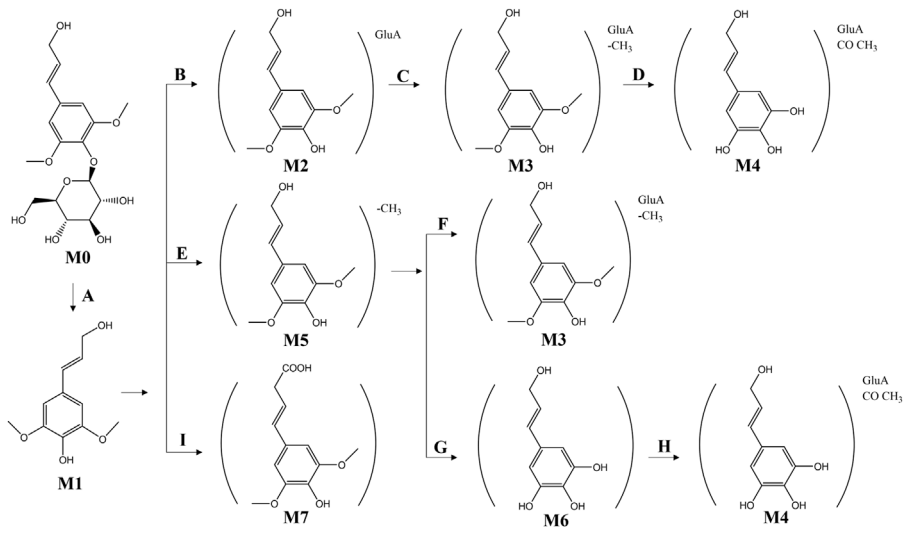

FIGURE 9
Potential metabolic biotransformation pathways and metabolites of syringin in rats. **(A)** (Deglycosylation); **(B)** (Glucuronidation); **(C)** (Demethylation); **(D)** (Demethylation + acetylation); **(E)** (Demethylation); **(F)** (Glucuronidation); **(G)** (Demethylation); **(H)** (Glucuronidation + acetylation); **(I)** (Hydroxylation + desaturation).

M7 through Process I. Simultaneously, demethylation and glucuronidation of M0 led to the production of demethylated glucuronide erucicol M3 through Processes E and F; this could then undergo further glucuronidation and acetylation via Processes G and H to yield a new product. The *in vivo* metabolic pathway of syringin is illustrated in Figure 9. The low bioavailability of syringin may be closely related to its metabolic processes in organisms. Further consideration and research are warranted to explore the transformation of its relationship with mustelianol and dextrose resinoid phenol and whether its metabolism mode affects its efficacy (Wang F. et al., 2021).

10 Discussion

Based on the rich pharmacological effects of syringin, its further development and application require efforts from multiple aspects.

10.1 Need for standardization in pharmacological research

Many polyphenols including phenylpropanoids like syringin have been reported may interfere with formazan formation critical to the MTT or MTS methods, resulting in inaccurate

positive outcomes (Wang P. et al., 2010). CCK-8 and other methods have been used to test the pharmacological activities of *in vitro* experiments.

In silico analysis has shown potential in predicting the pharmacological effects and mechanisms of syringin. However, the databases upon which network pharmacology relies require improvement in terms of accuracy, adaptability, and reliability. It is important to note that while this method is not fabricated, its evidence level is lower compared to experimental and clinical research (Fatima et al., 2022).

Since a long time ago, the activities of syringin have been tested in a lot of experiments relating to mice, rats, and rabbits. Unfortunately, there were some design defects in the early pharmacological studies, including the absence of positive control drugs and excessively high dose settings. To better promote the development of syringin, standardized pharmacological studies are necessary.

10.2 The mechanism of syringin relating to immune regulation and anti-tumor effect needs to be exposed

Over the past decade, immunotherapy, such as programmed death-1 (PD-1), supplied a breakthrough in cancer treatment (Chow et al., 2022). *Fuzheng* and *quxie* is a fundamental approach in traditional Chinese medicine for tumor therapy. It focuses on enhancing the body's immunity in contrast to the tumor. More and more research has shown that many tonic Chinese medicines have anti-tumor effects by boosting immune cell activity and exhibiting collaborative and enhancement effects with PD-1 (Chen F. Q. et al., 2021). As to syringin, this kind of experiment was rare and highly recommended for further study.

10.3 Clinical data should be collected in standardized experiments

Compared to cell and animal experiments, the clinical trial is more convincing to confirm the effectiveness and safety of a new drug. Despite the Aidi injection which contains syringin as the main component, has annual sales of more than one billion in China (Ding, 2024), it still lacks a large population, and multi-species randomized, double-blind, controlled clinical trials. Therefore, further improvement of clinical data is crucial to promote its application in cancer patients worldwide.

11 Conclusion

Since Tu Youyou was awarded the "2015 Nobel Prize in Physiology or Medicine", there has been significant progress in developing natural products extracted from Chinese herbs, which have contributed to treating diseases. Syringin exhibits rich pharmacological effects and high safety, making its further development and utilization of great significance for medicine and clinical applications. Therefore, stronger support is needed for its further development and utilization to promote economic growth while safeguarding public health. This paper summarizes relevant research reports on syringin as a reference for addressing the issues above.

Author contributions

QQ: Writing–original draft. JP: Writing–original draft. JY: Writing–review and editing. RW: Writing–review and editing. KL: Writing–review and editing. ZW: Writing–review and editing. SM: Writing–review and editing. YW: Writing–review and editing. ML: Funding acquisition, Supervision, Writing–review and editing. YG: Funding acquisition, Writing–review and editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This work was supported by Traditional Chinese Medicine Innovation Team and Talent Support Program Project from National Administration of Traditional Chinese Medicine (ZYYCXTDD-202207).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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frontiers | Frontiers in Pharmacology

TYPE Original Research
PUBLISHED 24 July 2024
DOI 10.3389/fphar.2024.1428817

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OPEN ACCESS

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RECEIVED 07 May 2024
ACCEPTED 01 July 2024
PUBLISHED 24 July 2024

CITATION
Cai H, Luo S, Cai X, Lai T, Zhao S, Zhang W, Zhuang J, Li Z, Chen L, Chen B and Ye Y (2024), Effect of Fu Zheng Jie Du Formula on outcomes in patients with severe pneumonia receiving prone ventilation: a retrospective cohort study. *Front. Pharmacol.* 15:1428817. doi: 10.3389/fphar.2024.1428817

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Effect of Fu Zheng Jie Du Formula on outcomes in patients with severe pneumonia receiving prone ventilation: a retrospective cohort study

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Background: The effect of combining prone ventilation with traditional Chinese medicine on severe pneumonia remains unclear.

Objective: To evaluate the effect of Fu Zheng Jie Du Formula (FZJDF) combined with prone ventilation on clinical outcomes in patients with severe pneumonia.

Methods: This single-center retrospective cohort study included 188 severe pneumonia patients admitted to the ICU from January 2022 to December 2023. Patients were divided into an FZJD group (receiving FZJDF for 7 days plus prone ventilation) and a non-FZJD group (prone ventilation only). Propensity score matching (PSM) was performed to balance baseline characteristics. The primary outcome was the change in PaO2/FiO2 ratio after treatment. Secondary outcomes included 28-day mortality, duration of mechanical ventilation, length of ICU stay, PaCO2, lactic acid levels, APACHE II score, SOFA score, Chinese Medicine Score, inflammatory markers, and time to symptom resolution.

Results: After PSM, 32 patients were included in each group. Compared to the non-FZJD group, the FZJD group showed significantly higher PaO2/FiO2 ratios, lower PaCO2, and lower lactic acid levels after treatment ($p < 0.05$ for all). The FZJD group also had significantly lower APACHE II scores, SOFA scores, Chinese Medicine Scores, and levels of WBC, PCT, hs-CRP, and IL-6 ($p < 0.05$ for all). Time to symptom resolution, including duration of mechanical ventilation, length of ICU stay, time to fever resolution, time to cough resolution, and time to resolution of pulmonary rales, was significantly shorter in the FZJD group ($p < 0.05$ for all). There was no significant difference in 28-day mortality between the two groups.

Conclusion: FZJDF as an adjuvant therapy to prone ventilation can improve oxygenation and other clinical outcomes in severe pneumonia patients. Prospective studies are warranted to validate these findings.

KEYWORDS

Fu Zheng Jie Du Formula, prone ventilation, severe pneumonia, retrospective cohort study, clinical efficacy

Introduction

Severe pneumonia remains a significant cause of morbidity and mortality worldwide, particularly among critically ill patients in intensive care units (ICUs) (Mandell et al., 2007). Despite advances in medical care, the mortality rate for severe pneumonia ranges from 20% to 50% (Fine et al., 1996; Restrepo et al., 2008). Mechanical ventilation is often required in the management of severe pneumonia, but it can lead to ventilator-induced lung injury and worsen patient outcomes (Acute Respiratory Distress Syndrome Network et al., 2000; Slutsky and Ranieri, 2013).

In recent years, prone ventilation has emerged as a promising strategy to improve oxygenation and outcomes in patients with severe pneumonia (Guérin et al., 2013). By positioning patients in the prone position, prone ventilation can optimize ventilation-perfusion matching, reduce ventilator-induced lung injury, and facilitate drainage of secretions (Gattinoni et al., 2013; Guerin et al., 2014). Several studies have demonstrated that prone ventilation can significantly improve oxygenation and reduce mortality in patients with acute respiratory distress syndrome (ARDS) (Sud et al., 2014; Mora-Arteaga et al., 2015; Munshi et al., 2017; Matthay et al., 2019; Meyer et al., 2021). However, the effect of prone ventilation on outcomes in severe pneumonia remains less well characterized (Thompson et al., 2017).

Traditional Chinese medicine (TCM) has been widely used in the management of respiratory diseases in China for thousands of years (Wu et al., 2008; Li et al., 2020; Li BH. et al., 2021; Wang et al., 2022). According to TCM theory, the pathogenesis of severe pneumonia can be attributed to the deficiency of Yang Qi and the invasion of pathogenic toxins into the lungs. The interaction between these two aspects forms a vicious cycle, leading to the aggravation of the disease and the manifestation of various symptoms, such as impaired lung function, intense pathogenic toxins, and blood stasis obstructing the collaterals (Luo et al., 2020). Fu Zheng Jie Du Formula (FZJDF), a classical TCM formula, has gained increasing attention for its potential in treating respiratory infections.Fu Zheng Jie Du Formula (FZJDF) is composed of eight herbs, *Aconiti Lateralis Radix* (Fuzi), *Zingiberis Rhizoma* (Ganjiang), *Glycyrrhizae Radix* (Gancao), *Lonicerae Flos* (Jinyinhua), *Gleditsiae Spina* (Zaojiaoci), *Ipomoeae cairicae herba* (Wuzhaolong), *Pogostemon cablin (Blanco) Benth. herba.* (huoxiang), *and Citri Reticulatae Pericarpium* (Chenpi).FZJDF has the efficacy of benefiting Qi and warming Yang, resolving dampness and detoxifying toxins (Liu et al., 2022).FZJDF has been empirically employed as an adjuvant therapy for COVID-19 (Wang et al., 2021) and is particularly beneficial for patients presenting with severe deficiency syndrome complicated by pneumonia.Recent studies have highlighted the therapeutic potential of FZJDF in the management of COVID-19 (Li G. et al., 2021; Wang et al., 2021) and acute lung injury (ALI) (Liu et al., 2022), with minimal herbal toxicity and adverse effects reported. Mechanistically, FZJDF has been shown to exert its anti-inflammatory effects through the modulation of key signaling pathways and immune cell functions. For instance, FZJDF can inhibit the activation of the PI3K/Akt signaling pathway, suppress the polarization of pro-inflammatory M1 macrophages, and enhance the barrier integrity of type II alveolar epithelial cells (Liu et al., 2022). These findings provide valuable insights into the pharmacological basis of FZJDF in treating respiratory diseases.However, its efficacy in severe pneumonia, particularly in combination with prone ventilation, has not been well established. Considering the complex pathophysiology of severe pneumonia and the potential synergistic effects of FZJDF and prone ventilation, further investigation is warranted to evaluate the clinical benefits of this integrative approach.

In this study, we hypothesized that FZJDF combined with prone ventilation could improve oxygenation and clinical outcomes in patients with severe pneumonia. We conducted a retrospective cohort study to compare the outcomes between patients receiving prone ventilation with or without FZJDF. Understanding the mechanisms underlying the potential synergistic effects of FZJDF and prone ventilation, particularly in the context of the gut-lung axis, may lead to more targeted and effective therapies for this critical condition (Yu-Jie et al., 2020; Shelhamer et al., 2021).

Methods

Study design and setting

This was a single-center, retrospective cohort study conducted at the Emergency Intensive Care Unit (EICU) of Guangdong Provincial Hospital of Traditional Chinese Medicine. We screened all adult patients (aged ≥18 years) with severe pneumonia admitted to the EICU between January 2022 and December 2023.The diagnosis of severe pneumonia was based on the American Thoracic Society and Infectious Diseases Society of America guidelines (Haukoos and Lewis, 2015). The inclusion criteria were: 1) met the diagnostic criteria for severe pneumonia; 2) receiving mechanical ventilation within 24 h of admission; 3) received at least one session of prone ventilation; 4) adult patients aged ≥18 years.The exclusion criteria were: 1) pregnancy; 2) malignant tumors; 3) immunocompromised status; 4) received extracorporeal membrane oxygenation (ECMO); 5)history of or current malignancy with an expected survival <28 days; 6)HIV infection; 7)Severe immunodeficiency (congenital immunodeficiency, organ transplantation, autoimmune diseases, *etc.*); 8) incomplete medical records.

Propensity score matching (PSM) (Haukoos and Lewis, 2015) was performed to minimize potential confounding factors. A 1: 1 nearest neighbor matching algorithm with a caliper width of 0.04 was used. The covariates included in the PSM model were: age, gender, APACHE II score, SOFA score, PaO2/FiO2 ratio, and comorbidities at admission.

A total of 188 patients with severe pneumonia were admitted to the EICU during the study period. After screening for eligibility and propensity score matching, 64 patients were included in the final analysis, with 32 patients in each group (Figure 1).

Interventions

All patients received standard treatment for severe pneumonia according to the international guidelines (Metlay et al., 2019). The antimicrobial therapy was initiated empirically and then adjusted based on the results of bacterial culture and antimicrobial

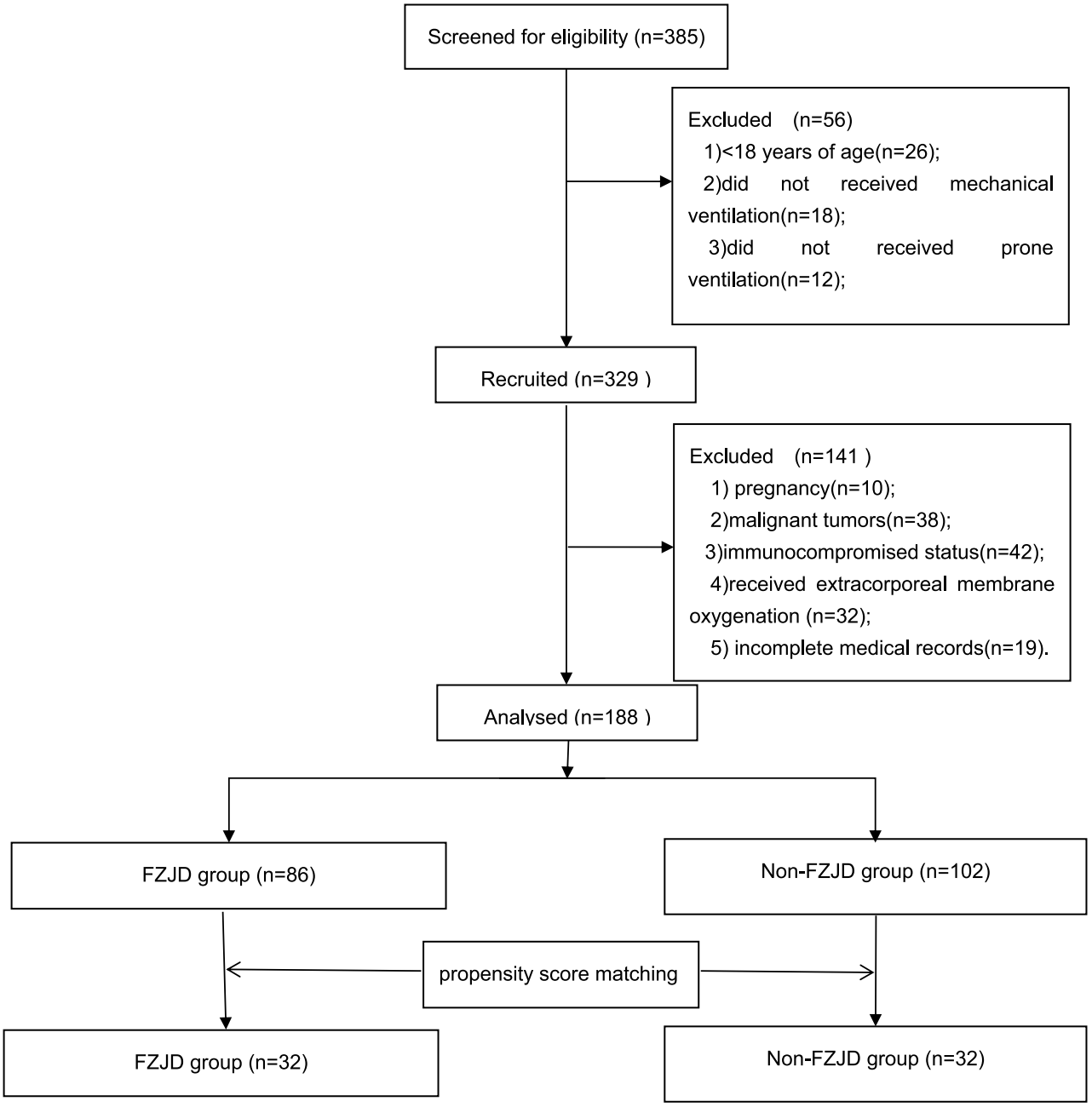

FIGURE 1
Enrollment flowchart of this study.

susceptibility testing.For most patients with severe community-acquired pneumonia (CAP), initial treatment included a combination of β-lactam antibiotics and either macrolides or fluoroquinolones. For patients without risk factors for methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa*, the β-lactam could be cefotaxime, ceftriaxone. For patients with risk factors for *P. aeruginosa*, β-lactams such as piperacillin/tazobactam, Cefoperazone sodium sulbactam sodium, imipenem, or meropenem were used to achieve pathogen coverage. For patients allergic to β-lactams, aztreonam was an alternative. Empiric treatment options for MRSA included vancomycin 15 mg/kg every 12 h (adjusted based on levels) or linezolid 600 mg every 12 h. The duration of antimicrobial therapy was 7–10 days. Antiviral agents, such as oseltamivir were administered when influenza infection was suspected or confirmed. Intravenous fluids were given to maintain adequate tissue perfusion and organ function, with the type and rate of fluids adjusted based on the patient's hemodynamic status and fluid responsiveness. Enteral nutrition was preferred and started as early as possible, with a target energy intake of 25–30 kcal/kg/day and a protein intake of 1.2–2.0 g/kg/day. Parenteral nutrition was used when enteral nutrition was contraindicated or insufficient. Organ function support, including vasopressors, inotropes, renal replacement therapy, was provided as

TABLE 1 Composition of FZJDF.

Herb name	Herbs name in Latin	Abbreviation	Family	Dosage (g)
Fuzi	*Aconiti Lateralis Radix*	FZ	Ranunculaceae	10
Ganjiang	*Zingiberis Rhizoma*	GJ	Zingiberaceae	15
Wuzhaolong	*Ipomoeae cairicae herba*	WZL	Convolvulaceae	20
Zaojiaoci	*Gleditsiae Spina*	ZJC	Fabaceae	15
Jingyinhua	*Lonicerae Flos*	JYH	Caprifoliaceae	15
Huoxiang	*Pogostemon cablin (Blanco) Benth. Herba*	HX	Lamiaceae	15
Chenpi	*Citri Reticulatae Pericarpium*	CP	Rutaceae	5
Gancao	*Glycyrrhizae Radix*	GC	Fabaceae	20

needed based on the patient's condition and the judgment of the attending physician. In addition to the above treatments, all patients received intravenous ambroxol (30 mg) in 0.9% sodium chloride injection, administered three times daily. Inhaled medications, including budesonide suspension (1 mg) and compound ipratropium bromide solution (2.5 mL), were given *via* nebulization three times daily, diluted with 3 mL of 0.9% sodium chloride injection.

Prone ventilation was initiated when patients met the following criteria: 1) PaO2/FiO2 ratio <150 mmHg; 2) FiO2 ≥0.6; 3) PEEP ≥5 cmH2O. The prone position was maintained for at least 16 h per day, with the duration determined by the attending physician based on the patient's response and tolerance. The ventilator settings were adjusted to maintain a tidal volume of 6–8 mL/kg of predicted body weight, a plateau pressure ≤30 cmH2O, and a respiratory rate of 20–35 breaths/min (Albert, 2000; Mancebo et al., 2006; Guérin, 2014). The FiO2 and PEEP were titrated to target a PaO2 of 60–80 mmHg or SpO2 of 88%–95% (Petrone et al., 2021). Lung-protective ventilation strategies, including low tidal volume, permissive hypercapnia, and recruitment maneuvers, were applied to minimize ventilator-induced lung injury.

Patients in the FZJD group additionally received FZJDF, which was prepared by the hospital's TCM pharmacy. The composition of FZJDF was as follows: *Aconiti Lateralis Radix* (Fuzi) 10g, *Zingiberis Rhizoma* (Ganjiang) 15g, *Glycyrrhizae Radix* (Gancao) 20g, *Lonicerae Flos* (Jinyinhua) 15g, *Gleditsiae Spina* (Zaojiaoci) 15g, *Ipomoeae cairicae herba* (Wuzhaolong) 20g, *P. cablin (Blanco) Benth. herba* (huoxiang) 15g, *and Citri Reticulatae Pericarpium* (Chenpi) 5 g.Source and Authentication:All herbs used in the FZJDF were provided by the Chinese Medicine Pharmacy of Guangdong Provincial Hospital of Chinese Medicine (The second Clinical Medical College of Guangzhou University of Chinese Medicine). The herbs were purchased from Kangmei Pharmaceutical Co., Ltd. Authentication of the herbs was performed by senior licensed Traditional Chinese Medicine pharmacists.Preparation Method:The FZJDF was prepared using a standardized method combining traditional Chinese medicine practices with modern pharmaceutical techniques. Herbs, sourced from the Chinese Medicine Pharmacy of Guangdong Provincial Hospital of Chinese Medicine and authenticated by senior licensed Traditional Chinese Medicine pharmacists, were combined according to Table 1. The mixture underwent two sequential decoctions: first in 1,000 mL of purified water for 30 min, then in 800 mL for 20 min, both at simmering temperature. The combined decoctions were concentrated to approximately 200 mL, yielding a 4:1 (w/v) concentration ratio of raw materials to final product. Each 200 mL dose, representing the extract from 90 g of raw herbs, was sealed in sterile containers and stored at 4°C with a 7-day shelf life. Quality control measures included organoleptic evaluation and sample retention for each batch. This rigorous process ensured consistency and potency, providing a reliable basis for clinical application.Quality Control and Batch Consistency Measures:To ensure consistency and quality across different batches of FZJDF, a comprehensive set of control measures was implemented. All herbs, sourced exclusively from Kangmei Pharmaceutical Co., Ltd., were authenticated by senior licensed Traditional Chinese Medicine pharmacists through organoleptic evaluation in accordance with the Chinese Pharmacopoeia standards. The preparation adhered to a standardized protocol, including precise weighing of herbs (±1% tolerance), controlled decoction parameters, and consistent concentration procedures to achieve a final volume of 200 mL per dose (±2% deviation). Each batch underwent rigorous organoleptic assessment for color, odor, and taste, as well as physical inspection for viscosity and absence of visible contaminants. The density of the final preparation was measured using a calibrated hydrometer (acceptable range: 1.05–1.10 g/mL). Batch-to-batch consistency was primarily ensured through strict adherence to the standardized preparation protocol and comprehensive documentation of each batch, including raw material sources, preparation details, and pharmacist observations. Prepared decoctions were stored at 4°C and used within a 7-day period. While quantitative analysis of individual compounds was not performed for each batch in this clinical setting, the rigorous standardization of the preparation process, combined with organoleptic and physical evaluations, provided a practical approach to maintaining consistent quality and composition. This method balances traditional practices with modern quality assurance principles, ensuring reliable production of FZJDF for clinical use.

Patients in the non-FZJD group received standard treatment and prone ventilation without FZJDF. The use of other TCM therapies, such as herbal injections, oral herbal preparations, or

TABLE 2 Metabolite of FZJDF.

Metabolite of Chinese herbs	Herb	Diabetes
sitosterol	CP	TCMSP
naringenin	CP	TCMSP
5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl) chroman-4-one	CP	TCMSP
Citromitin	CP	TCMSP
nobiletin	CP	TCMSP
11,14-eicosadienoic acid	FZ	TCMSP
Delphin_qt	FZ	TCMSP
Deltoin	FZ	TCMSP
Demethyldelavaine A	FZ	TCMSP
Demethyldelavaine B	FZ	TCMSP
Deoxyandrographolide	FZ	TCMSP
karakoline	FZ	TCMSP
Karanjin	FZ	TCMSP
Neokadsuranic acid B	FZ	TCMSP
benzoylnapelline	FZ	TCMSP
6-Demethyldesoline	FZ	TCMSP
deoxyaconitine	FZ	TCMSP
(R)-Norcoclaurine	FZ	TCMSP
ignavine	FZ	TCMSP
isotalatizidine	FZ	TCMSP
jesaconitine	FZ	TCMSP
Carnosifloside I_qt	FZ	TCMSP
hypaconitine	FZ	TCMSP
1-Monolinolein	GJ	TCMSP
Sexangularetin	GJ	TCMSP
beta-sitosterol	GJ	TCMSP
Diop	HX	TCMSP
Genkwanin	HX	TCMSP
patchoulan 1,12-diol	HX	TCMSP
pachypodol	HX	TCMSP
5-Hydroxy-7,4′-dimethoxyflavanon	HX	TCMSP
irisolidone	HX	TCMSP
phenanthrone	HX	TCMSP
quercetin 7-O-β-D-glucoside	HX	TCMSP
Acanthoside B	HX	TCMSP
3,23-dihydroxy-12-oleanen-28-oic acid	HX	TCMSP
quercetin	HX	TCMSP
Mandenol	JYH	TCMSP

(Continued in next column)

TABLE 2 (*Continued*) Metabolite of FZJDF.

Metabolite of Chinese herbs	Herb	Diabetes
Ethyl linolenate	JYH	TCMSP
phytofluene	JYH	TCMSP
Eriodyctiol (flavanone)	JYH	TCMSP
(−)-(3R,8S,9R,9aS,10aS)-9-ethenyl-8-(beta-D-glucopyranosyloxy)-2,3,9,9a,10,10a-hexahydro-5-oxo-5H,8H-pyrano [4,3-d]oxazolo [3,2-a]pyridine-3-carboxylic acid_qt	JYH	TCMSP
secologanic dibutylacetal_qt	JYH	TCMSP
beta-carotene	JYH	TCMSP
ZINC03978781	JYH	TCMSP
Chryseriol	JYH	TCMSP
kryptoxanthin	JYH	TCMSP
7-epi-Vogeloside	JYH	TCMSP
Caeruloside C	JYH	TCMSP
Centauroside_qt	JYH	TCMSP
Ioniceracetalides B_qt	JYH	TCMSP
XYLOSTOSIDINE	JYH	TCMSP
dinethylsecologanoside	JYH	TCMSP
kaempferol	JYH	TCMSP
Stigmasterol	JYH	TCMSP
luteolin	JYH	TCMSP
alizarin	WZL	Herb
amygdalin	WZL	Herb
citric acid	WZL	Herb
dihydromollugin	WZL	Herb
ergocornine	WZL	Herb
ergocorninine	WZL	Herb
furomollugin	WZL	Herb
l-arctigenin	WZL	Herb
malic acid	WZL	Herb
munjistin	WZL	Herb
muricatin b	WZL	Herb
muricatocin a	WZL	Herb
(?)-nortrachelogenin	WZL	Herb
oleanolic acid	WZL	Herb
prunasin	WZL	Herb
purpurin	WZL	Herb
purpuroxanthin	WZL	Herb
ruberythric acid	WZL	Herb
rubiadin	WZL	Herb

(Continued on following page)

TABLE 2 (*Continued*) Metabolite of FZJDF.

Metabolite of Chinese herbs	Herb	Diabetes
rubimaillin	WZL	Herb
succine acid	WZL	Herb
tartaric acid	WZL	Herb
trachelogenin	WZL	Herb
tracheloside	WZL	Herb
fisetin	ZJC	TCMSP
Fustin	ZJC	TCMSP
(−)-taxifolin	ZJC	TCMSP
Stigmast-4-ene-3,6-dione	ZJC	TCMSP
ent-Epicatechin	ZJC	TCMSP
Inermine	GC	TCMSP
DFV	GC	TCMSP
Mairin	GC	TCMSP
Glycyrol	GC	TCMSP
Jaranol	GC	TCMSP
Medicarpin	GC	TCMSP
isorhamnetin	GC	TCMSP
Lupiwighteone	GC	TCMSP
7-Methoxy-2-methyl isoflavone	GC	TCMSP
formononetin	GC	TCMSP
Calycosin	GC	TCMSP
(2S)-2-[4-hydroxy-3-(3-methylbut-2-enyl)phenyl]-8,8-dimethyl-2,3-dihydropyrano [2,3-f]chromen-4-one	GC	TCMSP
euchrenone	GC	TCMSP
glyasperin B	GC	TCMSP
glyasperin F	GC	TCMSP
Glyasperin C	GC	TCMSP
Isotrifoliol	GC	TCMSP
(E)-1-(2,4-dihydroxyphenyl)-3-(2,2-dimethylchromen-6-yl)prop-2-en-1-one	GC	TCMSP
kanzonols W	GC	TCMSP
(2S)-6-(2,4-dihydroxyphenyl)-2-(2-hydroxypropan-2-yl)-4-methoxy-2,3-dihydrofuro [3,2-g]chromen-7-one	GC	TCMSP
Semilicoisoflavone B	GC	TCMSP
Glepidotin A	GC	TCMSP
Glepidotin B	GC	TCMSP
Phaseolinisoflavan	GC	TCMSP
Glypallichalcone	GC	TCMSP
8-(6-hydroxy-2-benzofuranyl)-2,2-dimethyl-5-chromenol	GC	TCMSP

(Continued in next column)

TABLE 2 (*Continued*) Metabolite of FZJDF.

Metabolite of Chinese herbs	Herb	Diabetes
Licochalcone B	GC	TCMSP
licochalcone G	GC	TCMSP
3-(2,4-dihydroxyphenyl)-8-(1,1-dimethylprop-2-enyl)-7-hydroxy-5-methoxy-coumarin	GC	TCMSP
Licoricone	GC	TCMSP
Gancaonin A	GC	TCMSP
Gancaonin B	GC	TCMSP
licorice glycoside E	GC	TCMSP
3-(3,4-dihydroxyphenyl)-5,7-dihydroxy-8-(3-methylbut-2-enyl)chromone	GC	TCMSP
5,7-dihydroxy-3-(4-methoxyphenyl)-8-(3-methylbut-2-enyl)chromone	GC	TCMSP
2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-6-(3-methylbut-2-enyl)chromone	GC	TCMSP
Glycyrin	GC	TCMSP
Licocoumarone	GC	TCMSP
Licoisoflavone	GC	TCMSP
Licoisoflavone B	GC	TCMSP
licoisoflavanone	GC	TCMSP
shinpterocarpin	GC	TCMSP
(E)-3-[3,4-dihydroxy-5-(3-methylbut-2-enyl)phenyl]-1-(2,4-dihydroxyphenyl)prop-2-en-1-one	GC	TCMSP
liquiritin	GC	TCMSP
licopyranocoumarin	GC	TCMSP
Glyzaglabrin	GC	TCMSP
Glabridin	GC	TCMSP
Glabranin	GC	TCMSP
Glabrene	GC	TCMSP
Glabrone	GC	TCMSP
1,3-dihydroxy-9-methoxy-6-benzofurano [3,2-c] chromenone	GC	TCMSP
1,3-dihydroxy-8,9-dimethoxy-6-benzofurano [3,2-c] chromenone	GC	TCMSP
Eurycarpin A	GC	TCMSP
glycyroside	GC	TCMSP
(−)-Medicocarpin	GC	TCMSP
Sigmoidin-B	GC	TCMSP
(2R)-7-hydroxy-2-(4-hydroxyphenyl)chroman-4-one	GC	TCMSP
(2S)-7-hydroxy-2-(4-hydroxyphenyl)-8-(3-methylbut-2-enyl)chroman-4-one	GC	TCMSP
Isoglycyrol	GC	TCMSP
Isolicoflavonol	GC	TCMSP

(Continued on following page)

TABLE 2 (*Continued*) Metabolite of FZJDF.

Metabolite of Chinese herbs	Herb	Diabetes
HMO	GC	TCMSP
1-Methoxyphaseollidin	GC	TCMSP
Quercetin der	GC	TCMSP
3′-Hydroxy-4′-O-Methylglabridin	GC	TCMSP
licochalcone a	GC	TCMSP
3′-Methoxyglabridin	GC	TCMSP
2-[(3R)-8,8-dimethyl-3,4-dihydro-2H-pyrano [6,5-f] chromen-3-yl]-5-methoxyphenol	GC	TCMSP
Inflacoumarin A	GC	TCMSP
icos-5-enoic acid	GC	TCMSP
Kanzonol F	GC	TCMSP
6-prenylated eriodictyol	GC	TCMSP
7,2′,4′-trihydroxy-5-methoxy-3-arylcoumarin	GC	TCMSP
7-Acetoxy-2-methylisoflavone	GC	TCMSP
8-prenylated eriodictyol	GC	TCMSP
gadelaidic acid	GC	TCMSP
Vestitol	GC	TCMSP
Gancaonin G	GC	TCMSP
Gancaonin H	GC	TCMSP
Licoagrocarpin	GC	TCMSP
Glyasperins M	GC	TCMSP
Glycyrrhiza flavonol A	GC	TCMSP
Licoagroisoflavone	GC	TCMSP
18α-hydroxyglycyrrhetic acid	GC	TCMSP
Odoratin	GC	TCMSP
Phaseol	GC	TCMSP
Xambioona	GC	TCMSP
dehydroglyasperins C	GC	TCMSP

external application of herbal medicines, was not allowed in either group during the study period. The concomitant use of Western medications was permitted as clinically indicated and recorded in the case report form.

Outcomes

The primary outcome was the change in PaO2/FiO2 ratio from baseline to day 7. The secondary outcomes included:

1) 28-day mortality;
2) Duration of mechanical ventilation;
3) Length of ICU stay;
4) PaCO2 and lactic acid levels on days 1 and 7;
5) APACHE II score and SOFA score on days 1 and 7;
6) Chinese Medicine Score (Jiang et al., 2012) on days 1 and 7; The Chinese Medicine Score was a self-designed scoring system based on TCM syndrome differentiation, which evaluated the severity of Yang deficiency, Qi deficiency, Damp-Heat and Evil-Toxins. Each item was scored from 0 (none) to 3 (severe), with a total score ranging from 0 to 12. Higher scores indicated more severe TCM syndromes.
7) Inflammatory markers (WBC, PCT, Hs-CRP, IL-6) on days 1 and 7;
8) Time to fever resolution;
9) Time to cough resolution;
10) Time to resolution of pulmonary rales.

Data collection

Demographic and clinical data were extracted from the electronic medical records by trained research staff using a standardized data collection form. The collected data included age, sex, comorbidities (cerebral infarction, hypertension, coronary heart disease, diabetes, chronic kidney disease, chronic lung disease), laboratory tests (white blood cell count, lymphocyte count, C-reactive protein, procalcitonin), and clinical scores (Acute Physiology and Chronic Health Evaluation II [APACHE II] score, Sequential Organ Failure Assessment [SOFA] score).Data on interventions, including mechanical ventilation parameters (ventilation mode, tidal volume, positive end-expiratory pressure [PEEP], fraction of inspired oxygen [FiO2]), prone positioning, and medication administration (antibiotics, antivirals, glucocorticoids) were recorded daily.The primary outcome, PaO2/FiO2 ratio, was recorded at baseline and on day 7 after enrollment. Secondary outcomes, including inflammatory markers (interleukin-6 [IL-6], tumor necrosis factor-α [TNF-α]) and Chinese medicine syndrome scores, were also collected at these two time points.All data were entered into a secure electronic database and checked for accuracy and completeness by a second researcher. Any discrepancies were resolved through discussion with the principal investigator. The database was backed up regularly to ensure data integrity and security.

Statistical analysis

Missing data were first analyzed for their missing mechanisms, and the possibility of non-random missingness was excluded. For data with missing completely at random (MCAR) or missing at random (MAR), multiple imputation using chained equations (MICE) was performed to generate five complete datasets. Analyses were then conducted on each imputed dataset, and the results were combined using Rubin's rules. All missing data analyses were completed before the main outcome analyses.Continuous variables were expressed as mean ± standard deviation or median (interquartile range) and compared using the independent sample *t*-test or Mann-Whitney *U* test, as appropriate. Categorical variables were

TABLE 3 Presents the baseline characteristics of the two groups.

Characteristic	FZJD group (n = 86)	Non-FZJD group (n = 102)	Statistical values	*P*
Sex,n (%)				
Male	58 (67.442)	70 (68.627)	0.030	0.862
Female	28 (32.558)	32 (31.373)		
cerebral infarction,n (%)	19 (22.093)	20 (19.608)	0.175	0.675
high blood pressure,n (%)	23 (26.744)	12 (11.765)	6.910	0.009
coronary heart disease,n (%)	20 (23.255)	29 (28.431)	1.533	0.216
diabetes,n (%)	29 (33.721)	36 (35.294)	0.051	0.821
chronic kidney disease,n (%)	6 (6.977)	10 (9.804)	0.479	0.489
Chronic lung disease,n (%)	10 (11.628)	15 (14.706)	0.383	0.536
age,years	75.953 ± 14.333	70.137 ± 15.602	2.628	0.009
heat generation time, days	4.000 [3.000,5.000]	5.000 [4.000,6.000]	−1.621	0.097
T,°C	39.125 ± 4.861	39.500 ± 3.332	−0.601	0.548
SBP,mmHg	107.221 ± 20.651	97.392 ± 19.347	3.347	<0.001
DBP,mmHg	76.616 ± 14.626	72.451 ± 10.804	2.174	0.031
HR,bpm	113.965 ± 22.285	121.225 ± 22.971	−2.177	0.031
R,bpm	26.977 ± 3.267	27.490 ± 3.469	−1.033	0.303
SPO2,%	84.698 ± 6.595	84.059 ± 7.222	0.625	0.533
BMI,kg/m^2	28.384 ± 3.383	27.480 ± 3.572	1.760	0.080
CPIS, scores	9.000 [7.000,10.000]	8.000 [7.000,10.000]	1.158	0.243

presented as numbers (percentages) and compared using the chi-square test or Fisher's exact test.To minimize potential confounding factors, propensity score matching (PSM) was performed. The propensity score was estimated using a multivariable logistic regression model, with treatment assignment as the dependent variable and baseline characteristics as covariates. The covariates included in the propensity score model were age, gender, APACHE II score, SOFA score, PaO2/FiO2 ratio, and comorbidities at admission.Patients in the FZJD group were matched 1:1 to those in the non-FZJD group using the nearest neighbor matching algorithm with a caliper width of 0.04. The balance of baseline covariates between the two groups before and after PSM was assessed using standardized mean differences (SMDs), with an absolute SMD <0.1 indicating a negligible difference.The primary outcome, the change in PaO2/FiO2 ratio, and secondary outcomes, including inflammatory markers and Chinese medicine syndrome scores, were compared between the two groups using the independent sample *t*-test or Mann-Whitney *U* test, as appropriate. The paired *t*-test or Wilcoxon signed-rank test was used to compare the changes in these outcomes within each group before and after treatment.All statistical analyses were performed using SPSS 26.0 (IBM Corp., Armonk, NY, United States of America) and R 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria). A two-sided *p*-value <0.05 was considered statistically significant.

Results

Composition and active ingredients of FZJDF

FZJDF consists of eight traditional Chinese herbs, as detailed in Table 1. To elucidate its potential mechanism of action, we performed a network pharmacology analysis.Through screening of the TCMSP and Herb databases, we identified 172 potential active ingredients in FZJDF (Table 2). These active ingredients were predicted to act on 462 gene targets (Supplementary Table S1).Using the GeneCards and OMIM databases, we identified 4,483 genes associated with severe pneumonia. By comparing the herb-related targets with disease-related targets, we identified 221 common targets, which may be crucial for the therapeutic effects of FZJDF (Supplementary Table S1).

Baseline characteristics of patients before propensity score matching

A total of 188 patients with severe pneumonia were admitted to the EICU during the study period. After screening for eligibility and propensity score matching, 64 patients were included in the final analysis, with 32 patients in each group. The baseline characteristics of the two groups were well balanced after matching (Table 3).

TABLE 4 Baseline characteristics of patients after propensity score matching.

Characteristic	FZJD group (n = 32)	Non-FZJD group (n = 32)	Statistical values	*P*
Sex,n (%)				
Male	18 (56.250)	17 (53.125)	0.063	0.802
Female	14 (43.750)	15 (46.875)		
cerebral infarction,n (%)	10 (31.250)	9 (28.125)	0.075	0.784
high blood pressure,n (%)	9 (28.125)	10 (31.250)	0.075	0.784
coronary heart disease,n (%)	9 (28.125)	9 (28.125)	0.000	1.000
diabetes,n (%)	11 (34.375)	9 (28.125)	0.291	0.590
chronic kidney disease,n (%)	4 (12.500)	3 (9.375)	0.000	1.000
Chronic lung disease,n (%)	5 (15.625)	7 (21.875)	0.410	0.522
age,years	76.314 ± 12.205	72.938 ± 10.565	−1.251	0.215
heat generation time, days	4.000 [3.000,4.000]	4.000 [3.000,4.000]	−0.832	0.373
T,°C	38.804 [38.242,39.561]	38.839 [38.593,39.255]	0.752	0.456
SBP,mmHg	120.125 ± 12.869	115.219 ± 13.747	−1.451	0.152
DBP,mmHg	77.500 ± 11.164	78.250 ± 7.814	0.306	0.760
HR,bpm	122.281 ± 19.189	117.625 ± 16.101	−1.035	0.305
R,bpm	27.219 ± 4.519	27.906 ± 5.083	0.563	0.576
SPO2,%	85.531 ± 10.695	86.906 ± 6.939	0.601	0.551
BMI,kg/m^2	27.761 ± 6.292	27.439 ± 4.223	−0.237	0.814
CPIS, scores	8.000 [7.000,11.000]	8.000 [6.000,10.000]	−0.483	0.630

TABLE 5 28-Day mortality.

Characteristic	FZJD group (n = 32)	Non-FZJD group (n = 32)	Statistical values	*P*
28-d Death,n (%)	5 (15.625)	7 (17.910)	0.410	0.522

TABLE 6 Blood gas analysis.

Characteristic	FZJD group (n = 32)	Non-FZJD group (n = 32)	Statistical values	*P*
PaO2/FiO2,mmHg				
pretreatment	284.531 ± 77.246	258.719 ± 56.855	−1.498	0.139
7th day after treatment	391.281 ± 61.727	321.344 ± 72.050	−4.104	<0.001
PaCO2,mmHg				
pretreatment	56.031 ± 11.126	57.875 ± 8.241	0.741	0.461
7th day after treatment	38.844 ± 8.650	45.844 ± 7.807	3.345	0.001
LAC,mmol/L				
pretreatment	1.988 ± 0.492	2.143 ± 0.687	1.018	0.313
7th day after treatment	0.538 ± 0.207	1.165 ± 0.362	8.383	<0.001

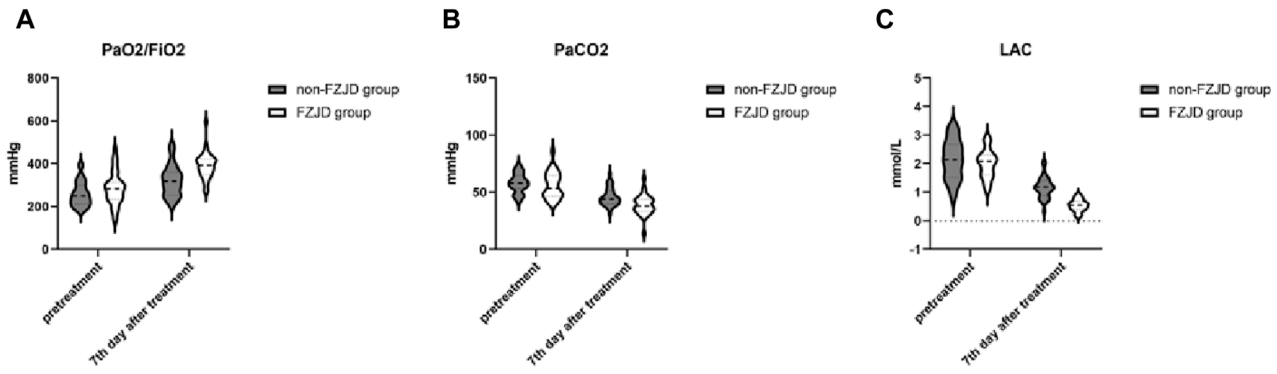

FIGURE 2
Changes in blood gas parameters over the treatment period. **(A)** PaO2/FiO2 ratio. **(B)** Partial pressure of arterial carbon dioxide (PaCO2). **(C)** Lactic acid (LAC) levels.

TABLE 7 Clinical scores.

Characteristic	FZJD group (n = 32)	Non-FZJD group (n = 32)	Statistical values	*P*
APACHEII,scores				
pretreatment	18.781 ± 3.974	20.844 ± 3.850	2.075	0.042
7th day after treatment	11.625 ± 2.274	14.719 ± 3.145	4.438	<0.001
SOFA, scores				
pretreatment	4.000 [3.000,6.000]	5.000 [4.000,5.000]	0.040	0.973
7th day after treatment	1.000 [1.000,2.000]	2.000 [2.000,2.000]	3.216	<0.001
Chinese Medicine Score, scores				
pretreatment	20.406 ± 4.422	21.219 ± 5.097	0.670	0.505
7th day after treatment	10.688 ± 2.984	14.344 ± 4.254	3.918	<0.001

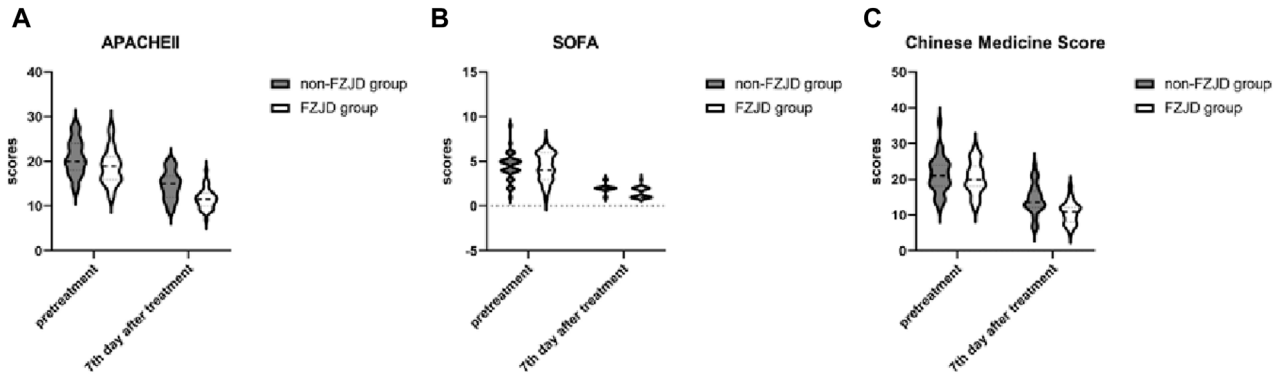

FIGURE 3
Changes in severity scores during treatment. **(A)** APACHE II (Acute Physiology and Chronic Health Evaluation II) score. **(B)** SOFA (Sequential Organ Failure Assessment) score. **(C)** Chinese Medicine Score.

Baseline characteristics of patients after propensity score matching

To reduce confounding bias arising from differences in baseline characteristics between the two groups, propensity score matching (PSM) was performed using 1:1 nearest-neighbor matching. A caliper value of 0.04 was used. After PSM, 32 patients were matched from each group. Table 4 presents the baseline characteristics of the two matched groups. No significant differences were observed in baseline characteristics between the two groups after matching.

TABLE 8 Inflammatory markers.

Characteristic	FZJD group (n = 32)	Non-FZJD group (n = 32)	Statistical values	*P*
WBC,×10^9/L				
pretreatment	17.178 ± 6.654	16.836 ± 4.399	−0.238	0.813
7th day after treatment	11.763 ± 3.560	13.778 ± 3.475	2.255	0.028
PCT,ng/mL				
pretreatment	10.589 ± 2.907	11.378 ± 4.189	0.862	0.392
7th day after treatment	3.781 ± 0.548	4.317 ± 0.868	2.907	0.005
Hs-CRP,mg/L				
pretreatment	109.670 [86.524,131.148]	122.622 [87.997,142.407]	0.819	0.417
7th day after treatment	30.172 ± 9.057	47.453 ± 10.662	6.878	<0.001
IL-6,ng/mL				
pretreatment	195.511 ± 93.130	174.695 ± 81.946	−0.934	0.354
7th day after treatment	44.864 ± 18.460	59.092 ± 24.067	2.612	0.011

28-Day mortality

No significant difference in 28-day mortality was observed between the FZJD group (5 [15.625%]) and the non-FZJD group (7 [17.910%]) (p = 0.522) (Table 5).

Blood gas analysis

After treatment, compared to the non-FZJD group, the FZJD group had a significantly higher PaO2/FiO2 ratio (391.281 ± 61.727 vs. 321.344 ± 72.050 mmHg, p < 0.001), lower PaCO2 (38.844 ± 8.650 vs. 45.844 ± 7.807 mmHg, p = 0.001), and lower lactic acid levels (0.538 ± 0.207 vs. 1.165 ± 0.362 mmol/L, p < 0.001) (Table 6; Figure 2).

Clinical scores

The FZJD group had significantly lower APACHE II score (mean ± SD, 11.625 ± 2.274 vs. 14.719 ± 3.145 scores; p < 0.001), SOFA score (median [IQR], 1.000 [1.000,2.000] vs. 2.00 [2.00, 2.00] scores; p < 0.001), and Chinese Medicine Score (mean ± SD, 10.688 ± 2.984 vs. 14.344 ± 4.254 scores; p < 0.001) compared to the non-FZJD group after treatment (Table 7; Figure 3).

Inflammatory markers

After treatment, the FZJD group had significantly lower levels of WBC (mean ± SD, 11.763 ± 3.560 vs. 13.778 ± 3.475 ×10^9/L; p = 0.028), PCT (mean ± SD, 3.781 ± 0.548 vs. 4.317 ± 0.868 ng/mL; p = 0.005), Hs-CRP (mean ± SD, 30.172 ± 9.057 vs. 47.453 ± 10.662 mg/L; p < 0.001), and IL-6 (mean ± SD, 44.864 ± 18.460 vs. 59.092 ± 24.067 ng/mL; p = 0.011 compared to the non-FZJD group (Table 8; Figure 4).

Symptom relief time

The FZJD group had significantly shorter mechanical ventilation time (median [IQR], 9.000 [7.000,13.000] vs. 14.000 [11.000,17.000] days; p = 0.002), ICU stay (median [IQR], 12.000 [10.000,16.000] vs. 18.000 [13.000,23.000] days; p = 0.007), fever duration (mean ± SD, 5.875 ± 3.049 vs. 8.375 ± 5.079 days; p = 0.025), cough disappearance time (mean ± SD, 8.906 ± 3.617 vs. 12.688 ± 7.086 days; p = 0.008), and disappearance time of pulmonary rales (mean ± SD, 10.156 ± 3.784 vs. 13.063 ± 5.722 days; p = 0.022) compared to the control group (Table 9; Figure 5).

Discussion

Before delving into a detailed discussion of our findings and their implications, we present a glossary of key terms used in this study (Table 10). Clarifying these terms will facilitate a better understanding of the subsequent discussion. In the theoretical framework of Traditional Chinese Medicine (TCM), Fu Zheng Jie Du Formula is categorized as a combination of "qi-tonifying and yang-warming" and "heat-clearing and toxin-eliminating" herbs. This classification reflects the formula's dual therapeutic approach within TCM theory. It is important to note that while these traditional concepts provide valuable historical context and insight into the formula's intended effects, they cannot be directly evaluated or validated through contemporary biological science methods. These TCM classifications do not correspond directly to modern biomedical understandings of physiology and pharmacology. Nevertheless, they remain relevant as background information, offering perspective on the theoretical basis that guided the formula's composition and its historical application in TCM practice. The clinical efficacy and mechanisms of action of FZJDF are subject to ongoing scientific investigation using evidence-based research methodologies, which aim to elucidate its effects in terms of modern medical understanding.In this retrospective cohort study, we found that the

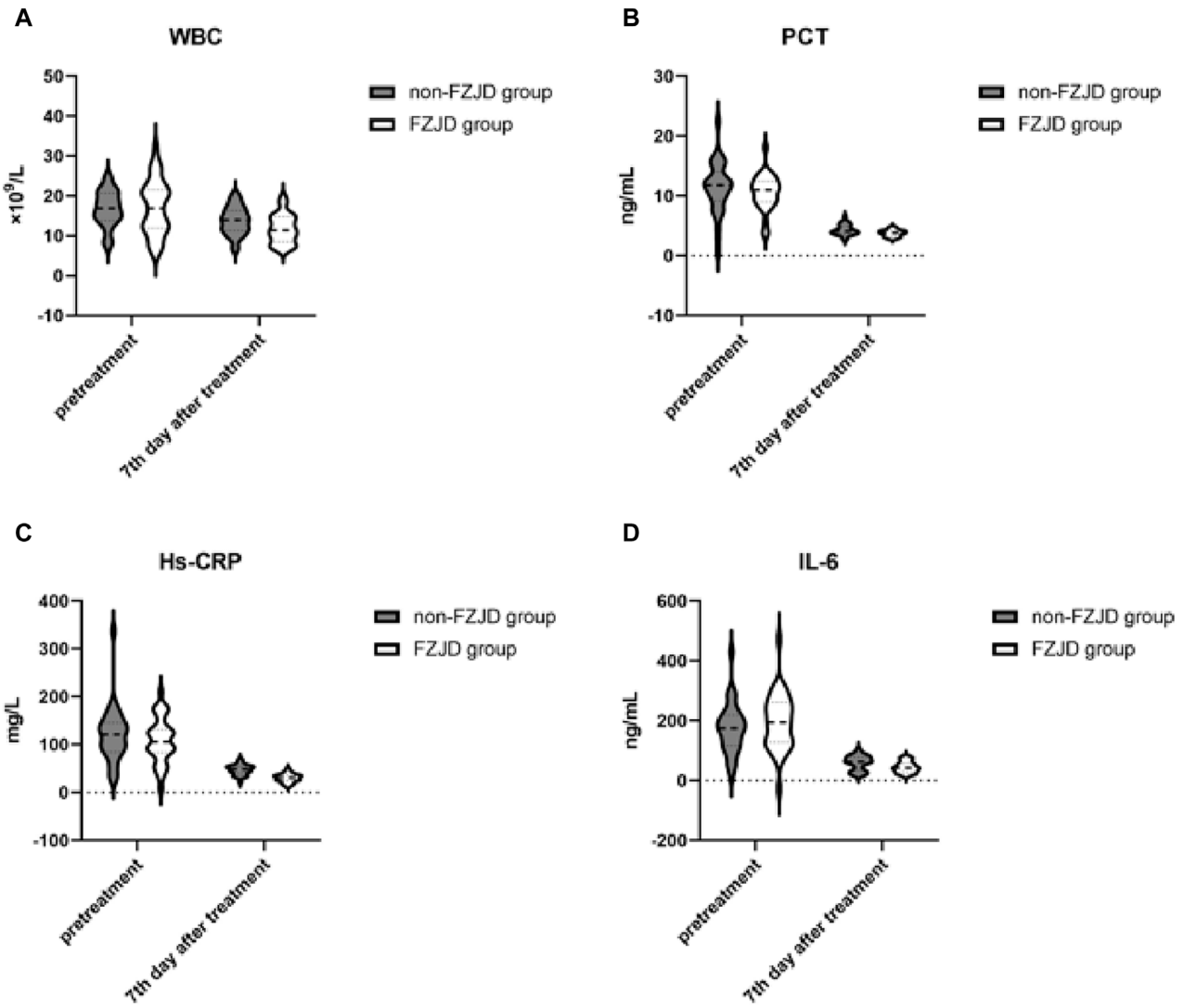

FIGURE 4
Changes in inflammatory markers during treatment. **(A)** White Blood Cell (WBC) count. **(B)** Procalcitonin (PCT) levels. **(C)** High-sensitivity C-Reactive Protein (Hs-CRP) concentration. **(D)** Interleukin-6 (IL-6) levels.

combination of FZJDF with conventional treatment and prone ventilation significantly improved oxygenation, reduced inflammatory response, and shortened the duration of mechanical ventilation and ICU stay in patients with severe pneumonia. Compared to the non-FZJD group, the FZJD group showed a significantly higher PaO2/FiO2 ratio, lower PaCO2, and lower lactic acid levels after treatment. The FZJD group also had significantly lower APACHE II score, SOFA score, Chinese Medicine Score, and levels of inflammatory markers, including WBC, PCT, Hs-CRP, and IL-6. These findings suggest that FZJDF may be an effective adjuvant therapy for severe pneumonia, potentially improving patient outcomes (Ren et al., 2021). Given the high mortality and complex pathophysiology of severe pneumonia, identifying effective comprehensive treatment strategies is of great importance (Phua et al., 2009; Zhang et al., 2020; Liu et al., 2021).

The rationale for combining FZJDF with prone ventilation is based on their complementary mechanisms of action. Prone ventilation improves oxygenation by optimizing ventilation-perfusion matching and reducing ventilator-induced lung injury (Li et al., 2020; Li BH. et al., 2021). FZJDF, on the other hand, targets the underlying pathophysiology of severe pneumonia from the perspective of TCM. According to TCM theory, severe pneumonia is caused by the invasion of pathogenic factors, such as wind, cold, heat, and dampness, which lead to deficiency of Yang Qi and the invasion of pathogenic toxins into the lungs. FZJDF contains a combination of herbs that can benefiting Qi and warming Yang, resolving dampness and detoxifying toxins, thereby restoring the balance of Qi and Yang in the lung (Liu et al., 2022).

The improvement in oxygenation with FZJDF may be attributed to its anti-inflammatory and anti-oxidative effects. Previous studies have demonstrated that FZJDF can inhibit the production of pro-inflammatory cytokines, such as TNF-α, IL-1β, and IL-6, and reduce oxidative stress in lung tissue (Butt et al., 2016; Li G. et al., 2021; Liu et al., 2022). These findings are consistent with our observation of a significant reduction in inflammatory markers, including WBC, PCT, Hs-CRP, and IL-6, in the FZJD group compared to the non-FZJD group. The alleviation of inflammation and oxidative

TABLE 9 Symptom Relief time.

Characteristic	FZJD group (n = 32)	Non-FZJD group (n = 32)	Statistical values	*P*
mechanical ventilation time, days	9.000 [7.000,13.000]	14.000 [11.000,17.000]	2.732	0.006
admission time to the monitoring room, days	12.000 [10.000,16.000]	18.000 [13.000,23.000]	2.652	0.008
fever reduction time, days	5.875 ± 3.049	8.375 ± 5.079	2.350	0.023
cough disappearance time, days	8.906 ± 3.617	12.688 ± 7.086	2.646	0.011
disappearance time of pulmonary wet rhonchi, days	10.156 ± 3.784	13.063 ± 5.722	2.359	0.022

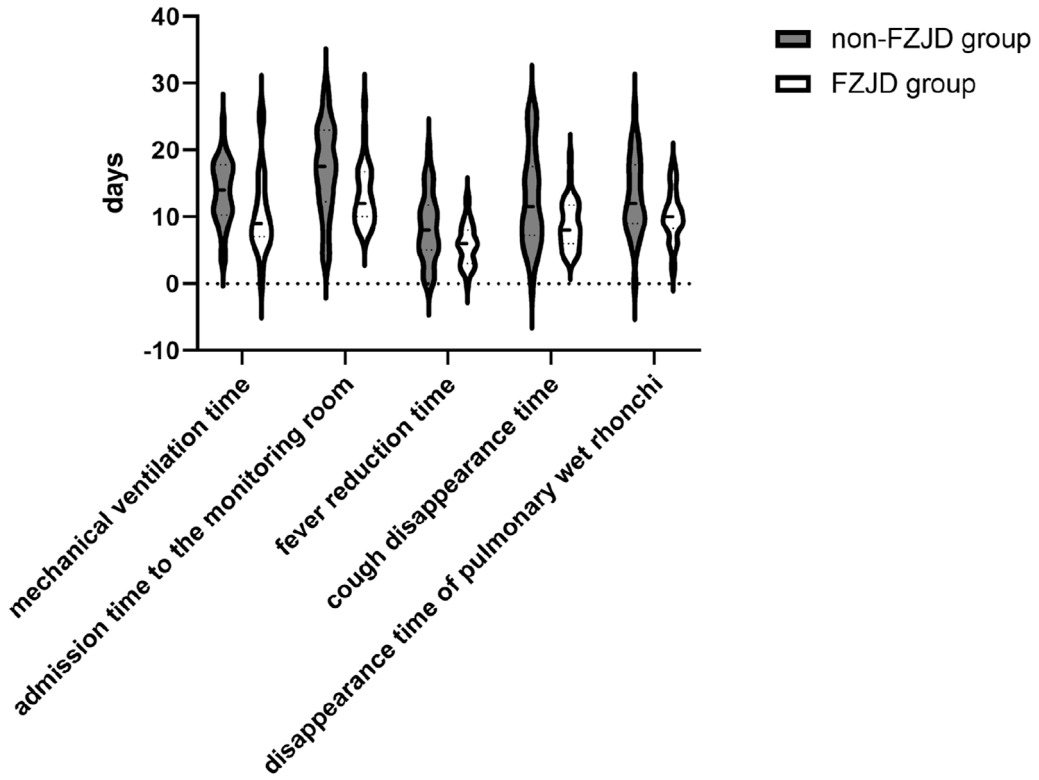

FIGURE 5
Symptom relief time.

stress may contribute to the preservation of alveolar-capillary membrane integrity, reduction of pulmonary edema, and improvement of gas exchange (Ware, 2006; Matthay et al., 2019).

The shortened duration of mechanical ventilation and ICU stay in the FZJD group may be a result of the faster resolution of symptoms and improvement in overall condition. We found that patients in the FZJD group had significantly lower APACHE II score, SOFA score, and Chinese Medicine Score compared to the non-FZJD group, indicating a better response to treatment (Knaus et al., 1985; Vincent et al., 1996). The faster resolution of fever, cough, and pulmonary rales in the FZJD group also suggests that FZJDF may promote the recovery of lung function and reduce the risk of ventilator-associated complications (Cavallazzi et al., 2015; Béduneau et al., 2017). These findings highlight the potential of FZJDF as an adjuvant therapy to enhance the efficacy of conventional treatment and prone ventilation in patients with severe pneumonia (Liu et al., 2004; Guo et al., 2022).

A recent study by Lu et al. (2024) has suggested that FZJDF may exert its therapeutic effects in severe pneumonia, at least in part, by modulating the gut-lung axis. Using a lipopolysaccharide-induced acute lung injury mouse model, they found that FZJDF treatment alleviated intestinal barrier dysfunction, reduced systemic and pulmonary inflammation, and attenuated lung histopathological changes, which were associated with the regulation of key signaling pathways, such as NF-κB and MAPK, in both the gut and the lung. These findings provide new insights into the potential mechanisms of action of FZJDF in severe pneumonia, highlighting the importance of the gut-lung axis in the pathogenesis and treatment of respiratory diseases (Sencio et al., 2021). However, further clinical studies are needed to validate this hypothesis and guide the optimal use of FZJDF in patients with severe pneumonia.

Our study has several limitations. First, it was a single-center retrospective study with a relatively small sample size, which may

TABLE 10 Glossary of terms used in the study.

Term	Definition
Fu Zheng Jie Du Formula (FZJDF)	A traditional Chinese medicine formula composed of eight herbs, used in this study to treat COVID-19 patients
Qi	A vital energy or life force that flows through the body according to traditional Chinese medicine concepts
Yang	A concept in traditional Chinese medicine that represents the active, warming, and drying aspects of the body
Prone ventilation	A mechanical ventilation strategy where patients are positioned face-down to improve oxygenation and respiratory mechanics
Decoction	A method of extraction by boiling herbal materials to dissolve the chemicals of the material in the water
Propensity score matching	A statistical technique used to construct an artificial control group by matching treated and untreated subjects on their propensity scores
Chinese Medicine Score	A scoring system used to quantify the severity of COVID-19 symptoms based on traditional Chinese medicine principles
Herbal medicine	A medical system based on the use of plants or plant extracts to treat illnesses or promote health
Pathogenic toxins	In traditional Chinese medicine, pathogenic toxins refer to harmful substances or influences that can cause disease or exacerbate existing conditions. These toxins are believed to disrupt the balance of Qi, Yin, and Yang in the body
Wind	In traditional Chinese medicine, wind is considered one of the six external pathogens that can invade the body and cause disease. Wind is associated with symptoms such as sudden onset, moving pain, and rapid changes in symptoms
Cold	In traditional Chinese medicine, cold is another external pathogen that can cause disease. Cold is associated with symptoms such as chills, cold limbs, and a preference for warm drinks and environments
Heat	In traditional Chinese medicine, heat is an internal pathogen that can result from external factors or internal imbalances. Heat is associated with symptoms such as fever, thirst, and a preference for cold drinks and environments
Dampness	In traditional Chinese medicine, dampness is an external or internal pathogen that can cause disease. Dampness is associated with symptoms such as a feeling of heaviness, sticky secretions, and a preference for dry environments

limit the generalizability of the findings. Second, the use of a self-designed Chinese Medicine Score may not be validated and could be subject to bias. Third, we did not assess the long-term outcomes or quality of life of the patients after discharge. Fourth, the mechanism of action of FZJDF in severe pneumonia remains to be fully elucidated.

In conclusion, our study suggests that FZJDF combined with prone ventilation may be an effective adjuvant therapy for patients with severe pneumonia. It can improve oxygenation, reduce inflammation, and shorten the duration of mechanical ventilation and ICU stay. However, prospective, multicenter, randomized controlled trials are needed to confirm these findings and further evaluate the safety and efficacy of FZJDF in severe pneumonia. The underlying mechanisms of FZJDF should also be investigated in future studies to better guide its clinical application.

Conclusion

Our study demonstrates that FZJDF combined with prone ventilation is superior to prone ventilation alone in improving oxygenation, reducing inflammation, and shortening the duration of mechanical ventilation and ICU stay in patients with severe pneumonia. These findings suggest that FZJDF is a promising adjuvant therapy for severe pneumonia.

The beneficial effects of FZJDF may be attributed to its unique composition and multifaceted pharmacological actions, which could alleviate the inflammatory storm, regulate immune dysfunction, and inhibit viral replication. This hypothesis is supported by previous studies on the efficacy of traditional Chinese medicine formulas in severe pneumonia.

However, our study has limitations, including the retrospective design, single-center setting, and lack of long-term follow-up. Further high-quality research is needed to validate our findings and guide the evidence-based use of FZJDF in clinical practice. Future studies should include large-scale, prospective, randomized controlled trials, investigate the mechanisms of action, evaluate long-term effects, optimize dosage and administration regimens, and identify subgroups of patients who may benefit most from this intervention.

In conclusion, our study highlights the potential of FZJDF as an adjuvant therapy for severe pneumonia and supports the integration of traditional Chinese medicine and Western medicine in treating critically ill patients. Further research is warranted to validate these findings and guide evidence-based clinical practice.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The requirement of ethical approval was waived by Medical Ethics Committee of Guangdong Hospital of Traditional Chinese Medicine for the studies involving humans because This study was a retrospective study and was exempted from ethical review by the Ethics Committee of our hospital, as per their

requirements for retrospective studies using anonymized data. Informed consent was waived due to the retrospective nature of the study and the use of anonymized data. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin because Informed consent was waived due to the retrospective nature of the study and the use of anonymized data.

Author contributions

HC: Writing–original draft, Writing–review and editing. SL: Writing–original draft, Writing–review and editing. XC: Writing–original draft, Writing–review and editing. TL: Writing–original draft, Writing–review and editing. SZ: Conceptualization, Data curation, Formal Analysis, Writing–original draft, Writing–review and editing. WZ: Formal Analysis, Project administration, Validation, Writing–original draft, Writing–review and editing. JZ: Data curation, Methodology, Writing–original draft, Writing–review and editing. ZL: Data curation, Formal Analysis, Writing–original draft, Writing–review and editing. LC: Data curation, Software, Writing–original draft, Writing–review and editing. BC: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing–original draft, Writing–review and editing. YY: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing–original draft, Writing–review and editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2024.1428817/full#supplementary-material

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frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 09 August 2024
DOI 10.3389/fphar.2024.1443667

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OPEN ACCESS

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RECEIVED 04 June 2024
ACCEPTED 23 July 2024
PUBLISHED 09 August 2024

CITATION
Liu L, Wang B, Ma Y, Sun K, Wang P, Li M, Dong J, Qin M, Li M, Wei C, Tan Y, He J, Guo K and Yu X-a (2024), A review of *Phyllanthus urinaria* L. in the treatment of liver disease: viral hepatitis, liver fibrosis/cirrhosis and hepatocellular carcinoma.
Front. Pharmacol. 15:1443667.
doi: 10.3389/fphar.2024.1443667

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A review of *Phyllanthus urinaria* L. in the treatment of liver disease: viral hepatitis, liver fibrosis/cirrhosis and hepatocellular carcinoma

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Due to the pathological production of liver disease in utility particularly complexity, the morbidity and mortality of liver disease including viral hepatitis, liver fibrosis/cirrhosis and hepatocellular carcinoma (HCC) are rapidly increasing worldwide. Considering its insidious onset, rapid progression and drug resistance, finding an effective therapy is particularly worthwhile. *Phyllanthus urinaria* L. (*P. urinaria*), an ethnic medicine, can be applied at the stages of viral hepatitis, liver fibrosis/cirrhosis and HCC, which demonstrates great potential in the treatment of liver disease. Currently, there are numerous reports on the application of *P. urinaria* in treating liver diseases, but a detailed analysis of its metabolites and a complete summary of its pharmacological mechanism are still scarce. In this review, the phytochemical metabolites and ethnopharmacological

Abbreviations: HCC, Hepatocellular Carcinoma; *P. urinaria*, *Phyllanthus urinaria* L.; HSCs, Hepatic Stellate Cells; HBV, Hepatitis B Virus; HCV, Hepatitis C Virus; NAFLD, Nonalcoholic fatty liver disease; HBx, Hepatitis B virus x Protein; EBV, Epstein-Barr virus; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; IGS, Intergenic spacer; HPLC, High performance liquid chromatography; SIMCA, Soft independent modeling of class analogy; TCM, Traditional Chinese Medicine; CHB, Chronic Hepatitis B; HBeAg, Hepatitis Be antigen; HBsAg, Hepatitis B surface antigen; TNF-α, Tumor Necrosis Factor Alpha; TGF-β, Transforming Growth Factor Beta; ERK, Extracellular signal-regulated kinase; STAT, Signal Transducer and Activator of Transcription; Shh, Sonic Hedgehog; TGF, Transforming growth factor; TLR4, Toll-like receptor 4; ECM, Extracellular matrix; IL-6, Interleukin 6; IL-10, Interleukin-10; IL-17, Interleukin 17; IL-1β, Interleukin 1β; Cyt-c, Cytochrome c; DEN, Diethylnitrosamine; DNA, Deoxyribonucleic Acid; CAT, Cationic amino acid transporter protein; NF-κB, Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells; PI3K, Phosphoinositide 3-Kinase; SOD, Superoxide Dismutase; VEGF, Vascular Endothelial Growth Factor; VEGFR-2, Vascular Endothelial Growth Factor Receptor 2; α-SMA, Alpha Smooth Muscle Actin; HepG2, Hepatoma G2; EGF, Endothelial Growth Factor; GSH, Glutathione; MAPK, Mitogen-Activated Protein Kinase; MMP, Matrix Metalloproteinases; PPAR, Peroxisome proliferator-activated receptor; HMGB1, High mobility group box 1 protein; SMO, Smoothened; PTEN, Phosphatase and tensin homology deleted on chromosome ten; TLR4, Toll-like receptor 4; SD rats, Sprague Dawley rats; TAA, Thioacetamide; CCL4, Carbon tetrachloride; PLGA, Polylactic-co-glycolic acid; ROS, Reactive oxygen species; EMT, Epithelial-to-mesenchymal transition; MET, Mesenchymal-to-epithelial transition.

applications of *P. urinaria* are summarized. Briefly, *P. urinaria* mainly contains flavonoids, lignans, tannins, phenolic acids, terpenoids and other metabolites. The mechanisms of *P. urinaria* are mainly reflected in reducing surface antigen secretion and interfering with DNA polymerase synthesis for anti-viral hepatitis activity, reducing hepatic stellate cells activity, inflammation and oxidative stress for anti-liver fibrosis/cirrhosis activity, as well as preventing tumor proliferation, invasion and angiogenesis for anti-HCC activity via relevant signaling pathways. Accordingly, this review provides insights into the future application of natural products in the trilogy of liver diseases and will provide a scientific basis for further research and rational utilization of *P. urinaria*.

KEYWORDS

Phyllanthus urinaria L., liver disease, viral hepatitis, liver fibrosis/cirrhosis, hepatocellular carcinoma

1 Introduction

Liver disease, a globally widespread disease accounting for 4% of the global death toll each year, is a serious challenge to people's health in different regions with numerous risk factors such as viral infections, excessive alcohol consumption, unhealthy diet, and drug injury (Devarbhavi et al., 2023). The early symptoms of liver disease may not be obvious, whereas the symptoms of abdominal pain, fatigue, jaundice and other concomitant symptoms are present following the aggravation of the infection (Neshat et al., 2021). The progression of liver disease usually passes through three stages including viral hepatitis, liver fibrosis/cirrhosis and hepatocellular carcinoma (HCC), which is called the trilogy of liver disease (Zeng et al., 2023). Infection with Hepatitis B virus (HBV) and Hepatitis C virus (HCV) is a known risk factor for HCC and the major contributor to the progression of chronic viral hepatitis to liver fibrosis/cirrhosis, ultimately leading to HCC (Fan et al., 2020). While vaccination and anti-viral therapy offer potential in decreasing the incidence of viral hepatitis, the emergence of resistance from prolonged use presents a significant obstacle to eradicating the disease (Thomas, 2019). Furthermore, the lack of effective supervision in vaccination, negligence in ensuring injection safety during the vaccination process, potential side effects of oral medications like headaches and fever, and the economic burden of long-term drug use all highlight the ongoing difficulty in finding a definitive cure for the progression of liver disease (Sarin et al., 2020). Consequently, there is a pressing demand for the exploration and development of alternative therapies to comprehensively address the trilogy of liver disease.

Recently, there has been a growing focus on natural products as complementary and alternative medicine for both preventing and treating liver diseases (Calixto et al., 1998). Natural products have potential biotherapy activities including anti-viral, balancing oxidative stress and anticancer effects, protecting the liver, and are widely used in the treatment of liver diseases, thus they are more readily available and widely used in the treatment of diseases (Xu et al., 2024). The *Phyllanthus* encompasses a vast array of species, with over 1,000 species contain more than 500 bioactive metabolites. They have been reported to included alkaloids, flavonoids, lignans, phenol, tannins, and terpenes, as well as others. Among them, about 16 most commonly used *Phyllanthus* species have a significant impact on liver disease, *contains Phyllanthus emblica L., Phyllanthus niruri L., Phyllanthus amarus Schumach,* and *Phyllanthus urinaria L.,* as well as others. *Phyllanthus emblica* L. extract metabolites have been shown to reduce oxidative stress injuries and suppress inflammatory responses, resulting in beneficial therapeutic effects on various liver diseases such as liver injury, fatty liver, viral hepatitis, and liver fibrosis (Jantan et al., 2019). Additionally, the 50% methanol extract of *P. niruri* L. has demonstrated hepatoprotective effects against the progression of nonalcoholic fatty liver disease (NAFLD) in rats. This extract also led to reduced visceral adiposity, improved liver enzyme abnormalities, and decreased hepatic lipid peroxidation and fat accumulation (Kaur et al., 2017). Various species of *Phyllanthus*, including *P. amarus* L. (Reddy et al., 2018), *P. niruri* L. (Venkateswaran et al., 1987), *Phyllanthus nanus* L. (Lam et al., 2006), and *Phyllanthus acidus* L. (Lv et al., 2014) have demonstrated inhibitory effects on hepatitis viruses through *in vitro*, *in vivo* experiments (Thyagarajan et al., 1988), and in clinical trials (Mandal and Hazra, 2023). Meanwhile, *Phyllanthus urinaria* L. (*P. urinaria*) is a well-known member of the *Phyllanthus* family recognized for its therapeutic potential in treating liver disease. Numerous studies have emphasized its effectiveness in managing HBV infection and its utilization in conjunction with traditional medicines for liver disease treatment in ethnomedicine (Qi et al., 2014). Moreover, *P. urinaria* has exhibited hepatoprotective properties by reducing elevated levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in animal models of liver injury (Geethangili and Ding, 2018).

The literature lacks a comprehensive on the treatment of the trilogy of liver diseases by *P. urinaria*. This review aims to provide a comprehensive summarized of the metabolites of *P. urinaria* and their therapeutic effects on liver diseases, specifically focusing on anti-viral hepatitis, anti-liver fibrosis/cirrhosis, and inhibition of HCC. This review includes findings from *in vitro*, *in vivo* experiments as well as insights from clinical applications, as illustrated in Figure 1. Additionally, it provides the morphology, geographic distribution, extraction method, and future challenges in the clinical utilization of *P. urinaria*. The metabolites can be found in Supplementary Table S1 and Supplementary Figure S1–S6, a thorough understanding of the therapeutic mechanisms of *P. urinaria* is essential for optimizing its bioactivity and bioavailability.

2 Methods

Literature for this review was gathered from various search engines and databases, including Web of Science, PubMed,

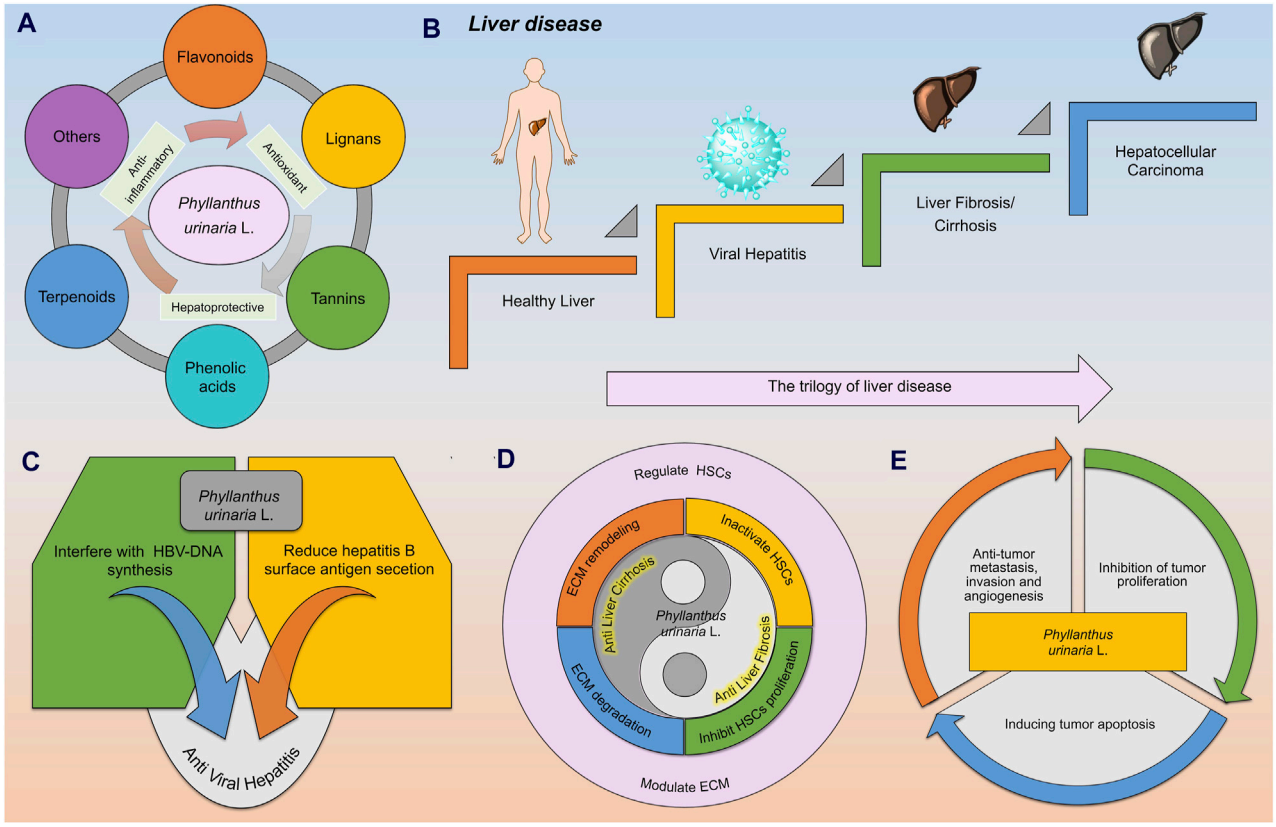

FIGURE 1
The metabolites of *Phyllanthus urinaria* L. (*P. urinaria*) have been identified for their anti-hepatitis properties, potential in reversing liver fibrosis/cirrhosis, and treatment of HCC. **(A)** The metabolites of *P. urinaria* L. include flavonoids, lignans, tannins, phenolic acids, terpenoids, and others (coumarins, steroids, and alkaloids). **(B)** In the progression of liver disease, a healthy liver can be impacted by a hepatitis virus, leading to conditions like liver fibrosis/cirrhosis and HCC. **(C)** *P. urinaria* combats viral hepatitis by interfering with HBV DNA synthesis and reducing the secretion of hepatitis B surface antigen (HBsAg). **(D)** *P. urinaria* shows properties that can help combat liver fibrosis/cirrhosis by regulating hepatic stellate cells (HSCs) and improving extracellular matrix (ECM) function. **(E)** *P. urinaria* exhibits effects against HCC by inhibiting tumor proliferation, promoting tumor apoptosis, and suppressing tumor invasion, metastasis, and angiogenesis.

SciFinder, Science Direct, Embase, Springer Link, and EBSCO. Articles published before May 2024 on the phytochemical and ethnopharmacological applications of *P. urinaria*, its use in treating viral hepatitis, liver fibrosis/cirrhosis, and HCC were reviewed. The search terms used were "*Phyllanthus urinaria* L.", "*Phyllanthus*", "*P. urinaria*", "Liver disease", "Viral hepatitis", "Liver fibrosis/cirrhosis", 'Hepatocellular Carcinoma'. Full-text access had no time limit, and there were no language restrictions on the literature. Additional articles were gathered through literature citations, related sources, and journal websites to ensure comprehensive coverage.

3 Results

3.1 Botanical description, distribution and quality control

3.1.1 Botanical characteristics of *Phyllanthus urinaria* L.

The botanical classification of *P. urinaria* includes its generic name and unique characteristics, an annual plant categorized under the *Plantae*, *Angiospermae*, *Magnoliopsida*, *Malpighiales*, *Phyllanthaceae*, and *Phyllanthus*. The plant features dark reddish-brown fibrous roots, upright stems ranging from 10–60 cm with a brown-red surface, leaves arranged in two rows, oval-shaped capsules that are oblate-spherical and sessile, female flowers are located at the proximal end, while male flowers are found at the distal end (Rossignol et al., 1987). The entire plant including leaves, stems, fruits, and roots, is utilized for medicinal purposes, as depicted in Figure 2, its distinct characteristics include brown roots, leaves, and fruits, with small easily broken brown leaves, and unique microscopic features in roots, leaves, and powder (Gama et al., 2016). This plant is valued by diverse ethnic groups, with its medicinal properties acknowledged in various traditional healing practices in countries like China, India, Indonesia, Japan, and the United States. For example, in India, it is commonly known as "shatter stone", "leaf flower", and "chamber bitter", plays a significant role in ayurvedic treatments for a range of ailments (Sharma et al., 2012). In Brazil, it is referred to as 'arrebenta pedra' and is utilized in local folk medicine to address liver and kidney diseases (Calixto et al., 1998). In China, *P. urinaria* is known as "yexiazhu", "pearl grass", "leaf pearl", or "dragon pearl grass" due to its red and green fruits, and it is used medicinally for conditions such as diarrhea, enteritis, jaundice, and liver ailments, with Traditional

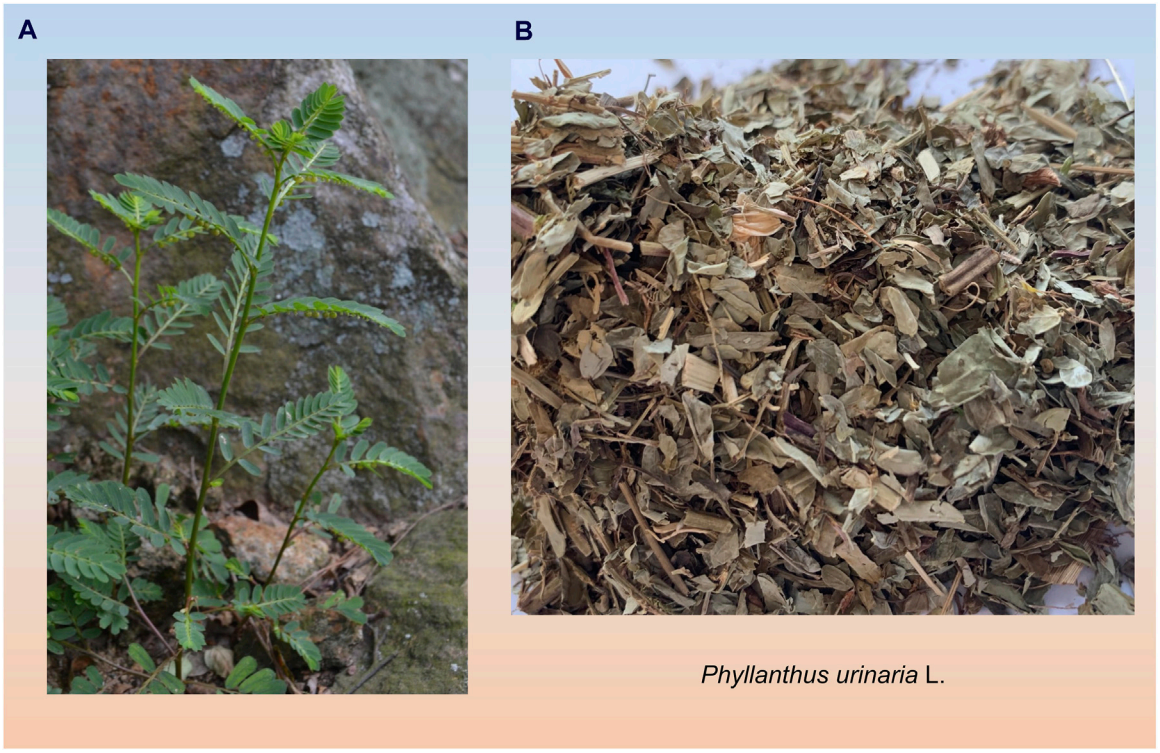

FIGURE 2
Morphology and medicinal parts of *P. urinaria* are depicted in photographs. **(A)** Photographs of the whole botanical drug of *P. urinaria* **(B)** Photographs of the medicinal use of *P. urinaria*.

Chinese Medicine (TCM) recognizing its liver-protective properties (Du et al., 2018).

3.1.2 Distribution and identification of *Phyllanthus urinaria* L.

P. urinaria, commonly found in tropical and subtropical regions such as Asia, Africa, and South America, has been traditionally used for treating a variety of conditions including diabetes (Gunawan-Puteri et al., 2012), heart disease (Chularojmontri et al., 2005; Chularojmontri et al., 2009), bone and joint issues (Buddhachat et al., 2017), inflammation, and neuropathic pain (Dias et al., 1995). *P. urinaria* is vulnerable to contamination by common adulterants, Fang et al. (Fang et al., 2022) employed molecular marker technology to enhance the genome resources of *P. urinaria*, identifying genetic differences through regions such as petA-psbJ, trnS-GCU-trnG-UCC, and trnT-UGU-trnL-UAA. They utilized the intergenic spacer (IGS) to distinguish between different species within the genus and species of *P. urinaria*. Viaene et al. (Viaene et al., 2015) used reversed-phase high performance liquid chromatography with ultraviolet detection (RP-HPLC-UV) to create fingerprints for distinguishing *P. urinaria* from *Phyllanthus,* they recommended the use of soft independent modeling of class analogy (SIMCA) to effectively gather sensitive and specific information on various herbal medicines. Orman and colleagues (Orman et al., 2023) developed a method called Quantitative assessment of multi-components by single markers (QAMS) to monitor the quality of *P. urinaria*. This approach utilizes rutin as a reference standard. The relative correction factors (RCFs) for isoquercitrin, geraniin, and phyllanthin were calculated to assess the levels of metabolites in *P. urinaria* sourced from various locations and harvested at different time points.

3.2 The trilogy of liver disease

Viral hepatitis, resulting from infection with hepatitis viruses, is a significant contributor to liver fibrosis/cirrhosis, and HCC. The types of viral hepatitis, namely, A, B, C, D, and E, are determined by the specific virus causing the infection (Zeng et al., 2021). Worldwide, the primary risk factors for HCC are HBV and HCV infections. HBV and HCV are classified as human carcinogens, with HBV being the predominant cause in Asia and Africa, while HCV is more prevalent in HCC cases in Europe and the Americas (Villanueva, 2019). HBV, a partially double-stranded DNA virus, contains the oncoprotein hepatitis B virus x protein (HBx) in its genome, which plays a crucial role in driving the progression of HCC, sometimes bypassing the end-stage liver disease phase (Ringelhan et al., 2017). Liver fibrosis is a common pathological process in the progression of various chronic liver diseases to cirrhosis. Early diagnosis and treatment of liver fibrosis can prevent the advancement of chronic liver diseases to cirrhosis and HCC, leading to improved outcomes. Viral hepatitis is a well-known risk factor for liver fibrosis, as chronic liver inflammation can result in widespread fibrosis and eventual cirrhosis. Liver fibrosis occurs when the liver is damaged, leading to the deposition of extracellular matrix proteins during the body's

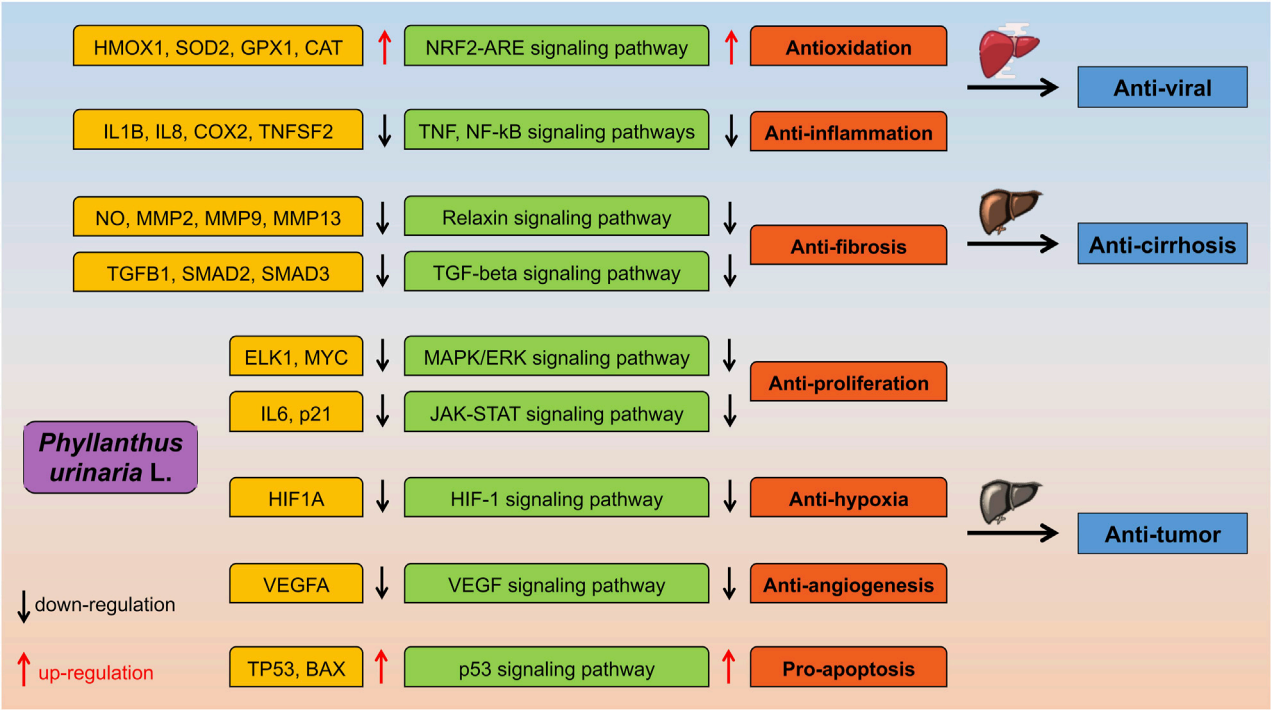

FIGURE 3
The proposed molecular mechanisms of *P. urinaria* suggest its potential anti-viral, anti-fibrosis/cirrhosis, and anti-tumor activities.

repair process (Caligiuri et al., 2021). The development of liver fibrosis/cirrhosis, and subsequent HCC (Gnyawali et al., 2022) from hepatitis B is associated with DNA damage caused by oxidative stress resulting from persistent inflammation, hepatocyte senescence, and alterations in the tumor microenvironment (Karakousis et al., 2020). The HBx gene transactivates the virus during infection and plays a role in influencing the proliferation, differentiation, and apoptosis of hepatocytes through protein interactions, various signaling pathways, and induction of immune imbalance. This is a crucial factor in the development of HCC. The HBx protein is involved in regulating cccDNA, controlling HBV-DNA damage, and disrupting HBV replication within cells. The stability of HBx is strengthened by TNF-α, NF-kB, Wnt/β-catenin, and other pro-inflammatory pathways, which in turn regulate the proliferation and apoptosis of HCC cells (Han et al., 2014). Inflammation plays a crucial role in the progression of liver disease through various mechanisms, the inflammatory environment can contribute to the development of liver fibrosis by activating TNF-β. Persistent inflammation can lead to alterations in ROS levels, which in turn impact oxidation, extracellular matrix (ECM), and collagen synthesis, increasing the likelihood of liver cirrhosis. Furthermore, inflammation and excessive oxidative stress can disrupt the liver microenvironment, triggering abnormal molecular signaling pathways like JNK, PPAR, and AMPK that promote tumor proliferation, metastasis, and progression to HCC. Overall, the interconnected signaling pathways and responses triggered by inflammation, oxidative stress, and microenvironmental changes are key features in the progression of liver diseases, as shown in Figure 3, with both innate and adaptive immunity playing a significant role in the development of chronic liver diseases. (Zhang et al., 2023).

3.3 Application status of *Phyllanthus urinaria* L. in anti-viral hepatitis

3.3.1 Epidemiology and treatment of viral hepatitis

Research indicates that by 2020, deaths from liver diseases linked to viral hepatitis B and C are expected to exceed 1.1 million, surpassing those caused by malaria and Acquired Immune Deficiency Syndrome (Devarbhavi et al., 2023). The majority of these deaths are attributed to hepatitis B, with China, India, and Nigeria carrying the highest burden of the disease (Sarin et al., 2020). Chronic hepatitis B (CHB) can manifest in various ways, including asymptomatic cases, liver fibrosis, advanced cirrhosis, and HCC. It is estimated that there are 296 million individuals living with CHB (Dusheiko et al., 2023), the prevalence of hepatitis C is similar to that of hepatitis B, with a global rate of 0.7% in 2020. Countries with the highest burden of hepatitis C include China, Pakistan, and India (Polaris Observatory, 2022). While preventive vaccination has limited impact on reducing hepatitis B burden (Yuan et al., 2022), nucleotide analogs and antiviral drugs have shown efficacy in treatment, although they may not completely eliminate the risk of HCC development (Roade et al., 2021). Hence, there is a necessity to explore additional complementary and alternative antiviral therapies. Baruch Blumberg conducted both *in vitro* and *in vivo* experiments using an extract from *P. niruri* L., demonstrating a preliminary inhibitory effect on HBV-DNAP (Venkateswaran et al., 1987). Additionally, Thyagarajan conducted clinical trials using *P. amarus* L. (Thyagarajan et al., 1988). Experimental data suggests that *Phyllanthus* has the potential to inhibit HBV virus replication, reverse transcriptase, and DNA polymerase. Initial clinical trials have demonstrated positive outcomes of *P. urinaria* against HBV

(Chen and Zhang, 2023). The pharmacological activities of *P. urinaria*, including extraction methods, mechanisms of action, and effects, are summarized in Supplementary Table S2.

3.3.2 The metabolites of anti-viral hepatitis

The anti-viral hepatitis metabolites of *P. urinaria* are detailed in Supplementary Table S1, with its biological activities primarily linked to antioxidant properties stemming from hydroxyl groups (Sarin et al., 2014). The use of the entire plant for medicinal purposes or extracting it with polar solvents like methanol, ethanol, or aqueous has demonstrated positive physiological effects. Various extracts of *P. urinaria*, including aqueous, methanol, ethanol, and acetone, have exhibited anti-bacterial, anti-diabetic, anti-malarial, antioxidant, and anti-tumor properties, suggesting potential benefits for treating conditions such as viral infections, jaundice, hypertension, diabetes, and cancer. For example, research by Yang et al. (Yang et al., 2005) revealed that methanol, ethanol, and acetone extracts of *P. urinaria* could hinder HSV-2 infection at the early stages. HSV-2, a large double-stranded DNA virus causing herpes infections, was effectively inhibited by the aqueous extract of *P. urinaria*, with metabolites like trigalloylglucopyronoside playing a key role in preventing virus entry and disrupting host protein manipulation (Tan et al., 2013). Ellagitannins (1-Galloyl-3,6-hexahydroxydiphenoyl-4-O-brevifolin-carboxyl-β-D-glucopyranose, geraniin, corilagin) isolated from the ethanol extract of *P. urinaria* were found to inhibit Epstein-Barr virus (EBV) activity effectively (Zeng et al., 2021). Furthermore, the methanol and chloroform extracts of *P. urinaria* demonstrated significant inhibition of *Helicobacter pylori* infection (Lai et al., 2008), potentially through the regulation of the NF-κB pathway by *P. urinaria* to reduce inflammation. *P. urinaria* is utilized for treating hepatitis. Various metabolites such as trimethyl-3,4-dehydrochebulate, methyl gallate (Fang et al., 2008), gentisic acid 4-O-β-D-glucopyranoside, syringin, ascorbic acid, arbutin, brevifolin carboxylic acid, gallic acid, and protocatechuic acid (Xu et al., 2007) have demonstrated significant biological activity in inhibiting tyrosinase (Hu et al., 2014). Moreover, metabolites like cucurbic acid, α-methyl cucurbate (Urakawa et al., 2004), and phyllanthurinolactone, a glycoside metabolite isolated from *P. urinaria*, have shown promise for the development of bioactive molecular probes. Guo et al. (Guo et al., 2021) utilized mechanochemical-assisted extraction (MCAE) to successfully isolate and identify ellagic acid in the ethanol extract of *P. urinaria*, achieving a rate of 10.2 mg/g. Additionally, gallic acid ethyl ester (GAEE) (Santos et al., 1999) was detected in the ethanol extract of *P. urinaria*, exhibiting analgesic and other effects (Paulino et al., 1999).

3.3.3 Anti hepatitis C

Hepatitis C is caused by a single-stranded RNA virus, Chung et al. (Chung et al., 2016a) demonstrated that the acetone extract of *P. urinaria*, a monoterpene lactone named loliolide, can inhibit HCV infection in a dose-dependent manner. Subsequent research by Chung et al. (Chung et al., 2016b) revealed that (4R, 6S)-2-dihydromenisdaurilide (DHMD), isolated from the acetone extract of *P. urinaria*, has the potential to treat hepatitis C by inhibiting HCV infection in Huh7 cells and preventing HCV entry into cells during the initial infection stage. Interestingly, DHMD exhibits selective inhibition towards HCV by targeting viral glycoprotein E1/E2, hindering glycoprotein-receptor interaction and conformational changes. This suggests DHMD's potential as a small molecule inhibitor, particularly for application in transplantation settings. Another metabolite, corilagin, identified from the methanol extract of *P. urinaria*'s whole plant, has also shown effectiveness in inhibiting HCV activity. The mechanism of action may involve corilagin binding to HCV N53 protease, forming an enzyme-inhibitor complex that can alter or deactivate the enzyme's activity structure (Yue et al., 2005).

3.3.4 Interfere with HBV DNA synthesis

In patients with Hepatitis Be antigen (HBeAg) positivity, higher HBV DNA levels in individuals with CHB are linked to a high risk of liver fibrosis and HCC (Liu et al., 2014). The analytical review conducted by Chen et al. (Chen and Zhu, 2013) revealed that *P. urinaria* inhibitory effect on plasma DHBV DNA could reach to over 45%. Moreover, ellagic acid, a phenolic metabolite, demonstrated a protective effect on HBV infection by interfering the secretion of HBeAg rather than interfering with the secretion of HBV DNA, highlighting the significance of quantitatively evaluating HBV DNA in future research. (Aishwarya et al., 2021). Shin et al. (Shin et al., 2005) isolated ellagic acid from *P. urinaria* extracts and demonstrated reduced HBeAg secretion in HepG2 2.2.15 cells post-treatment. Kang et al. (Kang et al., 2006) conducted *in vivo* experiments on HBeAg-producing transgenic mice, indicating that ellagic acid can boost immune responses, decrease HBeAg levels, and enhance cytokine release, thereby confirming its effectiveness against hepatitis B.

3.3.5 Reduce hepatitis B surface antigen secretion

P. urinaria demonstrates a strong anti-HBV effect, with phenolic acid, and other metabolites playing a crucial role in its anti-viral properties. Extracts and metabolites from *P. urinaria* have been found to reduce the secretion of HBsAg and HBeAg, as well as decrease serum enzyme levels (Upadhyay and Tiwari, 2023). Monitoring HBeAg and HBsAg levels is essential during anti-viral treatment to evaluate treatment efficacy and disease prognosis due to the burden of HBsAg leading to T cell and B cell depletion (Loggi et al., 2022). Wu et al. (Wu et al., 2015) utilized a recombinant eukaryotic expression plasmid pHBV1.1 to transfect HepG2 cells and establish an *in vitro* model of HBV for assessing the direct anti-viral impact of *P. urinaria* on HBV. Their study revealed that *P. urinaria* extract at concentrations of 0.8 and 0.2 g/L significantly interfered with HBsAg levels. Lamivudine (LMV), a common nucleotide analog for hepatitis B treatment, often leads to drug resistance in 80% of patients after 5 years of treatment. Lam et al. (Lam et al., 2006) engineered a mutant of HBV P protein and introduced it into HepG2 cells to create an *in vitro* model of LMV resistance. Exposure to varying concentrations of *P. urinaria* aqueous extract resulted in a dose-dependent decrease in HBsAg and HBcAg secretion levels. This effect may be attributed to the activation of immunity and induction of COX-2 by *P. urinaria* extract through the ERK1/2 and JNK signaling pathways, leading to increased IL-6 mRNA expression and subsequent interfere of HBV replication. Liang et al. (Liang et al., 2019) identified 16 phenolic compounds in the ethanol extract of *P. urinaria* and evaluated their ability to inhibit HepG2.2.15 cells *in vitro*. The results showed that

emodin-8-O-β-D-glucopyranoside, catechin, 3-O-methylgallic acid, ethyl gallate, and protocatechuic acid significantly reduced HBsAg secretion, potentially due to their antioxidant properties. Conversely, Fu et al. (Fu et al., 2023) utilized network pharmacology, *in vitro* experiments, protein interaction networks, and functional enrichment analysis to identify key metabolites in *P. urinaria*, such as gallic acid, kaempferol, quercetin, corilagin, and rutin. The signaling pathways are mainly related to TNF, VEGF, and JAK/STAT. *In vitro* assays have shown that *P. urinaria* extract can reduce HBsAg, HBeAg levels in a dose-dependent and time-dependent manner. This effect may be linked to the increased expression of NFE2L2 and HMOX1 proteins by *P. urinaria*.

3.3.6 Clinical applications

A systematic review indicated that *P. urinaria* had beneficial effects on CHB and improved liver function. The findings revealed a decrease in serum HBsAg secretion in patients treated with *P. urinaria* compared to those receiving a placebo. Moreover, *P. urinaria* was found to be more effective in interfering HBV DNA synthesis and reducing HBeAg levels compared to other botanical drug treatments, as well as superior to interferon monotherapy (Liu et al., 2001a). In a separate systematic review focusing on the treatment of CHB using Chinese herbal medicine, it was found that *P. urinaria* showed a comparable therapeutic effect to interferon. Additionally, *P. urinaria* demonstrated a significant impact in reducing HBeAg levels and interfering with HBV DNA synthesis (Liu et al., 2001b). In a meta-analysis of 53 randomized controlled trials, *P. urinaria* demonstrated superior therapeutic effects compared to interferon alone. *Phyllanthus urinaria* showed OR values of 2.05, 2.17, and 3.7 in interfering with HBV DNA synthesis, lowering HBeAg levels, and improving abnormal ALT values, indicating its potential anti-HBV activity (Zhang et al., 2010). In a clinical trial conducted by Xing et al. (Xing et al., 2020), the Bushen Qingtou prescription, a TCM compound, was administered to CHB patients with normal ALT levels. The study involved 395 patients in a 96-week trial, where those receiving oral TCM experienced a significant reduction in HBV DNA levels by 4 log10 IU/mL at week 48% and 10.05% at week 96, as opposed to 2.55% in the placebo group. These findings suggest that the TCM compound is more effective in decreasing HBV DNA and HBeAg values, potentially through *P. urinaria*'s modulation of host immune function. In a phase II clinical trial by Chan et al. (Chan et al., 2003), varying doses of *P. urinaria* extract were administered to patients with HBeAg-positive status, elevated ALT levels, and high HBV DNA levels for 24 weeks. The results indicated a reduction in HBV DNA levels in the treatment group compared to the placebo group.

3.4 Application status of *Phyllanthus urinaria* L. in anti-liver fibrosis/cirrhosis

3.4.1 Epidemiology and treatment of liver fibrosis/cirrhosis

The age-standardized rate of cirrhosis decreased from 25.7 per 100,000 in 1990 to 25.3 per 100,000 in 2019, with a higher prevalence among men than women. Cirrhosis is characterized by liver damage and fibrosis, the primary causes of liver fibrosis/cirrhosis include HBV and HCV infections, nonalcoholic steatohepatitis, and alcoholism, with an increasing incidence of HCV and alcoholism. Chronic liver damage often results from a combination of factors such as chronic viral hepatitis, alcohol consumption, obesity, and others, leading to the development of cirrhosis (Wang et al., 2024). Patients with compensated cirrhosis have a 4.7 times higher risk of death compared to the general population, which increases to 9.7 times higher in those with decompensated cirrhosis. The economic impact of cirrhosis and its complications, such as hemorrhage and ascites, is significant worldwide. Therefore, research on novel drug therapies for liver fibrosis/cirrhosis remains a crucial area of study (Ge and Runyon, 2016). The progression of liver fibrosis/cirrhosis involves various processes including the activation of Hepatic Stellate Cells (HSCs), apoptosis of liver parenchymal cells, capillarization of liver sinusoidal endothelium, epithelial-mesenchymal transition (EMT), and ECM deposition (El Awdan and F. Asaad, 2021). HSCs activation, marked by increased expression of cell phenotype markers like α-SMA and collagen, plays a central role in this progression (Zhang et al., 2022). Treatment with antiviral agents and anti-inflammatory medications can be beneficial in the prevention and management of liver fibrosis and cirrhosis.

Various approaches include inhibiting HSCs activation, collagen fiber deposition, promoting ECM matrix protein degradation, and antagonizing cytokines involved in liver fibrosis. While anti-viral therapy can slow down the progression of liver fibrosis/cirrhosis, its effectiveness is limited in preventing cirrhosis from advancing to HCC (Chang et al., 2010). Currently, there are no approved drugs specifically designed for liver fibrosis treatment, emphasizing the necessity for diverse strategies to address the intricate pathology of liver cirrhosis. Zhao et al. (Zhao et al., 2022) utilized systems biology and network informatics to pinpoint targets and key metabolites in natural drugs for liver fibrosis treatment. KEGG analysis unveiled connections with inflammation and energy metabolism, with drug targets associated with EGFR, PTGS2, ICAM1, TNF, IL-2. Excessive oxidative stress can activate HSCs, and Li et al. (Li et al., 2023) suggest that TCM can regulate oxidative stress to alleviate liver fibrosis. The pharmacological activities of *P. urinaria* are summarized in Supplementary Table S3, detailing the mechanisms of action and effects.

3.4.2 The metabolites of anti-liver fibrosis/cirrhosis

The anti-liver fibrosis/cirrhosis metabolites of *P. urinaria* is detailed in Supplementary Table S1. Flavonoids, known for their antioxidant properties, have been successfully extracted from *P. urinaria*. With the help of ESI-MS and NMR techniques to isolate various flavonoids, such as astragalin and quercetin 3-rutinoside, from the methanol extract of *P. urinaria*. They also discovered a new flavonoid named urinariaflavone (Thanh et al., 2014). *P. urinaria*, commonly employed in treating inflammatory conditions, is known for its heat-clearing, diuretic, liver-calming, and eyesight-improving effects. Metabolites like rhamnocitrin, quercitrin, and rutin (Fang et al., 2008) have demonstrated anti-inflammatory properties. Deng et al. (Deng et al., 2012) used ultraviolet capillary zone electrophoresis (CZE) and electrochemical detection (ED) to accurately identify active ingredients like quercitrin and rutin in *P. urinaria* extract. Analysis through NMR, HR-ESI-MS, and IR techniques revealed the presence of four flavonoids in the ethanol extract of *P. urinaria*, including quercetin 3-O-α-L-rhamnopyranoside, quercetin, quercetin 3-O-α-L-(2,4-di-

O-acetyl)rhamnopyranoside-7-O-α-L-rhamnopyranoside, and quercetin 3-O-α-L-(3,4-di-O-acetyl)rhamnopyranoside-7-O-α-L-rhamnopyranoside (Wu et al., 2013). Among these metabolites, kaempferol 7-methyl ether, quercetin 3-O-β-D-glucoside, and rutin (Xu et al., 2007) exhibited free radical scavenging activity. Furthermore, epigallocatechin and quercetin 3-(4′-acetylrhamnoside) 7-rhamnoside were identified (Han N. et al., 2023).

Lignans metabolites in *P. urinaria* have been extensively researched, with a particular focus on one dominant species of the metabolites. Fang et al. (Fang et al., 2008) utilized NMR and ESI-MS techniques to identify nine anti-inflammatory and antioxidant metabolites in *P. urinaria* ethanol extract. Chang et al. (Chang et al., 2003) also conducted a similar analysis on lignans from *P. urinaria*, identifying known metabolites like phyllanthin, niranthin, phyltetralin, hypophyllanthin, nirtetralin, lintetralin, isolintetralin, heliobuphthalmin lactone, and virgatusin (Mao et al., 2016), as well as newly discovered 5-Demethoxyniranthin, urinatetralin, dextrobursehernin, and urinaligran. In addition, Han et al. (Han N. et al., 2023) isolated dihydrocubebin, lyoniresiol, (7S, 7′S, 8R, 8′R)-icariol A2, evofolin B, 4-oxopinoresinol, syringaresinol, episyringaresinol from *P. urinaria* extract. Boehmenan and repandusinic acid A are extracts from the roots, stems, and leaves of *P. urinaria*, known for their inhibitory effects on α-glucosidase. Using HPLC-SPE-NMR, seven structurally similar lignans were analyzed from the methanol extract of *P. urinaria* (Wang and Lee, 2005), with niranthin, phyllanthin, and phyltetralin being the main metabolites identified, alongside hypophyllanthin, virgatusin, nirtetralin, and lintetralin. Fan et al. (Fan et al., 2015) developed a precise and rapid HPLC-MS/MS method for the specific identification of four lignans found in rat plasma after oral administration of *P. urinaria* extract, which includes niranthin, nirtetralin, hypophyllanthin, and phyllanthin, this method shows potential for qualitative analysis of other lignans.

3.4.3 Inactivate HSCs and inhibit HSCs proliferation

The activation of HSCs is influenced by changes in the cell microenvironment, leading to metabolic reprogramming, increased collagen synthesis, decreased apoptosis, delayed senescence, and reduced immune clearance. These factors collectively contribute to the progression of liver fibrosis. In liver fibrosis diseases, the upregulation of lysyl oxidases (LOX), tissue inhibitor of metalloproteinase 1 (TIMP1) and α-smooth muscle actin (α-SMA) are observed alongside HSCs activation (Cai et al., 2020). Abnormal activation of signaling pathways such as TGF-β/Smad, Wnt/β-catenin, Notch, PI3K/Akt, Hedgehog, Hippo/YAP, MAPK, and Toll-like receptor 4 (TLR4) can further promote HSCs activation. Studies suggest that reducing levels of α-SMA, CTGF, PDGFA/B, VEGFA, IL-11, IL-6, and CXCL12 may help inactivate HSCs (Dou et al., 2018). Flavonoids, terpenoids, alkaloids, and other plant metabolites offer a natural approach with multi-target, multi-level, and multi-channel advantages to regulate related proteins and potentially reverse liver fibrosis (Zheng et al., 2023). Natural products provide advantages such as easy accessibility, potent pharmacological effects, and minimal side effects, showing promising therapeutic potential in the treatment of liver fibrosis.

Phenolic acids and flavonoids have been shown to possess anti-liver fibrosis properties by regulating hepatocyte apoptosis through specific signaling pathways (Xu et al., 2023). Kumaran et al. (Kumaran and Joel Karunakaran, 2007) conducted research demonstrating the antioxidant activity of five *Phyllanthus* plant species, with *P. urinaria* ranking second. Quercetin, known for its strong antioxidant and anti-inflammatory properties (Shen et al., 2008), has been found to inhibit HSCs activation by affecting the NF-κB/MAPK and Bcl-2/BAX pathways, thereby delaying the progression of liver fibrosis. In an induced liver fibrosis model in Sprague Dawley (SD) rats, treatment with quercetin resulted in reduced levels of serum markers, improved liver appearance, decreased collagen deposition, downregulated NF-κB/p38, and MAPK/BAX expression, increased Bcl-2 levels, and reduced marker of HSCs activation and inflammatory factors (Wang et al., 2017). Liposomal modification of quercetin has been shown to improve liver fibrosis induced by concanavalin A in mice. Treatment with liposomal quercetin led to enhanced collagen deposition in the liver, inhibition of HSCs activation, reduced expression of α-SMA, NF-κB, and TGF-β (Wan et al., 2014). Li et al. (Li et al., 2016) confirmed the effectiveness of quercetin in improving liver fibrosis through both *in vivo* and *in vitro* experiments. Using a mouse liver fibrosis model induced by intraperitoneal injection of CCL4, it was observed that oral quercetin treatment resulted in lower portal inflammation scores, decreased serum enzymes ALT and AST, as well as reduced collagen deposition and fibrosis scores. *In vitro* experiments demonstrated that quercetin treatment led to decreased expression of TGF-β1, α-SMA, HMGB1, TLR2/TLR4, and NF-κB p65 proteins in COL1α1 mRNA, suggesting that quercetin may inhibit HSCs activation and improve liver fibrosis by modulating the HMGB1/TLR/NF-κB signaling pathway. Aslam et al. (Aslam et al., 2022) also showed that quercetin has the potential to ameliorate liver fibrosis by antagonizing the hedgehog pathway. Their study induced liver fibrosis in SD rats using thioacetamide (TAA) and observed various indicators post-quercetin treatment. The results indicated an increase in serum SOD levels and a decrease in the expression of hedgehog pathway markers Shh, Ihh, Ptch-1, Smo, and Gli3, as well as inflammation-related markers TNF-α and NF-κB. This suggests that quercetin may regulate the hedgehog pathway and oxidative stress to improve liver fibrosis. Additionally, Hernandez-Ortega et al. (Hernandez-Ortega et al., 2012) found that quercetin reduced AST, ALT, and GGT levels in mice with liver fibrosis, decreased the area of liver fibrosis and collagen deposition, increased SOD, CAT serum enzymes and mRNA levels, inhibited inflammation by reducing the expression of markers TNF-α, IL-6, TGF-β1, COL1α1, CTGF, TIMP-1, and α-SMA, induced HSCs apoptosis, and increased the expression of anti-liver fibrosis molecules TGF-β1, MMP2, and MMP9. Quercetin has also been shown to combat liver fibrosis by modulating macrophage activation and polarization in both *in vitro* and *in vivo* experiments (Li et al., 2018). *In vivo* studies have shown that treatment with quercetin significantly improves necrotic inflammation damage and liver collagen deposition induced by CCL4. Quercetin also inhibits the expression of α-SMA, a marker of HSCs activation, reduces protein levels of desmin and vimentin, downregulates macrophage markers F4/80 and CD68, decreases mRNA expression of TNF-α, IL-1β, IL-6, and MCP-1, and reduces macrophage recruitment. Additionally, *In vitro* experiments have revealed that quercetin inhibits the polarization of M1 macrophages in RAW264.7 cells, resulting in

reduced levels of TNF-α, IL-1β, IL-6, and NOS2, as well as decreased expression of Notch1. Another flavonoid, kaempferol found in *P. urinaria*, has also shown potential in reversing liver fibrosis (Zhou et al., 2022). In a mouse model of CCL4-induced liver fibrosis, treatment with kaempferol led to improved collagen deposition and reduced expression of α-SMA and COL1α1. The mechanism underlying the inhibition of HSCs differentiation may involve the downregulation of Notch/Jag1 and the upregulation of miR-26b-5p expression. Xu et al. (Xu et al., 2019) investigated the therapeutic effects of kaempferol on mice with CCL4-induced liver fibrosis. The study demonstrated that kaempferol led to significant repair of liver damage, reduction in serum levels of HA, LN, AST, and ALT, decreased collagen deposition, and downregulation of COL1α1 and α-SMA RNA and protein expression. *In vitro* experiments suggested that kaempferol could potentially reduce the elevation of Smad2 and Smad3 induced by TGF-β1 by selectively binding to the ATP binding site of ALK5. Besides, gallic acid was found to possess anti-inflammatory and antioxidant properties. El-Lakkany et al. (El-Lakkany et al., 2019) confirmed through *in vivo* and *in vitro* experiments that gallic acid effectively regulates the proliferation and activation of HSCs for the treatment of liver fibrosis. Gallic acid exhibited the ability to inhibit HSCs cell proliferation in a dose-dependent and time-dependent manner, decrease α-SMA expression, lower the levels of liver injury markers such as PCNA, PDGF-BB, TIMP-1, HP, and reduce collagen deposition in a rat model of liver fibrosis.

3.4.4 ECM degradation and remodeling

ECM degradation and remodeling are crucial processes in the dynamic formation of liver fibers. Matrix metalloproteinases (MMPs), including collagenases (MMP1, MMP8, MMP13) and gelatinases (MMP2, MMP9), play a significant role in ECM degradation, with MMP12 also contributing. Elastase is essential for activating MMPs, maintaining a balance between MMP/TIMP levels and VEGF-mediated angiogenesis is vital for improving fibrosis and tissue remodeling (Rockey et al., 2015). The pathophysiological characteristics of liver cirrhosis involve liver damage and fibrosis. Treatment approaches for liver cirrhosis encompass the management of hepatitis infections, reduction of cirrhosis risk factors, and prevention of liver damage from harmful substances (Ge and Runyon, 2016). *Phyllanthus urinaria* L. exhibits hepatoprotective potential (Srirama et al., 2012) and may offer benefits in addressing liver damage conditions (Sharma et al., 2011). Existing literature suggests that *P. urinaria* can inhibit the cytochrome P450 enzyme, thus reducing liver toxicity and providing protective effects to the liver. Ellagic acid and quercetin, identified as the metabolites in *P. urinaria*, have demonstrated effective protection of rat livers from damage induced by TAA (Lee et al., 2014).

Research findings suggest that ellagic acid may regulate TAA-induced hepatitis by modulating the mitogen-activated protein kinase through the TLR/MAPK/NF-κB signaling pathway. *In vivo* experiments on TAA model mice showed elevated levels of serum enzymes ALT, AST, and ALP, indicative of liver damage. Additionally, increased MDA levels and decreased TAC and GPX levels pointed to lipid peroxidation, while upregulation of MMP2 and MMP9 indicated liver fibrosis progression. However, treatment with ellagic acid and quercetin resulted in decreased ALT, AST, and ALP levels, reduced MDA levels by 34.8% and 54.65%, significantly increased TAC and GPX levels, and decreased expression of MMP2 and MMP9. These results suggest the potential therapeutic use of ellagic acid and quercetin in mitigating liver fibrosis (Afifi et al., 2018). CCL4 has been demonstrated to induce liver damage in rats, while *P. urinaria* has been shown to have a liver-protective effect. In a study by Lee et al. (Lee et al., 2006), treatment with the methanol extract of *P. urinaria* resulted in decreased levels of GOT and GPT enzymes in liver fibrosis model rats. Additionally, the methanol extract of *P. urinaria* led to increased levels of SOD, GSH, and GPX, ultimately reducing liver tissue necrosis. Four lignans, namely, hypophyllanthin, phyllanthin, nirtetralin, and niranthin, have been identified as quality control markers for *P. urinaria* extract. Guo and colleagues (Guo et al., 2017) used metabolomics to investigate the protective mechanism of *P. urinaria* against CCL4-induced liver damage. Their findings suggest that *P. urinaria* can alleviate branched-chain amino acid catabolism disorders and enhance free radical scavenging activity by modulating L-carnitine, taurocholic acid, and amino acid metabolism to ameliorate liver damage.

Phyllanthin, a lignan metabolite found in *P. urinaria* extract, plays a crucial role in regulating the innate immune response of phagocytes, scavenging free radicals, acting as an antioxidant, and protecting the liver (Yuandani et al., 2013). Studies have also shown that phyllanthin can protect against liver damage by modulating reactive oxygen species (ROS) (Chirdchupunseree and Pramyothin, 2010) and reducing lipid peroxidation to alleviate ethanol-induced liver damage (Krithika et al., 2009). In a study by Krithika et al. (Krithika et al., 2016), female mice were orally administered CCL4 to induce a liver fibrosis model. Treatment with phyllanthin was found to improve liver fibrosis by reducing the levels of TNF-α, NF-κB, and TGF-β1 in liver tissue. Additionally, it decreased the levels of LPO, ALT, and AST, as well as the mRNA expression of liver fibrosis markers such as fibronectin, TGF-β1, and COL1α1. Moreover, phyllanthin decreased the protein expression of TNF-α and NF-κB p65, and reduced collagen deposition to counteract ECM synthesis.

Encapsulating phyllanthin in nanoparticles using polylactic-co-glycolic acid (PLGA) enhanced its bioavailability and mitigated liver fibrosis by reducing collagen deposition (Krithika et al., 2019). Phyllanthin nanoparticles, prepared with PLGA, acetone, and Tween via the nanoprecipitation method, were administered to a CCL4-induced liver fibrosis model. The results showed that treatment with phyllanthin nanoparticles resulted in decreased ALT and AST levels, repair of cell membrane damage from CCL4, and improvement in liver fibrosis. Krithika et al. (Krithika et al., 2015) employed molecular docking to identify the specific binding site of phyllanthin and ALK5, followed by *in vivo* experiments to validate the anti-fibrosis mechanism of phyllanthin. The molecular docking results indicated a strong binding affinity between phyllanthin and ALK5. After phyllanthin treatment, ALT, AST, and liver collagen levels decreased, reversing pathological liver tissue changes induced by CCL4. Furthermore, the expression of TGF-β1, ALK5, p-Smad2, and p-Smad3 decreased played a crucial role in ECM remodeling.

Huangjia Ruangan Granule (HJRG) is a compound preparation of TCM known for its anti-liver fibrosis properties (Cai et al., 2022).

It comprises various metabolites such as *P. urinaria*, Hedysarum Multijugum Maxim (Huangqi), Trionyx sinensis Wiegmann (Biejia), Radix Puerariae (Gegen), Radix Bupleuri (Chaihu), Ganoderma (Lingzhi), Radix Paeoniae Rubra (Chishao), Radix Salviae (Danshen), Panax Notoginseng (Sanqi), and Abri Herba (Jigucao). Through an HPLC test conducted on a CCl4-induced liver fibrosis model in SD rats, the presence of gallic acid, puerarin, tanshinone IIA, and other metabolites was confirmed. Treatment with HJRG led to improvements in liver tissue necrosis, inflammation, and fatty degeneration in the rats. There were significant reductions in parameters such as fiber intervals, severity scores, and collagen fiber-positive areas. Moreover, the levels of AST and ALT enzymes decreased, along with PCI, COL IV, LN, HA, and α-SMA, suggesting that HJRG has the potential to alleviate liver damage and fibrosis. The mechanism behind this effect is likely linked to HJRG's ability to reduce oxidative stress and inflammation, possibly through modulation of the TNF-α/MAPK and NF-κB signaling pathways. Corilagin, a vital metabolite found in *P. urinaria* extract, has demonstrated efficacy in treating liver fibrosis/cirrhosis caused by schistosomiasis. This therapeutic effect is attributed to its modulation of the IL-13 signaling pathway and suppression of GATA3 to regulate the Th1/Th2 balance (Huang et al., 2013). Moreover, corilagin displays hepatoprotective properties by reversing elevated levels of AST and ALT enzymes induced by liver injury, reducing liver MPO activity, and increasing inflammatory markers like CINC-1, CINC-3, ICAM-1, IL-6, and TNF-α. The administration of corilagin can counter these alterations, potentially through the inhibition of the PI3K/Akt signaling pathway (Liu et al., 2017).

3.4.5 Clinical applications

Clinical trials have demonstrated that anti-viral therapy can significantly enhance the clinical outcomes of liver fibrosis/cirrhosis by eradicating or managing the disease-causing factors (Wang et al., 2022). While nucleotide analogs alone may have limited efficacy in reversing liver fibrosis/cirrhosis induced by hepatitis, the addition of TCM can be a viable complementary treatment approach (Zhang and Schuppan, 2014). In a multi-center clinical controlled trial led by Rong et al. (Rong et al., 2022), the therapeutic benefits of Biejia Ruangan Tablets combined with nucleoside analogs were assessed in patients with CHB related liver fibrosis, revealing a higher rate of fibrosis/cirrhosis regression in those treated with Biejia Ruangan Tablets. The combination of Fuzheng Huayu (FZHY) - comprising Radix Salvia Miltiorrhizae (Danshen), Pollen Pini (Songhuafen), Semen Persicae (Taoren), Gynostemma Pentaphyllammak (Jiaogulan), Cordyceps (Chongcao) - and entecavir (ETV) has been proven to ameliorate liver fibrosis by collagen degradation without inducing hepatotoxicity (Gui et al., 2020). In a study by Xing et al. (Xing et al., 2023), a multi-center randomized controlled clinical trial was conducted to evaluate the efficacy of Ruangan Granules (*P. urinaria*, Trionycis Carapax, Notoginseng Radix et Rhizoma, Salviae Miltiorrhizae Radix et Rhizoma, Persicae Semen, Astragali Radix, Atractylodis Macrocephalae Rhizoma, Poria, Curcumae Radix, Aurantii Fructus, Lobeliae Chinensis Herba, Schisandrae Chinensis Fructus) in patients with CHB related liver fibrosis. The study included 240 patients with severe liver fibrosis/cirrhosis and CHB, who were treated with Ruangan Granules in addition to anti-HBV drugs for 48 weeks. Results indicated that the group receiving Ruangan Granules showed improved liver pathology, reduced fibrous tissue proliferation, lower Knodell HAI score, Ishak score, and ultrasound semi-quantitative score, liver stiffness measurement (LSM), suggesting an improvement in liver fibrosis. Furthermore, there was an increase in HBV DNA negative conversion rate, ALB levels, and a decrease in ALT and AST levels. Follow-up after 2 years revealed a lower incidence of HCC in the Ruangan Granule group with no significant adverse events, indicating a favorable safety profile of the drug.

3.5 Application status of *P. urinaria* in anti-HCC

3.5.1 The epidemiology and treatment of HCC

Primary liver cancers, particularly HCC, are a significant global public health concern, ranking sixth in incidence and third in mortality among tumors (Bray et al., 2024). Treatment approaches for HCC are determined by factors such as liver function, tumor burden, and cancer stage. Options include curative therapies like liver transplantation, resection, transarterial chemoembolization, and radiofrequency ablation, often used in combination with early-stage cases (Torimura and Iwamoto, 2022). Non-definitive therapies for HCC include first-line systemic treatments such as transarterial radioembolization, transarterial chemoembolization, antibody therapies, immune checkpoint inhibitors, and molecularly targeted agents. However, due to the insidious nature of HCC onset and constraints in diagnostic capabilities, only approximately 20% of patients qualify for curative treatment. The majority of HCC cases, over 80%, are detected at an advanced stage, precluding the opportunity for curative interventions (Zhou et al., 2023). The use of botanical drugs or natural products derived from botanical drugs in the prevention and treatment of HCC provides a complementary alternative therapy. Natural products have advantages in the treatment of HCC by reducing the side effects linked to radiotherapy and chemotherapy, improving long-term effectiveness, and catering to patients who are not suitable for surgery, radiotherapy, or chemotherapy. These treatments can assist in tumor control, symptom relief, and enhancing quality of life. Additionally, they can enhance the body's immune response, reduce metastasis and postoperative recurrence, and potentially prolong the survival of some patients with tumors. Several studies have demonstrated the potential anticancer properties of *P. urinaria*. For example, it has been found that the aqueous extract of *P. urinaria* can induce apoptosis in HL-60 cells through the ceramide-related pathway, leading to upregulation of caspase 3 and BAX expression while downregulation of the expression of Bcl-2, Fas receptor, and Fas ligand genes, thus showing promise in treating human myelogenous leukemia (Huang et al., 2004a). Additionally, research has shown that *P. urinaria* extract can induce apoptosis in Lewis cells and combat lung cancer through the mitochondria-related intrinsic pathway, with upregulation of caspase 3 expression and downregulation of Bcl-2 expression, all while demonstrating minimal toxicity to normal cells (Huang et al., 2003). Supplementary Table S4 summarizes the pharmacological activities of *P. urinaria* based on extraction methods, mechanisms applied, and effects.

3.5.2 The metabolites of anti-HCC

The anti-HCC metabolites of *P. urinaria* are highlighted in Supplementary Table S1. Utilizing a combination of tannin ultrasonic-assisted extraction (UAE) and response surface methodology (RSM) proves advantageous for the extraction and characterization of phenolic metabolites from *P. urinaria* extract (Liu et al., 2018). He et al. (He et al., 2022) successfully identified 15 phenolic metabolites through spectral analysis, 1D-NMR, and 2D-NMR techniques, including furosin, geraniin, corilagin, repandinin B, repandusinic acid A, and mallotinin. Corilagin, a polyphenolic metabolite derived from *P. urinaria*, has demonstrated anti-atherosclerotic properties both *in vitro* and *in vivo*. Jikai et al. (Jikai et al., 2002) efficiently separated corilagin and gallic acid in the methanol extract of *P. urinaria* using high-speed countercurrent chromatography (HSCCC). Corilagin was successfully isolated from the methanol extract of *P. urinaria* with a yield of 25 mg/g. Moreover, dehydrochebulic acid trimethyl ester was also identified (Hu et al., 2014). The acetone extract of *P. urinaria* contains geraniin and 1,3,4,6-tetra-O-galloyl-β-D-glucose (Zhong et al., 2013), which have exhibited inhibitory effects on the growth of HSV-1 and HSV-2 (Yang et al., 2007a). Additionally, gemin D and hippomanin A have shown anti-HSV-2 effects (Yang et al., 2007b). Geraniin, identified through NMR spectrum and mass spectrometry of the *P. urinaria* acetone extract, is a hydrolyzable tannin with antioxidant properties (Lin et al., 2008). Phyllanthusiin A and isostrictinin, both polyphenols, are also present in the *P. urinaria* extract (Han N. et al., 2023). Various terpenes (Hu et al., 2014), including triterpenes like glochidiol and oleanolic acid, diterpenes such as cleistanthol and spruceanol, sesquiterpenes like cloven-2β,9α-dio, and monoterpenes such as (6R)-menthiafolic acid, have been identified. Wu et al. (Wu et al., 2017) identified several triterpenoids in the ethanol extract of *P. urinaria*, including betulin, β-betulinic acid, 3-oxo-friedelan-28-oic acid, oleanolic acid, 28-norlup-20(29)-ene-3β, 17β-diol, and 3R-Z-coumaroyltaraxerol. These metabolites have demonstrated the ability to inhibit the production of NO in mouse macrophages, suggesting potential anti-inflammatory properties. Other metabolites found in *P. urinaria* extracts included montanoic acid ethyl, methyl brevifolin carboxylate (Calixto et al., 1998), sterols such as β-Sitosterol-3-O-β-D-glucopyranoside and β-Sitosterol (Fang et al., 2008), alkaloids like phyllurine (Ueda and Yamamura, 2000) and phyllanthurinolactone (Ueda et al., 1998), as well as 5-hydroxymethyl-2-furaldehyde and epigallocatechin-(4β->8)-catechin (Han N. et al., 2023). Furthermore, excoecarianin (Cheng et al., 2011) was identified in the acetone extract of *P. urinaria* and showed specific activity against HSV-2 virus infection. Phthalic acid bisester (Satyan et al., 1995) was also isolated from the petroleum ether extract of *P. urinaria*.

3.5.3 Inhibition of tumor proliferation

External factors, such as infection, can influence the inhibition of tumor proliferation. HBV infection has the potential to activate cancer-related signaling pathways and modify the immune microenvironment by triggering host gene instability and epigenetic remodeling through viral integration. This process can ultimately facilitate the development of HCC (Jiang et al., 2021). Flavonoids and tannins demonstrate cytotoxic effects that hinder tumor cell proliferation, prompt tumor cell cycle arrest, and curb HCC growth. Quercetin and corilagin have been recognized as metabolites with anti-HCC properties (Teodor et al., 2020b). Flavonoids can boost tumor cell apoptosis by up-regulating oncogenes, down-regulating pro-oncogenic genes, and modulating various signaling pathways like Wnt/β-catenin, MAPK, AP-1, and NF-κB. Moreover, they influence enzymes such as PTK, PKC, XO, iNOS, and COX-2 to impede tumor cell proliferation and regulate the cell cycle across different phases (S/G2, G1, S, and G2) (Hazafa et al., 2020).

Sorafenib, a chemotherapy drug used for treating HCC, may lose effectiveness over time due to acquired resistance. Research indicates that quercetin shows promise in reversing this acquired resistance to sorafenib. In a study conducted by Zhang et al. (Zhang et al., 2024), a drug-resistant HCC cell line named Huh7^{R} was established. *In vitro* experiments demonstrated that quercetin could maintain cell morphology in both Huh7 and Huh7^{R} cell lines and decrease the number of HCC cell clones, indicating its inhibitory effect on HCC cell growth. The analysis suggests that the mechanism behind this reversal of drug resistance may involve the protein kinase B signaling pathway and the EGFR signaling pathway. Molecular docking studies revealed a strong binding affinity between quercetin and Akt1. Treatment with quercetin led to decreased expression of p-EGFR/EGFR, p-Akt/Akt, p-ERK/ERK, and Bcl-2 proteins, while increasing Cleaved PARP1 and BAX expression. In a drug-resistant tumor model in nude mice, quercetin treatment significantly reduced tumor weight and volume, with protein expression patterns consistent with *in vitro* findings. Corilagin has been utilized in clinical studies, demonstrating no acute or subacute toxicity following safety assessment (Wang et al., 2023). *In vitro* experiments have demonstrated that corilagin inhibits the proliferation of HCC cells (Bel7402, SMMC7721) in a concentration-dependent manner. It causes cell cycle arrest in the G2/M phase, leading to the downregulation of key proteins cyclin B1 and ccdc2 in the G2/M phase, while also enhancing the inhibition of oncogenes p21, Cip1, and p-p53. Furthermore, *in vivo* studies using the Balb/c nude mouse xenograft model with MHCC97-H cells have shown that treatment with varying doses of corilagin (10, 20, or 30 mg/kg) results in a significant reduction in tumor mass and volume, demonstrating a dose-dependent inhibition of tumor growth. (Ming et al., 2013). Furthermore, corilagin has exhibited the ability to suppress the growth of liver tumors in athymic nude mice injected with Hep3B cells, with 15 mg/kg of corilagin leading to a notable decrease in tumor growth and volume compared to the control group. Liver function tests and blood markers AST and ALT have remained within normal ranges, indicating no observable toxicity associated with corilagin treatment (Hau et al., 2010).

3.5.4 Anti-tumor metastasis, invasion, and angiogenesis

Tumor metastasis and spread are the primary causes of mortality in cancer patients, closely associated with EMT and mesenchymal-to-epithelial transition (MET). These transitions enable tumor cells to escape the primary site and disseminate through the bloodstream to form new tumors nearby or in distant locations (Jassim et al., 2023). The activation of invasion and metastasis is a fundamental characteristic of cancer, an effective strategy to combat tumor metastasis involves combining therapies targeting ECM adhesion molecules (de Visser and Joyce, 2023),

intra-tumor interactions, EMT, and protease release (Fares et al., 2020). Given the highly vascular nature of HCC, the formation of blood vessels is essential for tumor growth, aiding in tumor cell invasion and metastasis. Consequently, inhibiting vascular endothelial growth factor receptor (VEGFR) through anti-angiogenesis therapy is a primary approach to prevent HCC invasion (Liu et al., 2022). Studies have shown that *P. urinaria* extract can hinder tumor growth by targeting the extracellular signal-related kinase 1/2 (ERK1/2) signaling pathway and angiogenesis (Tseng et al., 2012). Upon treatment with *P. urinaria* extract, there is a decrease in the protein expression of ELk-1, c-Raf, c-Myc, c-Jun/AP-1, Pan-Ras, HIF-1α, VEGF, and iNOS (Lee et al., 2016). In a study by Huang et al. (Huang et al., 2024), it was discovered that reducing levels of MMP 2 and MMP 9 in the MAPK family can impede tumor invasion and metastasis by targeting the ERK/JNK and hypoxia pathways. Quercetin has been shown to regulate SMURF2/RhoC, resulting in the inhibition of HCC invasion and metastasis. Experimental data have shown that RhoC protein and mRNA levels were elevated in highly metastatic and invasive HCC cell lines, but quercetin treatment reduced RhoC levels in a concentration-dependent manner, thereby inhibiting the invasion and migration abilities of HCC cells. Further analysis revealed that quercetin inhibits invasion and migration by suppressing RhoC expression through the protease pathway. In various mouse models, including those with subcutaneous transplanted tumors, orthotopic tumors, and patient tumor metastasis, quercetin treatment led to reduced tumor mass and volume, along with inhibition of tumor lung metastasis. Immunohistochemical staining indicated increased SMURF2 expression, suggesting that quercetin may hinder HCC invasion and metastasis by reducing RhoC expression via SMURF2 and the ubiquitination pathway. Inhibition of protease activity has been shown to suppress tumor metastasis, with studies demonstrating that targeting the PI3K/Akt and JNK1/2 signaling pathways reduces MMP 2, MMP 9, urokinase plasminogen activator (u-PA), NF-κB, and AP-1 mRNA and protein expression in tumor cells, thereby impeding tumor invasion and metastasis. Tang et al. (Tang et al., 2015) and Huang et al. (Huang et al., 2006) have both confirmed that *P. urinaria* inhibits tumor invasion and angiogenesis by suppressing MMP activity.

TCM formulations with spleen-strengthening and detoxification properties have shown promise in improving liver function and quality of life in HCC patients. The Yiqi Jianpi Jiedu (YQJPJD) prescription, containing botanical drugs like *P. urinaria*, was specifically developed for this purpose. Wu et al. (Wu et al., 2022) conducted a study to elucidate the mechanism of action of the YQJPJD prescription in treating HCC using network pharmacology and *in vitro* experiments. Their findings highlighted the role of quercetin and other active metabolites in the prescription in HCC treatment. Pathway analysis revealed associations with cancer and hepatitis B signaling pathways. Protein-protein interaction analysis identified key target proteins like TP53, Akt1, STAT3, and MAPK. Molecular docking results indicated strong binding of active metabolites to proteins such as MAPK3, RAC1, and β-catenin. *In vitro* experiments demonstrated that YQJPJD treatment led to a concentration-dependent increase in wound healing area in HepG2 and Hep3B cells. Transwell migration experiments confirmed the ability of YQJPJD to inhibit the invasion and migration of HCC cells. Moreover, YQJPJD was found to suppress the activation of the PI3K/Akt pathway, as evidenced by reduced levels of p-PI3K and p-Akt in treated cells, suggesting a potential mechanism for its anti-invasive effects. Studies have also shown that *Phyllanthus Urinaria* L. Anti-neoplastic Decoction (PAD) has the potential to suppress invasion and metastasis of HCC. Wei et al. (Wei et al., 2024) discovered that PAD, a key botanical drug in 'yiqi jianpi jiedu huayu', can combat HCC by targeting the PI3K/Akt pathway. The primary metabolites of PAD, such as quercetin and kaempferol, play a crucial role in this process. *In vitro* experiments showed that PAD effectively reduced levels of p-PI3K, Akt, and p-Akt proteins in Hep3B and HepG2 cells.

Huang et al. (Huang et al., 2021) investigated the TCM compound CP, consisting of *P. urinaria*, Astragalus mongholicus Bunge, Curcuma aromatica Salisb., Scutellaria barbata D., and Cremastra appendiculata Makino, commonly used in treating HBV-related HCC. Their study delved into the inhibitory mechanism of this metabolite on HCC through a combination of *in vitro* and *in vivo* experiments, along with bioinformatics analysis. Network pharmacology results revealed the pivotal pathways and targets of *P. urinaria* in HCC, namely, the Wnt/β-catenin signaling pathway and Cav-1 protein. In their *in vitro* experiments, the researchers utilized HepG2 cells infected with HBx-expressing lentiviral particles to establish HepG2-HBx cells, which displayed heightened migration and invasion capabilities compared to HepG2 cells. Treatment with *P. urinaria* attenuated the wound-healing ability of HCC cells and reduced cell migration through the transwell chamber. Western blot experiments demonstrated that the compound downregulation the expression of EMT marker proteins vimentin and N-cadherin, while upregulation E-cadherin expression. Subsequent investigations have revealed that *P. urinaria* exerts inhibitory effects on the Akt/GSK-3β/β-catenin pathway, thereby suppressing EMT, indicating its potential to impede the migration and invasion of HBV-related HCC cells. Utilizing an *in vivo* metastatic zebrafish xenograft model, the researchers demonstrated the ability of *P. urinaria* treatment to restrain tumor dissemination in zebrafish. The combined findings from both *in vitro* and *in vivo* studies substantiate the efficacy of *P. urinaria* in inhibiting HCC metastasis.

Exosomes and autophagy are critical factors in shaping the tumor microenvironment, and overcoming resistance to lenvatinib is a promising approach to improving treatment outcomes in HCC. In a recent study by Liao et al. (Liao et al., 2024), the effectiveness of a combination of lenvatinib and TCM compound CP was investigated in the context of HBV-related HCC. The results showed that the combination therapy had superior therapeutic effects compared to lenvatinib alone, leading to enhanced inhibition of cell proliferation in HepG2 cells. Moreover, *in vivo* experiments demonstrated a significant reduction in subcutaneous tumor mass and volume in mice treated with CP in combination with lenvatinib. Transcriptomic sequencing analysis revealed that this combined treatment altered the expression of exosomal miRNAs in HepG2 cells, specifically upregulating miR-181b-5p and miR-423-5p. PCR verification confirmed an increase in the expression of tumor suppressor genes like miR-193-3p and a decrease in proto-oncogenes such as miR-7704 following the combination therapy. Gene enrichment analysis indicated the involvement of differentially expressed

miRNAs in the autophagy pathway, while Western blot experiments showed increased levels of autophagy-related proteins beclin-1 and LC3-II, and a decrease in p62 expression. These findings suggest that *P. urinaria* has the potential to enhance the efficacy of chemotherapy drugs, thereby inhibiting invasion and metastasis in HCC.

3.5.5 Inducing tumor apoptosis

Induction of tumor apoptosis is a highly regulated cellular response that involves detection of extracellular signals, amplification of local signals, and integration of cellular information into the central processing unit for cell death. *Phyllanthus urinaria* has been investigated for its ability to trigger apoptosis (Huang et al., 2010) in various tumor cells through mechanisms such as loss of mitochondrial membrane potential (Wu et al., 2012), activation of the Fas receptor (Huang et al., 2004a)/ligand signaling pathway (Tang et al., 2010), and inhibition of telomerase enzyme activity (Huang et al., 2009). Mitochondria play a critical role in HCC metabolism, influencing processes like mitophagy and apoptosis through the regulation of mtDNA. Targeting Mdivi-1, BAPTA-AM, and microRNAs to suppress mitochondrial fission and induce apoptosis in HCC has demonstrated potential (Zhang et al., 2020).

Chudapongse et al. (Chudapongse et al., 2010) conducted research demonstrating that *P. urinaria* has the ability to regulate mitochondrial metabolism and induce apoptosis in HCC cells. The methanol extract of *P. urinaria* was found to contain 354 ± 27 mg/g of gallic acid, with experiments revealing a concentration-dependent inhibition of HepG2 cell proliferation and alterations in cell morphology. Treatment with *P. urinaria* was also observed to suppress mitochondrial oxidative phosphorylation, decrease intracellular ATP levels, modulate Ca^{2+} levels, trigger mitochondrial dysfunction, and ultimately result in cell apoptosis. Corilagin, a polyphenolic metabolite present in *P. urinaria*, is identified as one of its primary metabolites. Deng et al. (Deng et al., 2018) reported that corilagin can induce apoptosis in HCC cells through both the death receptor pathway and the mitochondrial apoptosis pathway. MTT assay demonstrated that corilagin inhibited the viability of various HCC cells (MHCC97-H, Bel-7402, and SMMC-7721) in a concentration-dependent manner, leading to morphological changes like nuclear fragmentation and chromatin condensation. These findings suggest that corilagin possesses the capability to impede the proliferation of HCC. Flow cytometry analysis confirmed that following corilagin treatment, there was an increase in the proportion of apoptosis in HCC cells, a decrease in the mitochondrial membrane potential ratio, elevation in apoptosis-related proteins such as Cyto c, caspase 8, and TP53, reduction in p-Akt and Bcl-2 protein expression, and cleavage of caspase 3, caspase 9, and PPAR.

Lignans, metabolites of *P. urinaria*, exhibit inhibitory effects on HepG2 cells (Teodor et al., 2020a). The proto-oncogenes c-myc and Bcl-2 are crucial in apoptosis. Giridharan et al. (Giridharan et al., 2002) isolated lignan metabolites from *P. urinaria* extract, identifying them as 7′-hydroxy-3′,4,5,9,9′-pentamethoxy-3,4-methylene dioxy. These metabolites upregulate c-myc, downregulation Bcl-2, activate caspase, and inhibit telomerase activity, inducing HepG2 cell apoptosis *in vitro*. Kaempferol, another metabolite from *P. urinaria*, triggers HepG2 cell apoptosis via the endoplasmic reticulum stress/CHOP pathway. It inhibits HepG2 activity depending on concentration and time, leading to increased LDH activity, upregulation of GRP94 and GRP78 endoplasmic reticulum stress markers at mRNA and protein levels, and induction of CHOP and caspase 3 apoptosis proteins in HCC cells (Guo et al., 2016). Furthermore, an aqueous extract of *P. urinaria* induces apoptosis in HepG2 cells in a concentration-dependent manner, displaying typical apoptotic characteristics like apoptotic bodies, cell shrinkage, membrane blebbing, and gelation. DNA fragmentation was evident through electrophoresis. Importantly, this extract shows no significant toxicity to normal liver cells WRL68 and endothelial cells HUVEC, indicating good safety (Huang et al., 2004b).

3.5.6 Modulate signaling pathways and immune microenvironment

Regulating cell transduction pathways and immune responses are crucial strategies in cancer treatment. Research has shown that *P. urinaria* can effectively influence various signaling pathways implicated in tumorigenesis (Tang et al., 2013), such as NF-κB, PI3K/Akt, and ERK/JNK/MAPK pathways, to combat HCC (Saahene et al., 2021). Jantan et al. (Jantan et al., 2019) conducted a review on the regulatory properties of *P. urinaria* and its metabolites, such as ellagic acid, quercetin, and corilagin on innate and adaptive immunity, suggesting its potential in HCC treatment through immune modulation. Diethylnitrosamine (DEN) is a well-known hepatotoxic carcinogen that reduces 8-OHdG levels, inhibits liver protein synthesis, generates free radicals, and induces lipid peroxidation. It is commonly used to establish animal models for HCC.

Previous studies (You et al., 2021) have shown that phyllanthin induces apoptosis through the PI3K/Akt/mTOR signaling pathway, providing resistance against HCC. Treatment with phyllanthin in HCC rat models induced by DEN led to a decrease in tumor count and incidence. Moreover, markers such as CEA, AFP, 8-OHdG, LDH, GGT, ALT, AST, and ALP showed elevated levels. Additionally, the mRNA expression of TP53, caspase 3, caspase 9, and BAX increased, while Bcl-2, mTOR, PI3K, and Akt expression decreased, phyllanthin treatment also induced apoptosis in HepG2 cells *in vitro*. Another study by Seydi et al. (Seydi et al., 2018) demonstrated that kaempferol can modulate ROS signaling pathways in HCC treatment. In an animal model induced with DEN and 2-acetylaminofluorene (2-AAF), kaempferol inhibited rat hepatocyte activity, increased apoptotic cell proportion, caspase 3 enzyme activity, ROS production, and cytochrome C release into the cytoplasm, suggesting its role in enhancing ROS production and regulating oxidative stress to induce apoptosis in HCC cells. Furthermore, Tan et al. (Tan et al., 2024) found that ellagic acid can overcome sorafenib resistance in HCC by targeting the MAPK and Akt/mTOR signaling pathways. Treatment with ellagic acid resulted in decreased cell viability in Huh7 and Hep3B cells, increased apoptosis ratio, and smaller tumor volumes and weights in nude mice with subcutaneously transplanted tumors. The study observed an increase in apoptotic proteins c-PARP, c-caspase 3, and BAX. Transcriptomics and KEGG analysis suggested suppression of the MAPK and Akt/mTOR pathways, as evidenced by decreased phosphorylation levels of ERK, JNK, MAPK, Akt, and mTOR proteins. Additionally, the combined treatment of ellagic acid and

sorafenib displayed enhanced anti-tumor effects, highlighting the importance of targeting MAPK and Akt/mTOR signaling pathways for improved therapeutic results. Li et al. (Li et al., 2019) conducted research on the anti-tumor efficacy of TCM compound CP, both *in vitro* and *in vivo*. *In vitro* experiments showed that *P. urinaria* could inhibit the proliferation, migration, and colony formation of HepG2 cells. *In vivo* studies demonstrated a dose-dependent inhibition of subcutaneously transplanted tumors in mice by *P. urinaria*. The research also indicated a reduction in mRNA and protein expression of HBx, PTCH-1, SMO, GLI-1, and GLI-2, suggesting that *P. urinaria* delays the progression of HBV-related HCC by inhibiting the HBx-SHH pathway.

Zhao et al. (Zhao et al., 2008) demonstrated that corilagin dose-dependently inhibited the inflammatory response of macrophages RAW264.7 induced by LPS. This inhibition led to reduced secretion of TNF-α, IL-1β, IL-6, NO, and IL-10, along with decreased mRNA and protein expression of NF-κB and COX-2. Conversely, corilagin increased the expression of the anti-inflammatory enzyme heme oxygenase-1 (HO-1). These findings suggest that *P. urinaria* possesses anti-inflammatory properties, can prevent macrophage M2 type polarization through the NF-κB signaling pathway, promote M1 type transformation, and provide resistance against HCC. Wan et al. (Wan et al., 2019) found that Qizhu Decoction, containing seven herbal ingredients including *P. urinaria*, effectively treated DEN-induced HCC. Primary hepatocytes isolated from the HCC model and treated with Qizhu Decoction revealed corilagin as the primary metabolite in the extract. CCK8 and cell colony formation assays showed that Qizhu Decoction suppressed HCC cell proliferation. Wound healing experiments indicated reduced cell migration with Qizhu Decoction treatment. Flow cytometry analysis showed an increase in apoptotic cells. Western blot analysis demonstrated elevated expression levels of EMT marker proteins E-cadherin and caspase 3, while N-cadherin and Bcl-2 expression decreased. Additionally, both mRNA and protein levels of NF-κB signaling pathway components p-p65, TNF-α, IL-1β, and COX-2 were downregulated, indicating that *P. urinaria* exerts its anti-HCC effects by modulating the NF-κB signaling pathway.

Qizhu Anticancer Recipe (QACR) is a herbal compound containing ten botanical drugs, including *P. urinaria*. Studies indicate that QACR can hinder the proliferation of HCC cells by targeting the c-Jun N-terminal kinase signaling pathway (Han Z. et al., 2023). *In vitro* studies, QACR has been shown to inhibit the growth of MHCC97-L and SK-Hep-1 cells, induce cell apoptosis, reduce levels of PCNA, Bcl-2, and CD31 proteins, enhance expression of apoptosis-related proteins like caspase 3, caspase 8, caspase 9, and DFF40, and decrease HUVEC cell angiogenesis. Experiments on orthotopic HCC mouse models with MHCC97-L cells revealed that QACR treatment can suppress tumor growth. The protein expression patterns observed *in vivo* align with the *in vitro* results, with QACR treatment resulting in increased p-JNK expression. Additionally, using the signaling pathway inhibitor SP600125 can reverse the effects on p-JNK expression induced by QACR treatment. High-resolution mass spectrometry identified metabolites of QACR, distributed in subcutaneously transplanted tumors of nude mice post oral administration, including Deacetyl asperulosidic acid methyl ester, paeoniflorin, liquiritin, and glycyrrhizic acid. Molecular docking, network pharmacology analysis, and western blot experiments confirmed the efficacy of QACR in HCC treatment. Potential targets of QACR in HCC were identified as VEGFA, TERT, ABCB1, CA9, JUN, AR, MMP7, MMP1, and ESR2. Both *in vivo* and *in vitro* experiments demonstrated that QACR can inhibit cell proliferation, slow tumor growth in nude mice, and arrest the cell cycle at the G0/G1 phase. These results suggest that QACR exerts its anti-HCC effects through the ERK/Akt pathways (Lu et al., 2013).

3.5.7 Clinical applications

In a clinical trial conducted by Tong et al. (Tong et al., 2014), the study investigated the effects of TCM Compound CP on patients with HBV-related cirrhosis. The treatment group, consisting of 52 patients, received additional treatment with CP over a 3-year observation period. Results showed that *P. urinaria* effectively inhibited liver disease progression and delayed the development of HBV-related cirrhosis to HCC. Post-treatment, reductions in HBV-DNA levels, lower AFP levels compared to the control group, decreased precancerous serum markers URG11 (β-catenin) and URG19 (vascular endothelial growth factor receptor 3, VEGFR3), and increased DRG2 (Suil) were observed. During the 5 year follow-up, only 3 new cases of HCC were reported in the treatment group, while 15 cases occurred in the control group. These findings suggest that *P. urinaria* may enhance the immune system by inducing cell cycle arrest in HCC cells, inhibiting angiogenesis, and impeding the progression of HCC.

4 Discussion

A thorough examination of toxicological effects is imperative to ensure the safety and efficacy of clinical medication, conducting toxicity tests on extracts and metabolites is essential to guarantee the clinical efficacy of *P. urinaria,* addressing issues related to heavy metals, pesticide residue, and mycotoxin is crucial during toxicity testing. Moreover, there has been no extensive exploration into the specificity of different metabolites in treating liver disease and their distinct impact on its progression, lack of a dearth of comprehensive investigations into the mechanism of action of the various metabolites of *P. urinaria*. To enhance the quality and therapeutic efficacy of *P. urinaria*, it is imperative to improve the extraction, purification, and preparation processes of *P. urinaria*, more stringent quality control standards should be implemented to mitigate the occurrence of adverse reactions. It is crucial to develop sensitive analytical methodologies to test the metabolites of *P. urinaria* and ensure their quality under various conditions like picking time, geographical regions, and other factors. Clinical studies are scarce on the treatment of liver disease, with a notable absence of high-quality clinical trials to substantiate its safety and efficacy. Existing clinical studies primarily consist of observational research, lacking sufficient observational indicators and robust experimental design. Prior to initiating randomized, placebo-controlled, double-blind clinical trials, it is essential to have sufficient pre-clinical testing data on *P. urinaria* and its metabolites. Furthermore, more studies are required to elucidate the relationship between the monomer metabolites and effective parts in *P. urinaria*'s disease treatment, offering insights for drug development and application. Additional research on anti-inflammatory, antibacterial, and other aspects is crucial to

support its clinical use. Existing literature on anti-liver fibrosis/cirrhosis shows consistency in most basic experimental detection indicators. However, the clinical study of liver fibrosis treatment targeting HSCs is lacking, highlighting the need for an effective integration of modern treatment and ethnic medicine. Limited research exists on the use of *P. urinaria* in treating liver cirrhosis, a critical stage in the progression from liver fibrosis to HCC, underscoring the necessity for further investigation. Further research is needed to fully understand the mechanisms behind liver protection and antioxidant effects to meet clinical demands. Utilizing computational chemistry techniques to study the structural modification of active metabolites in plant extracts is essential for future investigations. It is important to recognize that the clinical benefits are not solely attributed to a single metabolite, thus combining various extracts from *P. urinaria* may enhance the overall therapeutic effect.

5 Conclusion and future perspectives

A comprehensive review of the pharmacological and clinical effects of *P. urinaria* on viral hepatitis, liver fibrosis/cirrhosis, and HCC has demonstrated its anti-viral, anti-tumor, and liver-protective activities through basic experiments and clinical applications. In future research, it is recommended to combine modern techniques such as proteomics and transcriptomics to investigate the impact of *P. urinaria* metabolites on gene expression and signal pathways in immune cells and liver cells. This will help elucidate the role of these changes in the progression of liver disease. Furthermore, future randomized controlled trials and multi-center studies should be conducted to assess the therapeutic potential of *P. urinaria* in liver disease. The low metabolic stability and bio-availability of metabolites can limit their effectiveness. Further clinical evaluation of drug delivery strategies like nanoparticles, liposomes, and polymeric micelles is needed to address these limitations. Standardization is crucial in the drug development process, including exploring various dosage forms like pills, tablets, granules, and capsules. Developing new technologies and optimizing existing ones can enhance metabolite yield. *P. urinaria*, as an ethnic medicine, shows therapeutic potential and may serve as a natural product with minimal adverse reactions compared to modern medicine. Accurate identification and appropriate preparation methods are essential for utilizing *P. urinaria* and other species effectively. It is recommended to establish metabolite fingerprints for batch-to-batch quality control. Detailed studies on the anti-viral, immune tolerance regulation and liver fibrosis improvement mechanisms of *P. urinaria* are needed. Researchers should also consider the synergistic effects of *P. urinaria* with anti-viral, liver protection, and anti-tumor drugs, and explore the optimal dosage and sequence of administration in combination therapies. Additionally, documenting the therapeutic outcomes of *P. urinaria* in different disease stages and populations is essential for ensuring its safety and minimizing adverse reactions. It is important to identify the key metabolites responsible for their anti-tumor effects, evaluate the impact of different solvent extracts on efficacy and mechanism, and conduct additional clinical trials to assess *P. urinaria*'s therapeutic effects. These steps are crucial for enhancing its clinical efficacy and promoting widespread application.

Author contributions

X-aY: Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Validation, Visualization, Writing–review and editing. LL: Data curation, Formal Analysis, Investigation, Software, Writing–original draft. BW: Investigation, Project administration, Writing–original draft. YM: Investigation, Methodology, Writing–original draft. KS: Methodology, Writing–original draft. PW: Investigation, Writing–original draft. MfL: Methodology, Writing–original draft. JD: Resources, Writing–original draft. MQ: Methodology, Writing–original draft. MgL: Methodology, Writing–original draft. CW: Formal Analysis, Writing–original draft. YT: Methodology, Writing–review and editing. JH: Methodology, Writing–review and editing. KG: Methodology, Writing–review and editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was supported by the National Natural Science Foundation of China (82104357, 82074384), and Shenzhen Traditional Chinese Medicine Hospital "3030 plan" TCM clinical research project (G3030202102).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2024.1443667/full#supplementary-material

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frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 16 August 2024
DOI 10.3389/fphar.2024.1421662

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OPEN ACCESS

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RECEIVED 22 April 2024
ACCEPTED 05 August 2024
PUBLISHED 16 August 2024

CITATION
Sun Y, Zhang Y, Sun M, Gao W, He Y, Wang Y, Yang B and Kuang H (2024) Advances in *Eucommia ulmoides* polysaccharides: extraction, purification, structure, bioactivities and applications.
Front. Pharmacol. 15:1421662.
doi: 10.3389/fphar.2024.1421662

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Advances in *Eucommia ulmoides* polysaccharides: extraction, purification, structure, bioactivities and applications

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Eucommia ulmoides (EU) is a precious tree species native to China originating during the ice age. This species has important economic value and comprehensive development potential, particularly in medicinal applications. The medicinal parts of EU are its bark (*Eucommiae cortex*) and leaves (*Eucommiae folium*) which have been successively used as a traditional Chinese medicine to treat diseases since the first century BC. During the last 2 decades, as natural polysaccharides have become of increasing interest in pharmacology, biomedicine, cosmetic and food applications, more and more scholars have begun to study polysaccharides derived from EU as well. EU polysaccharides have been found to have a variety of biological functions both *in vivo* and *in vitro*, including immunomodulatory, antioxidant, anti-inflammatory, anticomplementary, antifatigue, and hepatoprotective activities. This review aims to summarize these recent advances in extraction, purification, structural characteristics, pharmacological activities and applications in different fields of EU bark and leaf polysaccharides. It was found that both *Eucommiae folium* polysaccharides and *Eucommiae cortex* polysaccharides were suitable for medicinal use. *Eucommiae folium* may potentially be used to substitute for *Eucommiae cortex* in terms of immunomodulation and antioxidant activities. This study serves as a valuable reference for improving the comprehensive utilization of EU polysaccharides and further promoting the application of EU polysaccharides.

KEYWORDS

Eucommia ulmoides polysaccharides, extraction and purification, structural characteristics, pharmacological activities, applications

Abbreviations: AE, Alkali Extraction; Ara, Arabinose; EU, Eucommia ulmoides; Fru, Fructose; Fuc, Fucose; Gal, Galactose; GC, Gas Chromatography; Glc, Glucose; HPLC, High Performance Liquid Chromatography; MAE, Microwave-Assisted Extraction; Man, Mannose; Mw, Molecular Weight; NMR, Nuclear Magnetic Resonance Spectroscopy; Rha, Rhamnose; UAE, Ultrasound-Assisted Extraction; UV, Ultraviolet Visible Spectroscopy.

1 Introduction

Eucommia ulmoides (EU), also known as Formica, is a member of the monotypic family Eucommiaceae (Zhu et al., 2016). This species is a relic plant that survived the third glacial period and a dioecious perennial deciduous tree (2n = 34) grows up to approximately 20 m in height (Call and Dilcher, 1997; Wuyun et al., 2018; Jin et al., 2020; Liu et al., 2022). EU has the following typical botanical features: bark rough, beige or grayish-brown, with rubber inside, found to have strands of latex in bark openings; leaves simple ovate, 6–16 cm long and 3.5–6.5 cm wide, with serrate margins and acuminate apexes, found to have brown pilose when young, connected by elastic silvery white dense glue wires between the fracture surfaces and petioles; flower inconspicuous, small, greenish, male clustered, female axillary, with obovate bracts; flowering from April to May (He et al., 2014; Bao et al., 2024). EU is endemic and precious to China and is naturally distributed in southwest, southeast, central, and eastern southwest China to Zhejiang Province. The genuine producing areas of the EU are Sichuan, Shaanxi, Guizhou and Hubei provinces, and its habitats are protected by complex terrains (Dong et al., 2020; Xie et al., 2023). For a long time, EU has been a kind of tree with high economic value, which can be sold as Chinese medicinal materials and ornamental plants. At the same time, EU can also be used in the production of wood and its products. The "silver wire" found within the tree is a type of glial substance that exhibits adhesive properties and is resistant to corrosion by acids and alkalis. It is also characterized by its resistance to deformation, insulation, and heat insulation properties. In contemporary times, it can be refined into high-quality rubber or optical cable lines, submarine cables, oil pipelines, and other advanced materials. These attributes have earned it the moniker of "plant gold" (Zhao et al., 2024). In terms of medicine, EU has been used as a Chinese medicinal plant for over 2,000 years (Wang et al., 2011; Yao et al., 2012; Liu et al., 2016; Huang L. et al., 2021; Zhao et al., 2022), and it was first documented in Shen Nong Ben Cao Jing, a classical text on traditional Chinese medicine (Huang L. et al., 2021). Moreover, the Materia Medica Compendium systematically and comprehensively summarizes information related to EU (Xing et al., 2019). Currently, the Chinese Pharmacopoeia (2020 edition) includes EU bark (*Eucommiae cortex*) and EU leaves (*Eucommiae folium*) as two kinds of Chinese medicinal materials. Traditional Chinese medicine indicates that the properties and flavour of the *Eucommiae cortex* are sweet and warm, and the properties and flavour of *Eucommiae folium* are pungent and warm. Both materials are generally prepared as a general tonic to support liver and kidney health, promote muscle and bone strength, and alleviate hypertension. Importantly, the difference is that the *Eucommiae cortex* can also be used to calm the foetus (Liu et al., 2016; Huang L. et al., 2021). In recent years, the *Eucommiae cortex* has attracted much attention for its potential benefits in the treatment of diabetes mellitus, high blood pressure, hyperglycaemia, obesity, ageing, osteoporosis, Alzheimer's disease, sexual dysfunction and other activities. These beneficial physiological effects are ascribed to a diverse array of bioactive ingredients, including cycloethers, phenols, flavonoids, lignans, polysaccharides, sterols, and plant gum (Luo et al., 2010; He et al., 2014; Xu et al., 2015; Hussain et al., 2016; Xing et al., 2019; Liu P. et al., 2020), and *Eucommiae folium* also contains these substances (Xing et al., 2019).

Polysaccharides are important macromolecules in the human body, made up of multiple monosaccharides linked together in complex structures (Amicucci et al., 2019; Tan et al., 2020). Numerous studies have identified natural polysaccharides from diverse biological sources, including animals, plants, and microorganisms, as confirmed by scientific research. These polysaccharides exhibit a variety of beneficial activities. In addition, they exhibit low toxicity and remarkable safety, making them an attractive therapeutic option for disease (Zhou et al., 2022). Studies of active ingredients in EU have found that polysaccharides from EU (EU polysaccharides) are considered some of the important effective components. With increasing interest in natural plant polysaccharides, research has been conducted on EU polysaccharides. Studies have demonstrated their antioxidant (Hong et al., 2013), immunoregulatory (Feng et al., 2016), anticomplementary (Zhu et al., 2008), anti-inflammatory (Sun et al., 2021), antifatigue (Xia and Piao, 2010), and hepatoprotective (Gao et al., 2020) activities, among others. For decades, various polysaccharides have been extracted from different EU parts (*Eucommiae cortex* and *Eucommiae folium*) by different extraction and purification methods. Because biological activity is related to chemical structure, it is important to analyze the chemical structure diversity of polysaccharides in EU to understand their biological activities (Tomoda, et al., 1990; Zhu et al., 2009; Feng H. et al., 2021).

However, although many scholars have studied EU polysaccharides, there is no systematic review of the polysaccharides of EU. As a result, there is a lack of clear understanding of the activity of polysaccharides from EU, and the structure-activity relationship is not well defined, which hinders the development of their applications. In this paper, we systematically summarized the current research results on EU polysaccharides and detailed the extraction, purification, structural analysis and pharmacological activity methods of EU polysaccharides. Then, the composition and biological properties of polysaccharides from the bark and leaves of EU were compared. In addition, bark harvesting requires the growth of the tree for several years, and improper harvesting methods readily cause tree death. This paper explores the potential for substituting polysaccharides from *Eucommiae cortex* with those from *Eucommiae folium* to conserve resources and further study the similar pharmacological effects of *Eucommiae* polysaccharides.

2 Search strategy

This review was systematically conducted thorough databases such as Google Scholar, Web of Science, PubMed, Elsevier, Springer, and Wiley databases from inception till 2023. Keywords such as "*E. ulmoides* polysaccharides", "*E. ulmoides* bark polysaccharides", "*E. ulmoides* leaves polysaccharides", and "*E. ulmoides* leaf polysaccharides" were used to collect the literature on extraction, purification, structural characteristics, bioactivities, applications and other aspects of polysaccharides derived from *E. ulmoides*. Meanwhile, duplicate and irrelevant literatures were excluded.

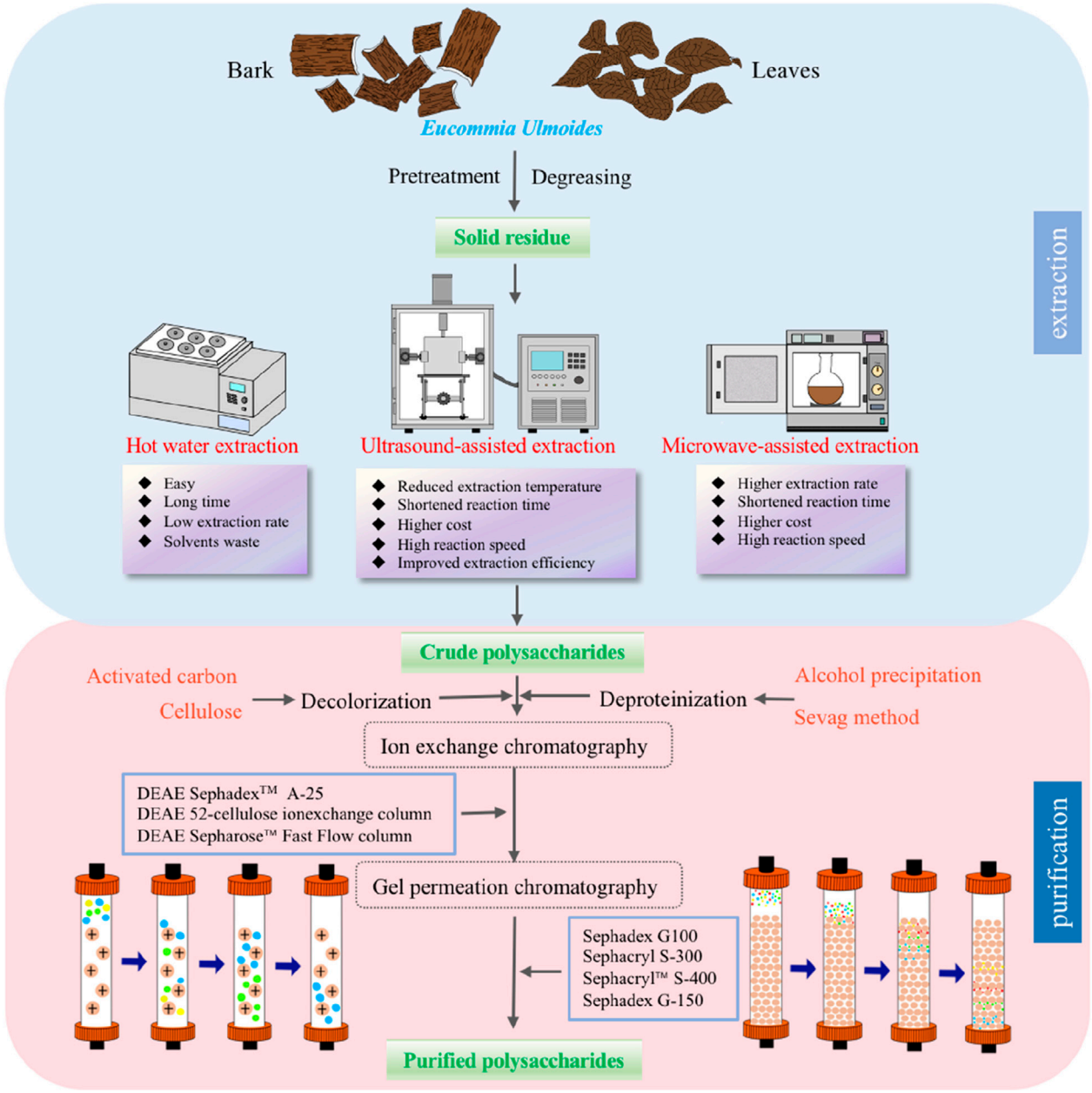

FIGURE 1
The flowchart of extraction methods and purification of *Eucommia ulmoides* polysaccharides.

3 Preparation of EU polysaccharides

Generally, the preparation methods for polysaccharides should be determined according to their composition and structure, and the selection of preparation methods should account for the chemical properties of components, extraction conditions, impurities and other factors (Sheng et al., 2021; Qin et al., 2022; Xu et al., 2022). Therefore, to efficiently and safely prepare polysaccharides without compromising their structural integrity, it is imperative to carefully select the appropriate preparation method. In recent years, technological and scientific advancements have improved the EU polysaccharide extraction and purification efficiencies (Zhu et al., 2009; Xia and Piao, 2010; Xu et al., 2018; Liu M. et al., 2020). Figure 1 briefly illustrates the preparation process of EU polysaccharides.

3.1 Extraction methods

The first step in studying natural plant polysaccharides is extraction, which is also the most important step in the isolation of bioactive substances (Shi, 2016; Mohan et al., 2020; Zeng et al., 2022). Specifically, polysaccharides with relatively high polarity are more soluble in water than ethanol (Li et al., 2021). Therefore, polysaccharides are normally extracted by the hot water extraction (HWE) method (Wang et al., 2019; Barbosa et al., 2020). In recent

TABLE 1 Different extraction methods of polysaccharides from *Eucommia ulmoides*.

Part	Source	Polysaccharide Name (fraction)	Extraction method	Conditions	Polysaccharide content	Yield	Reference
E. ulmoides bark	Purchased from Shanghai Hua-Yu Chinese Materia Medica Co. Ltd., China	EUPs(crude)	Hot water	-	Contained 69.80% of total carbohydrate and 28.86% of uronic acid	2.2%	Jiang et al. (2011)
E. ulmoides bark	-	EUP (crude)	Hot water	Time: 80min Solid and liquid ratio: 1:3 Extraction number 3	-	23.9%	Hong et al. (2013)
E. ulmoides bark	-	EUPS	Hot water	-	EUPS contained 97.4%, proteins content was 2.1%	-	Feng et al. (2016)
E. ulmoides bark	Purchased from China Pharmaceutical Corporation-Canton, China	EUP1	Hot water	-	Around 22.4% (EUP1), 9.2% (EUP2) and 37.5% (EUP3) of total EUP	-	Li et al. (2017)
E. ulmoides bark	China	Eucomman A	Hot water	-	-	0.22%	Gonda et al. (1990)
E. ulmoides bark	China	Eucomman B	Hot water	-	-	-	Tomoda et al. (1990)
E. ulmoides bark	Purchased from Huayu Materia Medica Co., Ltd, Shanghai, China	EWDS-1	Hot water	-	EWDS-1 contained 82.46% of total carbohydrate, 2.15% of uronic acid, 2.07% of sulfate, and 3.95% of protein	0.1‰	Zhu et al. (2008)
E. ulmoides bark	Purchased from Huayu Materia Medica Co., Ltd, Shanghai, China	EWDS-2	Hot water	-	Contained 92.32% of total carbohydrate, as well as 6.55% of protein	2.3%	Zhu et al. (2009)
E. ulmoides bark	Collected from the arboretum of Northwest Agricultural and Forestry University, Yangling, China	EUP1	Hot water	Temperature 60°C Time: 180min, liquid ratio: 1:20	-	5.99% of the original materials	Xu et al. (2015)
E. ulmoides bark	From a farm of the Northwest A&F University, Yangling, Shaanxi, China	FBWP	Hot water	-	Water-soluble polysaccharides (3.8%–8.6%)	4.13%	Zhu et al. (2016)
E. ulmoides bark	From a farm of the Northwest A&F University, Yangling, Shaanxi, China	FBAP	Alkali	-	Alkali-extractable polysaccharides (12.2%–26.1%)	12.19%	Zhu et al. (2016)
E. ulmoides bark	Collected from the arboretum of Northwest Agricultural and Forestry University, Yangling, China	EUP2	Ultrasound-assisted extraction	Room temperature Time: 60min, liquid ratio: 1:20	-	7.38% of the original materials	Xu et al. (2015)
E. ulmoides leaves	Collected from the arboretum of Northwest Agricultural and Forestry University, Yangling, China	EULP1	Hot water	Temperature 60°C Time: 180min, liquid ratio: 1:20	-	9.26% of the original materials	Xu et al. (2015)

(Continued on following page)

TABLE 1 (*Continued*) Different extraction methods of polysaccharides from *Eucommia ulmoides*.

Part	Source	Polysaccharide Name (fraction)	Extraction method	Conditions	Polysaccharide content	Yield	Reference
E. ulmoides leaves	Obtained from SPH Zunyi Pharmaceutical Co.,Ltd. (Zunyi, China)	PsEUL	Hot water	Temperature 60°C Time: 120min, liquid ratio: 1:20	Total sugar content was 96.36%, ratio of polysaccharide to protein was determined to be 12:25	5.7%	Feng et al. (2021a)
E. ulmoides leaves	From a farm of the Northwest A&F University, Yangling, Shaanxi, China	LWPE	Hot water	-	Water-soluble polysaccharides (3.8%–8.6%)	5.03%	Zhu et al. (2016)
E. ulmoides leaves	From a farm of the Northwest A&F University, Yangling, Shaanxi, China	LAP	Alkali	-	Alkali-extractable polysaccharides (12.2%–26.1%)	26.06%	Zhu et al. (2016)
E. ulmoides leaves	Collected from the arboretum of Northwest Agricultural and Forestry University, Yangling, China	EULP2	Ultrasound-assisted extraction	Room temperature Time: 60min, liquid ratio: 1:20	-	10.51% of the original materials	Xu et al. (2015)
E. ulmoides leaves	Purchased from SPH Zunyi Pharmaceutical Co. Ltd (Zunyi, China)	PsEUL	Ultrasound-assisted extraction	Temperature 60°C Time: 120min, liquid ratio: 1:20	-	-	Feng et al. (2022)
E. ulmoides leaves	Collected from *E. ulmoides* research base of Chinese Academy of Forestry, China	Cp	Ultrasound-assisted extraction	Temperature 60°C Time: 80min, liquid ratio: 1:30, Power 200 W	164.95 mg/g	10.49%	Liu et al. (2020a)
E. ulmoides leaves	Harvested from the arboretum of Northwest A & F University, Yangling, China	EULP	Microwave-assisted extraction	Temperature 74°C Time: 15min, liquid ratio: 1:29	Carbohydrates contained 84.2%–89.5%	12.31%	Xu et al. (2018)
E. ulmoides leaves and bark	From a farm of the Northwest A&F University, Yangling, Shaanxi, China	LBWP	Hot water	-	Water-soluble polysaccharides (3.8%–8.6%)	4.54%	Zhu et al. (2016)
E. ulmoides leaves and bark	From a farm of the Northwest A&F University, Yangling, Shaanxi, China	LBAP	Alkali	-	Alkali-extractable polysaccharides (12.2%–26.1%)	16.07%	Zhu et al. (2016)

-: information was not available.

years, new methods with improved speed and efficiency have been developed to improve production efficiency or obtain higher yields (Liu et al., 2018), including alkali extraction (AE) (Zhu et al., 2016), microwave-assisted extraction (MAE) (Xu et al., 2018), and ultrasound-assisted extraction (UAE) (Liu M. et al., 2020). Table 1 summarizes the different extraction methods for EU polysaccharides.

3.1.1 Hot water extraction method

The hot water extraction process, which uses abundant and environmentally friendly water as the principal solvent, is the most traditional method of extracting EU polysaccharides, which includes decoction, soaking, and reflux (Khoza et al., 2014). During this process, extraction conditions, such as the temperature, duration, frequency, and solvent-drug ratio, all significantly affect the extraction yield of polysaccharides (Zhang et al., 2021). Typically, herbs are pretreated prior to extraction, and then the extraction operation is carried out (Zhu et al., 2008; Zhu et al., 2009). For instance. Zhu et al. (2008), Zhu et al., 2009) crushed EU bark (9 kg) into powder, degreased it with 95% ethanol, and extracted it with hot water to obtain insoluble parts; the yield was 2% of the dry matter. In a similar manner. Jiang et al. (2011) ground dried bark into fine particles, defatted it using 95% ethanol, and extracted the insoluble part with hot water, and the yield of crude EU polysaccharides was 2.2% of the dry matter. To improve the extraction rate, Hong et al. (Hong et al., 2013) used five volumes of distilled water to extract EU powder (500 g) at 100°C several times. Through contour plot and variance analysis, the optimal extraction process conditions were found to consist of an 80-min extraction time, a water-material ratio of 3, and extraction times of 3, and the yield reached 23.9%. In addition to crushing medicinal materials, some scholars cut them into pieces or strips and then extracted them. For example. Li et al.

(2017) cut EU bark into strips and defatted it with 95% ethanol. They then boiled the bark in water three times for 3 h each to extract the necessary compounds. Moreover, Tomoda et al. (Gonda et al., 1990; Tomoda et al., 1990) extracted sliced bark (810 g) with hot water (8.1 L) under stirring for 30 min, and the yield was 0.22%. It follows that particle size significantly affects the extraction rate of EU polysaccharides (Li et al., 2023). The increased crushing degree can generate finer particles from the raw materials, leading to an initial increase in extraction yield due to a greater contact surface area, improved mass transfer rate, and lowered diffusional resistances (Gao et al., 2023).

Overall, hot or boiling water extraction is the most commonly adopted method in industrial and laboratory settings because it is inexpensive, environmentally friendly, and simple and minimizes damage to the polysaccharide structure (Hromádková et al., 2002; Zhang et al., 2020; Wang and Zhang, 2021; Jiang et al., 2022). However, its disadvantage lies in the prolonged extraction time, which leads to a slower extraction rate, and at the same time, the high solid–liquid ratio leads to a waste of resources. Furthermore, high temperature can cause the destruction of the polysaccharide structure, thereby reducing its pharmacological activity (Hromádková et al., 2002; Xing et al., 2013; Yan et al., 2013). To compensate for these shortcomings, alternative extraction methods, including UAE, MAE and AE, have been developed (Ying et al., 2011). Microwave and ultrasound extraction methods have less impact on polysaccharide extraction based on particle size compared to hot water extraction. However, extraction can still affect the biological activity of polysaccharides, so multiple factors should be considered when choosing a method.

3.1.2 Microwave-assisted extraction method

MAE has been widely used to extract bioactive compounds from natural materials. MAE technology can penetrate cell membranes and rupture cell walls, thereby releasing high-molecular weight (Mw) compounds such as polysaccharides into the solvent (Chen et al., 2017; Sharma et al., 2022). Briefly. Xu et al. (2018) mixed 2.0 g of the ground EU leaf sample with distilled water in a conical flask with a specific volume. The microwave generator was set to 74°C for 15 min for extraction. The resulting extracts were vacuum-filtered and concentrated at 40°C. Three volumes of ethanol (95%) were slowly added to the concentrated filtrate under continuous agitation. Through centrifugation and lyophilization, the precipitate was recovered, resulting in the isolation of the polysaccharide EULP-MAE with a yield of 12.31%. In addition, as seen in Table 1, significantly higher polysaccharide yields than with hot water extraction and UAE techniques were observed. Evidently, MAE of polysaccharides is an efficient method to increase yield (Lal et al., 2021). In contrast with the traditional method, MAE offers advantages such as a reduction in solvent consumption, a reduced extraction time, and an enhanced extraction yield (Zeng et al., 2012; Ekezie et al., 2017; Bagade and Patil, 2021). However, the drawbacks of this method are that the microwave extraction equipment is complex, and the extraction range is limited (Wen et al., 2018; Huang G. et al., 2021). Currently, MAE of EU polysaccharides is only used for *Eucommiae folium*, which may be due to the differences in composition and structure between *Eucommiae folium* and *Eucommiae cortex*, and the specific extraction conditions may need to be adjusted and optimized for *Eucommiae cortex*. In the future, it is necessary to further evaluate the use of microwave technology to extract polysaccharides from *Eucommiae cortex* to expand the application scope and value of this technology. Specifically, MAE equipment can be optimized to provide a more efficient, reliable, and operable microwave generator. New methods of MAE, such as cooperative MAE and adding extracts after extraction, can be studied to improve extraction efficiency and product purity.

3.1.3 Ultrasound-assisted extraction method

UAE is also a new extraction technique that harnesses the high-frequency vibration of ultrasound and thermal effects within cavities to rupture cell membranes to increase solvent penetration into the tissue (Ebringerová and Hromádková, 2010; Liu et al., 2021). EU polysaccharides can also be extracted using this method. For instance. Xia and Piao. (2010) dried and crushed EU leaves and sieved them with 60 mesh. EU powder was immersed in petroleum ether, and degreasing was carried out by ultrasonic treatment at 30°C for 40 min. The solution was then mixed with 95% alcohol and sonicated at 80°C for 20 min to further remove impurities. Treated samples were immersed in water for 12–18 h, and UAE was performed. The optimal extraction conditions were determined to be an extraction temperature of 80°C, an extraction time of 40 min, a solid–liquid ratio of 1:35, and two repeated extractions, and a polysaccharide extraction rate of 4.892%, and a polysaccharide purity of 86.76% were obtained. Furthermore, Liu et al. (Liu M. et al., 2020) dried EU leaves to constant weight in a thermostatically controlled blower at a temperature of 50°C. Then, the samples were crushed and filtered with a 60-mesh screen. Polysaccharides were extracted from EU leaves employing UAE technology. The four factors of extraction solution ratio (v/w), time (min), temperature (°C), and power (W) were optimized by orthogonal experiments to improve and enhance the extraction rate (%). Four independent variables were studied at three distinct levels (1, 2, and 3), and the supernatant obtained after centrifugation was the crude polysaccharide (Cp). The findings showed that the optimal extraction parameters encompassed an extraction liquid ratio of 30, an extraction time of 80 min, an extraction temperature of 60°C, an extraction power of 200 W, and the extraction yield increased from 4.82% to 10.49%. These results indicate that the UAE method has better extraction efficiency for EU polysaccharides than traditional methods such as the hot water extraction method (Gong et al., 2020). As a result, the extraction process and activity characteristics of EU polysaccharides is incomplete, and the comprehensive understanding and application of EU polysaccharides are limited. In future research, both EU leaf polysaccharides and EU bark polysaccharides should be studied to gain a thorough understanding of their extraction methods and properties for future applications.

Compared with the traditional hot water method, the extraction of polysaccharides can be enhanced by UAE, resulting in higher purity of extracted components at a lower temperature and reduced solvent usage (Hromádková et al., 2002; Zhang et al., 2009). However, this method is expensive and has not been widely adopted in industrial fields (Luft et al., 2021). Moreover, studies have shown that ultrasonic extraction can lead to polysaccharide degradation, molecular weight becomes smaller, thus affecting its biological activity (Li S. et al., 2022). The ultrasonic extraction of EU

polysaccharides also has this problem, so solving this problem is very important for the development of EU polysaccharides in the future. With the ongoing evolution of polysaccharide extraction techniques, several novel methods have emerged, including supercritical fluid extraction, supercritical CO_2 extraction, and ultrafiltration extraction. The exploration of these novel techniques can be employed to extract EU polysaccharides, effectively rectifying the shortcomings of the existing methodologies. Consequently, the necessity for further investigation and development of an optimized extraction technique for the isolation of EU polysaccharides cannot be ignored.

3.1.4 Alkali extraction method

Studies have shown that extraction under alkaline conditions causes cell wall swelling, and then the cell wall of the plant is destroyed, causing it to release additional polysaccharides. Thus, a specific concentration of an alkali solution can be employed to extract cell wall-bound polysaccharides or intracellular polysaccharides, with the aim of enhancing the yield of polysaccharides (Sun Y. et al., 2022; Chen et al., 2022). A scholar used this method to extract EU polysaccharides. Xu et al. (2015) treated EU powder with 1 M NaOH aqueous solution for 3 h at 75°C using a solid–liquid ratio of 1:20 g/mL. Subsequently, the base extraction filtrate was neutralized to pH 5.5 and concentrated to approximately 30 mL with 6 M HCl. Subsequently, the concentrate was removed with three volumes of 95% ethanol with vigorous stirring, filtered, washed twice with three volumes of 95% ethanol, and freeze-dried. Ultimately, the yield of polysaccharides extracted from different sources of EU ranged from 12.19% to 26.06% (Bai et al., 2022). Comparing hot water extraction and AE showed that the yield of AE was much higher than that of hot water extraction. It is known that AE has the advantages of short extraction times, mild reaction conditions, and high yields. Notably, the glycosidic bond and structure of polysaccharides will be destroyed by excess alkali concentrations, and polysaccharide degradation will occur (Nie and Xie, 2011; Huang and Huang, 2020). Future research could explore alternative reaction media, such as acidified solutions at specific pH levels, neutral solutions, or organic solvents, which may have varying effects on the extraction of cell wall polysaccharides, potentially leading to an increase in polysaccharide production. It is worth mentioning that in addition to obtaining polysaccharides by destroying cell walls by alkali extraction method, recently researchers found that plant polysaccharides can also be extracted by alkali precipitation method. Polysaccharide CGP-AP obtained by alkali precipitation from *Chaetomium globosum* has better anti-inflammatory and antioxidant activities (Wang S. et al., 2024), which suggests that we can study the extraction of polysaccharides from EU by alkali precipitation method in the future. It is of great significance to analyze whether the structure and biological activity of the polysaccharide obtained by alkali precipitation and alcohol precipitation are different, which will broaden the application range of EU polysaccharides.

In summary, the current research on polysaccharides extraction methods of EU is limited to some methods that have been widely used in polysaccharide extraction. With the development of biotechnology, the use of microbial fermentation to modify polysaccharides has become a research hotspot. Microbial fermentation can not only improve the bioavailability of polysaccharides, but also increase the extraction rate of polysaccharides to a certain extent (Wang G. et al., 2023). However, at present, there are few researches on the extraction of polysaccharides by microbial fermentation, and the extraction of EU polysaccharides by this method is in the blank stage. Therefore, the study on the extraction of polysaccharides by microbial fermentation is the focus of future research, which has positive significance to expand the application range of polysaccharides from EU and improve its commercial value. In addition, enzyme-assisted extraction of polysaccharides has been widely used in recent years. Compared with other extraction methods, the enzyme-assisted extraction method has the advantages of mild reaction conditions, easy deterioration of products, high extraction efficiency, low cost, environmental protection and energy saving. Through literature review, we found that there have been relevant studies on the extraction of chemical components of EU except polysaccharides by enzyme extraction method, which indicates that enzyme extraction method may be used for the extraction of polysaccharides. The further study of this method to extract polysaccharides from EU and whether the polysaccharides obtained have better biological activity will play an important role in the future development of related fields.

3.2 Isolation and purification methods

The crude polysaccharides contained more proteins than purified polysaccharides; therefore, in polysaccharide purification, it is often necessary to remove excessive proteins and peptides from the sample to ensure the accuracy of subsequent analysis. Several methods exist for protein elimination, with the Sevag and trichloroacetic acid methodologies being the most common (Long et al., 2020; Mohammed et al., 2020). After the treatment of the above steps, the protein content in the sample was effectively controlled, and the inorganic salts were then removed by dialysis, the preferred method used in most laboratories (Zhang C. et al., 2019). Additionally, for further purification and separation, column chromatography and fractional precipitation may be used (Tang and Huang, 2022). After the above steps are completed, polysaccharides can be purified by concentration and drying. See Table 2 for purification methods for EU polysaccharides.

The first step in isolating and purifying polysaccharides is to remove the protein (Yang et al., 2009; Rajauria et al., 2021), for which there are many methods. For example. Zhu et al. (2009) added trichloroacetic acid to a hot water extract to precipitate proteins. The water fraction was subsequently dialyzed in tap water for a period of 3 days, and the polysaccharide was precipitated by the addition of four volumes of 95% ethanol. Following centrifugation, the precipitate was extensively washed with ethyl alcohol anhydride and subsequently lyophilized to yield a crude polysaccharide. In addition. Feng et al. (2021a) evaporated the filtrate using the Sevag method at low pressure. Similarly, Jiang et al. used the same method for protein removal (Jiang et al., 2011). After extracting polysaccharides in boiling water, the polysaccharides were precipitated overnight at 4°C and centrifuged utilizing chloroform/n-butanol at a ratio of 4:1 by volume. Subsequently, the collected water phase was dialyzed at a cut-off point of 8,000–14000 Da, and the process was followed by lyophilization

TABLE 2 Purification, monosaccharide compositions, molecular weight, and possible structures of polysaccharides from *Eucommia ulmoides*.

Part	Compound name	Purification methods	Monosaccharide compositions	Molecular weight	Possible structures	Reference
E. ulmoides bark	EPs	Dialysis	Man, Rha, GalA, Glc, Gal, Xyl, Ara (1.13:2.18: 3.15:82.71:0.79: 2.02:1.99)	0.63–251 kDa	-	Sun et al. (2022b)
E. ulmoides bark	EUPs(crude)	-	Ara, Gal, Glc, Rha (6.35:3.15: 1.47:1)	-	-	Jiang et al. (2011)
E. ulmoides bark	EUP	-	Glc, Gal, Ara, Rha (53.9–63.2: 10.6–16.1: 6.7–18.7: 2.0–2.1)	-	-	Xu et al. (2015)
E. ulmoides bark	EUPS	Seriatim, dialysis, DEAE Sephadex™ A-25	Glc, Fru, Man, Fucose, Gal, Ara (36.6:16.6:14.2: 15.7:9.5:7.4)	1146.32 kDa	A pyranose sugar, contained a β-type glycosidic bond	Feng et al. (2016)
E. ulmoides bark	EUP1	DEAE 52-cellulose ion-exchange column, gel-filtration column (Sephadex G100)	Rha, Ara, Gal, Man, Glc (4.6:8.6: 6.1:1:2)	358.1 kDa	→3,4-rha–1→3-glc-1→, →4-Man–1→4-Glc-1→, →4-Glc–1→4-Glc-1→, →4-Glc–1→3-Glc-1→, →3-Glc–1→4, 3-Rha–1→, →3,4-Rha–1→3,6-Gal-1→, →3,6-Gal–1→3,6-Gal–1→6-Gal-1→, →6-Gal–1→6-Gal–1→3-Gal-1→, Man–1→3,6-Gal-1→	Li et al. (2017)
E. ulmoides bark	Eucomman A	DEAE Sephadex A-25	Ara, Gal, Glc, Rha, GalA (8:6:4: 5:8)	6 kDa	α-L-Ara*f* 1→, α-D-Glc*p* 1→, →5 α-L-Ara*f* 1→, →4 α-D-Glc*p* 1→, →3 α-L-Ara*f* 1→, α-L-Rha*p* 1→, β-D-Glc*p* 1→, →2 α-L-Rha*p* 1→, →3 β-D-Glc*p* 1→, →4 β-D-Glc*p* 1→, →6 β-D-Glc*p* 1→, →4 α-D-Glc*p*A 1→	Gonda et al. (1990)
E. ulmoides bark	Eucomman B	DEAE-Sephadex A-25, Sephacryl S-300	Ara, Gal, Rha, GalA (10:5:24:24)	-	α-1,2-linkedl-rhamno-α-1,4-linkedd-galacturonan, α-1,5-Linkedl-arabinofuranose, α-1,3-linkedl-arabinopyranose, β-1,3- and β-1,4-linkedd-galactose, 2,4-branchedl-rhamnose and 3,4-branchedd-galacturonic acid	Tomoda et al. (1990)
E. ulmoides bark	EWDS-1	DEAE Sepharose™ Fast Flow column, Sephacryl™ S-400	Gal, Glc and Ara (2.1:1.0:0.9)	2000 kDa	(1,3,4,5-tetra-O-acetyl-2-O-methyl-arabinose (1,3,5-linked α-L-Araf),1,4,5,6-tetra-O-acetyl-2-O-methyl-galactose (1,4,6-linked β-D-Galp)), 1,5-linked and 1,3,5-linked α-L-Araf, 1,4-linked, 1,6-linked, and 1,4,6-linked β-D-Galp, 1,3-linked and 1,4-linked β-D-Glcp	Zhu et al. (2008)
E. ulmoides bark	EWDS-2	DEAE Sepharose™ Fast Flow column, Sephacryl™ S-400	Glc, Gal, Ara, and Rha (2.2:1.0: 0.4:0.2)	1000–2000 kDa	1,3-linked, 1,4-linked, 1,2,6-linked, 1,3,6-linked Glc; 1,6-linked, 1,2,6-linked, 1,3,4-linked, 1,4,6-linked Gal; 1,5-linked, 1,3,5-linked Ara; terminal and 1,2,5-linked Rha	Zhu et al. (2009)
E. ulmoides bark	FBWP	Enzymatic hydrolysis	Glc, Gal, Ara (31.8–53.4: 16.5–23.7:15.6–30.2)	17,540 g/mol	-	Zhu et al. (2016)
E. ulmoides bark	FBAP	Enzymatic hydrolysis	Ara, Xyl, Gal, Rha, Glc, Man GlcA (43.4–59.9:14.2–24.7: 15.2–21.4:1.9–2.4:3.5–5.9: 0.8–1.0:2.9–3.7)	210.1 kDa	-	Zhu et al. (2016)
E. ulmoides leaves	PsEUL	Sephadex G-150 column, dialysis	Glc, Ara, Gal, Rha monohydrate (38.2–39.1:37.7:12.8: 11.8)	60–600 kDa	-	Feng et al. (2021b)

(Continued on following page)

TABLE 2 (*Continued*) Purification, monosaccharide compositions, molecular weight, and possible structures of polysaccharides from *Eucommia ulmoides*.

Part	Compound name	Purification methods	Monosaccharide compositions	Molecular weight	Possible structures	Reference
E. ulmoides leaves	LWPE	Enzymatic hydrolysis	Glc and Gal (77.5: 8.8)	49,870 g/mol	-	Zhu et al. (2016)
E. ulmoides leaves	LAP	Enzymatic hydrolysis	Ara, Xyl, Gal, Rha, Glc, Man GlcA (43.4–59.9: 14.2–24.7: 15.2–21.4:1.9–2.4:3.5–5.9: 0.8–1.0:2.9–3.7)	62.1 kDa	-	Zhu et al. (2016)
E. ulmoides leaves	EULP	-	Glc, Gal, Rha (53.9–63.2: 10.6–16.1:10.1–11.3)	-	-	Xu et al. (2015)
E. ulmoides leaves	EUP	-	Gul acid, ribose, Rha, Glucosamine, Glucuronic acid, Aminogalactose, Xyl (0.81:77.7: 4.44:2.07:1.15:7.76: 6.06)	3170 g/mol	-	Deng et al. (2019)
E. ulmoides leaves	PsEUL	D101 macroporous adsorption resin, DEAE SephadexTMA-25	-	-	A hexahedral and cubic structure, with a uniform distribution and relatively uniform size	Feng et al. (2022)
E. ulmoides leaves	Pp	Anion-exchange chromatography (DEAE-52 anion-exchange chromatography column), hierarchical alcohol sink method	-	-	-	Liu et al. (2020b)
E. ulmoides leaves	EULP	-	EULP-CHE, contained Rha, Ara, Gal, Glc, Xyl, Man, GlcA, and galacturonic acid (9:32:20: 55:19:2:14:1) EULP-MAE contained Rha, Ara, Gal, Glc, Xyl, Man, GlcA, and GalA (7:4:6:14:1:2:3:1)	EULP-MAE 38,830 g/mol, EULP-CHE 12,055 g/mol	Belonged to a structure of β-type acidic heteropolysaccharides with a glucan group and highly branched degree	Xu et al. (2018)
E. ulmoides leaves and bark	LBAP	Enzymatic hydrolysis	Ara, Xyl, Gal, Rha, Glc, Man GlcA (43.4–59.9: 14.2–24.7: 15.2–21.4:1.9–2.4:3.5–5.9: 0.8–1.0:2.9–3.7)	178.1 kDa	-	Zhu et al. (2016)
E. ulmoides leaves and bark	LBWP	Enzymatic hydrolysis	Glc, Gal, Ara (31.8–53.4: 16.5–23.7:15.6–30.2)	27,890 g/mol	-	Zhu et al. (2016)

-: information was not available.

to yield the crude extract (Li et al., 2017). Furthermore, Liu et al. (Li et al., 2015) fractionated the precipitate with 30%, 40%, 50%, 60%, 70%, and 80% ethanol concentrations at 4°C for 24 h, obtaining the polysaccharide precipitates by centrifugation. The Savag method exhibits mild reaction conditions, but its efficiency is limited (Shi, 2016). Following extraction, the protein is treated with hydrogen peroxide or activated carbon to achieve decolorization (Liu et al., 2020). Notably, the above methods may lead to the loss of polysaccharides when removing proteins, and there is the problem of confusion, so it is necessary to carry out multiple extraction and purification steps. In addition, the trichloroacetic acid method is suitable for samples with high polysaccharide content, while the Sevag method has limited efficiency in samples with high polysaccharide content, so it is necessary to choose according to the actual situation. Currently, enzymatic hydrolysis is also used to remove proteins. This method involves the use of enzymes to break proteins into soluble fragments, which are then removed from polysaccharides. This can reduce the precipitation or agglutination encountered in the extraction process of polysaccharides and at the same time can improve the purity and Mw determination results of polysaccharides. However, according to the literature, enzymatic hydrolysis has not been applied in the removal of proteins from EU polysaccharides and should be studied.

In the purification of polysaccharides from EU, column chromatography is often used after the removal of inorganic salts, including ion exchange columns (usually filled with DEAE) and gel filtration columns (including Sephacryl and Sephadex) (Zhu et al., 2009). In general, the initial purification of crude polysaccharides is typically carried out through anion-exchange column chromatography, followed by gel column chromatography for subsequent purification (Shi, 2016). For instance. Zhu et al. (2009) utilized a DEAE Sepharose Fast Flow column to fractionate crude polysaccharides. The polysaccharide was first washed with water and gradually treated with 0.4, 0.8, 1.2, and 2.0 M NaCl solution to yield five subfractions. Subsequently, Sephacryl S-400 was employed for repeated size-exclusion column chromatography. In addition, Tomoda et al. applied crude polysaccharide onto a DEAE-Sephadex A-25 column

(Pharmacia), eluted it with water and $(NH_4)_2CO_3$, collected the eluate, and then applied it to a Sephacryl S-300 column. After elution with the same buffer, the eluate was further processed using a Sephadex G-25 column, eluted with water, concentrated and lyophilized (Tomoda et al., 1990). Moreover. Li et al. (2017) applied crude polysaccharides in conjunction with a DEAE 52-cellulose ion-exchange column to extract distinct acidic polysaccharides. Later, gel filtration column (Sephadex G100) chromatography led to the isolation of pure polysaccharides, which included EUP1, EUP2, and EUP3. Currently, anion-exchange columns and gel filtration columns have been widely used for polysaccharide purification. However, importantly, these methods have slow flow rates and are time consuming, particularly for acidic polysaccharides (Shi, 2016). To solve this problem, high-performance liquid chromatography (HPLC) can be used instead of traditional column chromatography. To further improve the purification efficiency and speed up the purification and to provide a more efficient and accurate method for the research and application of polysaccharides.

Highly purified and uniform polysaccharides were successfully isolated and obtained through a meticulous series of separation and purification techniques, including protein removal, decolorization, and column chromatography. Nevertheless, these processes can be both intricate and time-consuming and may require the use of significant amounts of volatile organic solvents such as ethanol. The three-phase partitioning (TPP) system and ultrafiltration technology can compensate for these shortcomings. They also have the advantages of high separation efficiency, simple equipment requirements and no pollution. These two methods can be used in future research on the isolation and purification of EU polysaccharides. They can effectively improve the extraction efficiency and purity of polysaccharides, thereby providing more high-quality raw materials for EU polysaccharides for research and application in biology, medicine and other fields.

4 Physicochemical and structural features of EU polysaccharides

Polysaccharides are a class of biological macromolecular substances with intricate structures that play crucial roles in biochemical and biomechanical functions (Boddohi and Kipper, 2010; Grube et al., 2020). A variety of techniques have been used to study and identify the physicochemical and structural characteristics of polysaccharides. Commonly used techniques include high-performance ion chromatography (HPIC), HPLC, gas chromatography (GC), ultraviolet visible spectroscopy (UV), Fourier transform infrared spectroscopy (IR) and nuclear magnetic resonance spectroscopy (NMR) (Cui et al., 2020). Table 2 provides details of reported EU polysaccharides, including name, monosaccharide composition, Mw, structure, and relevant references.

4.1 Thermal stability

Owing to the necessity of industrial thermal sterilization, thermal stability as one of the physicochemical properties of polysaccharides is a key feature for their industrial application of biological materials (Nuerxiati et al., 2019). Additionally, practicality and usefulness of plant polysaccharides are directly correlated to their thermal stability to maintain the appearance, nutrition, and healthiness of food and medicine (Feng Y. et al., 2021). Deng et al. (2019) used Differential Scanning Calorimetry (DSC) and Thermo Gravimetry Analysis (TGA) to investigate the thermal stability of EU polysaccharides (EUP) and strontium EU polysaccharides (EUP-Sr). EUP with the incorporation of strontium increased the amount of adsorbed water and decreased the total weight losses. It is indicated that EUP-Sr was found to have better thermal stability than EUP due to a new complex formed with thermal-stable property. Sun et al. (2018) successfully prepared the nanocrystalline cellulose (NCC)/EU gum (EUG) nanocomposite films. However, the thermal stability of the incorporation of 2, 4, 6, and 8% NCC were decreased to varying degrees. Fortunately, among them the 4% NCC film exhibited the highest thermal stability. It suggests that NCC/EUG nanocomposite films are able to widely used in the future in packaging applications or dryland agriculture. Liu et al. (2023a) measured differential scanning calorimetry (DSC) curves to assess thermal stability of three components (EUP1, EUP2 and EUP3) purified from EU leaves. They all exhibited good thermal stability below 270 °C, but EUP1 was found to have the highest thermal stability. Differences among all the three in thermal stability might result from different molecular weight and monosaccharide composition. Physicochemical properties of polysaccharides are strongly associated with their biological effects (Meng et al., 2018), however, up to now a lack of knowledge persists concerning comprehensive studies on physicochemical properties of EU polysaccharides, such as rheological property, gelling property, and solubility.

4.2 Molecular weight (Mw)

Mw is a crucial index affecting the pharmacological activities of polysaccharides (Sun et al., 2012; Zhang J. et al., 2019). Recently, gel permeation chromatography (GPC) and high-performance gel permeation chromatography (HPGPC) have been employed to determine the Mw of polysaccharides from EU. As an example. Feng et al. (2016) extracted the polysaccharide EU polysaccharides from the bark of EU. These authors determined its Mw using GPC on a Sephadex G-100 column and established a calibration curve with a dextran standard. The result showed that the Mw of EU polysaccharides was 1146.32 kDa. In addition. Zhu et al. (2008), Zhu et al. (2009) isolated and refined two polysaccharides (designated EWDS-1 and EWDS-2) from *Eucommiae cortex* and subsequently determined their respective Mws using HPGPC. Their findings indicated that EWDS-1 had an average Mw of approximately 2000 kDa, while EWDS-2 had an average Mw ranging from 1000~2000 kDa. Moreover. Xu et al. (2014), Xu et al. (2018) utilized GPC with a refraction index detector (RID) to determine the Mw of polysaccharides from *Eucommiae folium* with the assistance of microwave extraction. The polysaccharides extracted with microwave extraction had a higher Mw and greater polydispersity than those prepared without microwave extraction. Simultaneously. Xu et al. (2015) found that the relative Mw of polysaccharides extracted from leaves (21,410–91,800 g/mol) was

generally higher than that extracted from bark (17,995–32,125 g/mol) by GPC and RID. In addition, the relatively high Mw also indicated that ultrasonic treatment did not significantly alter the macromolecular structure of EU polysaccharides but was conducive to the dissolution of macromolecular polysaccharides in water. It is clear that the Mw of polysaccharides varies with variable factors such as extraction and purification methods, solvents, raw materials and calibration methods (Prajapati et al., 2013; ShanChen et al., 2019; Liao et al., 2020), so these factors need to be considered during optimization and studied in depth. Second, the extraction and purification methods used by different researchers are quite different, making comparison of the obtained Mw data difficult. Studies have found that low-Mw polysaccharides have greater antioxidant activity than high-Mw polysaccharides due to the challenge high-Mw polysaccharides face in penetrating cell membranes (Qi et al., 2005; Zha et al., 2016). However, according to the EU polysaccharides reviewed in this paper, polysaccharides with a high Mw have strong antioxidant activity, and this difference needs to be further studied in the future.

4.3 Monosaccharide composition

It is well known that polysaccharides are formed by a few monosaccharides linked by glycosidic bonds. Many exciting studies have shown that the bioactivity of polysaccharides is intricately linked to the composition and proportion of individual monosaccharides; therefore, it is important to study the monosaccharide composition. In general, the first step to determine monosaccharide composition is acid hydrolysis, after which samples are analysed utilizing GC and HPLC (Yan et al., 2014; Ferreira et al., 2015; Cao et al., 2019; Fan et al., 2019). In this review, we found that most *Eucommia* polysaccharides contained glucose (Glc), galactose (Gal) and arabinose (Ara), but their contents differed. Zhu et al. (2009) determined the monosaccharide composition of EWDS-2 by GC analysis of alditol acetates, which consisted of Glc, Gal, Ara, and rhamnose (Rha) (2.2:1.0:0.4:0.2), accompanied by trace amounts of mannose (Man) and 6.55% protein. Additionally. Feng et al. (2016) hydrolysed EU polysaccharides with 2 M trifluoroacetic acid (TFA) at 100°C and then analysed the monosaccharide composition of the sample by GC. The monosaccharide components of EU polysaccharides were Glc, fructose (Fru), Man, fucose (Fuc), Gal and Ara (36.6:16.6: 14.2:15.7: 9.5%: 7.4%). In addition. Xu et al. (2015) identified the polysaccharide components of EU by sulfuric acid hydrolysis and found that leaf polysaccharides mainly contained Glc, Gal and Rha, while bark polysaccharides mainly contained Gal, Glc and Ara. Similarly. Xu et al. (2018) also found that two polysaccharides from *Eucommiae folium* obtained by conventional heat-reflux extraction (CHE) and MAE contained similar monosaccharides, but their contents were significantly different. At the same time, it is worth noting that both EULP-CHE and EULP-MAE contain large amounts of Glc, 38.2% and 39.1%, respectively. This suggests that some β-glucan may be present in the isolated polysaccharide, indicating that microwave-assisted extraction may be beneficial to the release of β-glucan. Furthermore. Zhu et al. (2016) found that water-soluble EU polysaccharides were primarily composed of Glc, while alkali-extracted polysaccharides were mainly composed of Ara. These results suggest that EU polysaccharides obtained from various plant parts and through various extraction processes have different chemical compositions, which can impact the medicinal properties and alter chemical structures (Huang et al., 2019; Wang et al., 2021; Wu et al., 2021). According to the study results of Xu et al., the antioxidant activity of leaf polysaccharides was higher than that of bark polysaccharides, which may be caused by the stronger antioxidant activity of Rha than of Ara, regardless of content. This indicates that monosaccharide composition may play a potentially crucial role in antioxidant activity. Further studies on the composition and proportion of monosaccharides in polysaccharides obtained from different parts and extraction processes are needed to clarify their biological activities. In addition, although the relationship between the biological activity of polysaccharides and the composition and proportion of monosaccharides has been studied, the mechanism by which the composition and proportion of monosaccharides affect biological activity has not been discussed in detail. Moreover, the biological activity of polysaccharides is also closely related to other components, so the existence and influence of these components need to be further studied. Investigating the interaction between the biological activity of polysaccharides and other components provides a scientific basis for the further development and utilization of polysaccharides.

4.4 Structural features

4.4.1 Functional groups

Growing evidence has proved that a variety of polysaccharides with specific functional groups, such as acetyl groups, sulfate groups and phosphate groups, whether by chemical modifications or derived from natural resources possess better bioactivities than those that are naturally occurring without these functional groups (Song et al., 2015).

Acetyl groups of polysaccharides play a key role in their immunostimulatory activities (Ferreira et al., 2015). Fourier transformed infrared (FT-IR) spectroscopy is an available tool for identifying the functional groups of polysaccharides (Zhao et al., 2020). Liu et al. (2023b) used FT-IR spectroscopy to characterize the functional groups of three polysaccharide fractions derived from EU eluted by a column of DEAE-52 cellulose. The results showed that these three fractions all had an absorption peak at 1262 cm^{-1}, suggesting that they contained acetyl groups. Likewise. Xu et al. (2018) indicated that under microwave irradiation decrease of acetyl groups linked to the polysaccharides were produced, and some acetyl groups attached to the polysaccharides derived from EU were confirmed by the presence of the weak peak at 1238 cm^{-1}. Besides. Xu et al. (2015) reported the presence of some acetyl groups attached to the polysaccharides, particularly in the fraction isolated from EU leaves. This was due to the presence of the two weak absorbances at 1715 and 1240 cm^{-1}. Nuclear magnetic resonance (NMR) spectroscopy is another important tool to obtain functional groups characteristic of polysaccharides (Huang et al., 2020). In ^{1}H-NMR spectrum of the polysaccharide derived from EU, Liu et al. confirmed the presence of the acetyl group by the signal at δH1.9–2.3 ppm corresponded. This result was consistent with

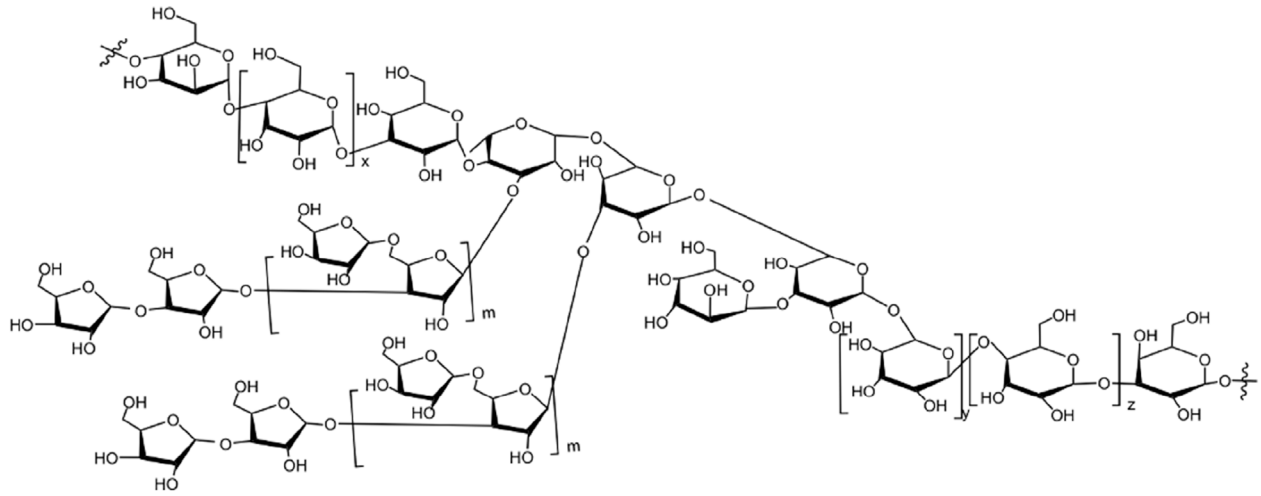

FIGURE 2
The hypothetical structure of EUP1.

that of the FT-IR spectrum analysis mentioned above (Liu et al., 2023a).

Additionally, some studies have shown that the sulfate-group content of polysaccharides is usually associated with their biological activities, especially antitumor and anticoagulant activities (Wang et al., 2018; Li et al., 2020; Li T. et al., 2022). Polysaccharides with higher sulfate group content exhibit such higher bioactivities. Zhu et al. (2009) measured the sulfate content of the polysaccharide EWDS-2 with anti-complementary activity derived from EU using a modification of $BaCl_2$ turbidimetric method. The result indicated that EWDS-2 was characterized with 2.82% sulfate content. It might be consequently proved right that the sulfate group of polysaccharides was found to be required for the exhibition of their anti-complementary activity.

In the present study, however, the mechanistic details of biological activities of polysaccharide functional groups derived from UC was not deeply discussed. Their structure-activity relationships with regard to functional groups remain undetermined.

4.4.2 Glycosidic bond

The polysaccharide's overall structure is influenced depending not only upon the monosaccharide composition/molecular weights, but also upon the other factors, especially the glycosidic bonds (Hou et al., 2021). To analyze the polysaccharide structure in more detail in glycosidic bonds, it is necessary to clarify type of glycosides linkage and branching of polysaccharides (Zou et al., 2020). The glycosidic linkage and link manner of polysaccharides derived from EU were investigated by complete or partial acid hydrolysis, methylation, together with GC-MS, IR and NMR spectra analysis.

Zhu et al. (2008), Zhu et al. (2009) obtained a homogeneous protein-bound polysaccharides EWDS-1 and EWDS-2 from the *Eucommiae cortex*. Through methylation analysis and NMR, the structure of their residues was deduced. EWDS-1 was a complex branched polysaccharide with an anti-complementary effect; the backbone was composed of Gal and Glc residues arranged in a pyranosidic form, while the side chains were enriched with Ara residues in the furanosyl configuration. Moreover, there were terminal, 1,5-linked and 1,3,5-linked α-L-Araf; terminal, 1,3-linked and 1,4-linked β-D-Glcp; and terminal, 1,4-linked, 1,6-linked, and 1,4,6-linked β-D-Galp. The main linkages of the residues of EWDS-2 that was also anti-complementary active include terminal, 1,6-linked, 1,2,6-linked, 1,3,4-linked, 1,4,6-linked Gal; 1,3-linked, 1,4-linked, 1,2,6-linked, and 1,3,6-linked Glc; 1,5-linked and 1,3,5-linked Ara; and terminal and 1,2,5-linked Rha. Similarly. Li et al. (2017) extracted a polysaccharide from EU bark, EUP1, which was characterized by methylation and NMR; the following linkages were identified: →3,4-Rha–1→3-Glc-1→, →4-Man–1→4-Glc-1→, →4-Glc–1→4-Glc-1→, →4-Glc–1→3-Glc-1→, →3-Glc–1→4,3-Rha–1→, →3,4-Rha–1→3,6-Gal-1→, →3,6-Gal–1→3,6-Gal–1→6-Gal-1→, →6-Gal–1→6-Gal–1→3-Gal-1→, and Man–1→3,6-Gal-1→. The possible chemical structure of EUP1 is shown in Figure 2. In addition, Tomoda et al. (Tomoda et al., 1990) found a glycan, eucomman B in the hot water extract of the bark of EU, and chemical and spectral studies have shown that this glycan predominantly constituted the characteristic structural subunits of α-1,2-linked L-Rha, α-1,4-linked D-galacturonan, α-1,5-linked L-arabinofuranose, α-1,3-linked L-arabinopyranose, β-1,3- and β-1,4-linked D-Gal, 2,4-branched L-Rha, and 3,4-branched D-galacturonic acid residues. This data shows that most polysaccharides obtained from EU are 1,3-linked, 1,4-linked, and 1,3,6-linked polysaccharides, but there are many different linkage sites and fragments, resulting in different chemical structures, which could be due to species differences and varying growth environments of EU, leading to different EU polysaccharide biological activities (Qiu et al., 2022). EU polysaccharides exhibit diverse types of glycosidic bonds and functional groups.

4.4.3 Spatial structure

Besides functional groups, glycosidic bond, et al., spatial structure is also one of the important parameters of plant polysaccharides. And spatial structure or conformation of polysaccharides was found to be decisive for the biological

activity (Kolsi et al., 2016; Hou et al., 2021). Increasing studies focus on exploring the advanced structure of polysaccharides to elucidate the relationship between their spatial structure and biological function (Lin and Huang, 2022). Morphologically, being soluble in aqueous solution, polysaccharide molecules may present diverse conformations, such as random coil, rod-lone, sphere-like shapes, and helical structures (Lin and Huang, 2022; Fu et al., 2024).

Deng et al. (2019) compared the particle sizes of EUP and EUP-Sr by scanning electron microscopy (SEM). Interestingly, EUP-Sr was found to have a more uniform and smaller particle size than EUP. This was probably because strontium incorporation had proved useful for optimization of the disordered structure of EUP. Deng et al. also observed the morphologies of EUP and EUP-Sr by atomic force microscope (AFM). It could be observed from the AFM images that height distributions of both EUP and EUP-Sr were fairly narrow with spherical morphology. But there was a slightly difference between the two by height distribution analysis. Strontium incorporation may cause the smaller particle size of EUP-Sr, resulting in the aggregation of the molecular chain in EUP. In addition to spherical ones, EUPs derived from EU were also found to display a spongy appearance by SEM 500-fold, 3000-fold and 5000-fold amplification, presenting a rough surface with pores and crevices (Feng et al., 2016). Compared with conventional SEM, field emission scanning electron microscopy (FE-SEM) observations have several advantages, including providing a clearer picture with 3–5 times better resolution (Das et al., 2022). Liu et al. (2023b) used FE-SEM at 10 K×multiples to observe the surface microstructures of three polysaccharide components purified from EU leaves. The three resulting component structures presented completely different surface morphologies. EUP1, EUP2 and EUP3 were honeycomb-shaped, rod-shaped and fake structure with smooth surface, respectively. These differences could be related to their presented different aggregation and bonding strength.

Overall, the available literature on information on the spatial structures of EU polysaccharides is quite inadequate. Due to complex structures of polysaccharides, it is very difficult to fully characterize spatial structures or conformation. Therefore, more attention should be paid to novel and more effective techniques. More details obtained in spatial structure will subsequently help reveal some structure-activity relationship of EU polysaccharides in the future.

5 Biological activities of EU polysaccharides

EU is a valuable Chinese herb for treating diseases, and EU polysaccharides are important active ingredients due to their pharmacological function. The bioactivity of EU polysaccharides has been studied extensively *in vivo* and *in vitro*. Numerous studies have proven that EU polysaccharides have a wide range of biological activities, including immunomodulatory (Feng et al., 2016), antioxidant (Hong et al., 2013), anti-inflammatory (Sun et al., 2021), anticomplementary (Zhu et al., 2008), antifatigue (Xia and Piao, 2010), and hepatoprotective (Gao et al., 2020) activities. The comprehensive bioactivity information of EU polysaccharides is shown in Figure 3; Table 3.

5.1 Immunostimulatory activity

Numerous studies have demonstrated that plant polysaccharides exert various effects on immune regulation but cause almost no adverse reactions (Chen et al., 2021b; Zhang et al., 2021). This phenomenon is mainly achieved through their impacts on the immune system, such as the activation of macrophages and dendritic cells (DCs). Macrophages have multiple functions, serving as crucial components of the immune system by actively participating in both specific and nonspecific immune responses (Artyomov et al., 2016). DCs are closely related to the development of tumors and are the most powerful antigen-presenting cells in the immune system (Kim et al., 2009). The activity of macrophages and DCs is a key indicator of immune activation and an important part of immune effect research (Yuan et al., 2015). Most scholars choose to detect DCs and macrophages to determine immune activity. Sun et al. (2021) found that EU polysaccharides, at concentrations of 50 μg/mL and 100 μg/mL, exhibited potent stimulatory effects on macrophage proliferation. This effect was accompanied by a reduction in M1-polarized macrophages and an accumulation of M2-polarized macrophages. Additionally. Feng et al. (2016) performed *in vitro* and *in vivo* experiments. The *in vitro* experiments showed that EU polysaccharides (1.2–75 μg/mL) induced DC maturity and not only increased MHC I/II, CD80, CD86 and CD40 expression but also significantly stimulated lymphocyte proliferation and effectively augmented the production of IL-4 and IFN-γ cytokines. During the *in vivo* study, it was found that EU polysaccharides could increase T-cell proliferation and production of IgG, IgG1, IgG2a, and IgG2b antibody titres. In summary, EU polysaccharides are strong immunostimulants that can potentially enhance the efficacy of vaccines.

Notably, modified polysaccharides enhance immunomodulatory activity. To develop a new bone immunomodulatory biomaterial based on EU polysaccharides. Deng et al. (2019) successfully synthesized strontium-EU polysaccharide conjugates (EUP-Sr). The results of the CCK8 assay showed that EUP-Sr exerted a positive influence on the proliferation of macrophages and could inhibit the production of inflammatory factors and osteoclasts. The aim of the study was to enhance the adjuvant activity of polysaccharides isolated from *Eucommiae folium* (PsEUL) in potentiating an efficient immune response against ovalbumin (OVA). Feng et al. accomplished conjugation of PsEUL to OVA by employing the one-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) method. Experimental *in vitro* and *in vivo* studies demonstrated that the PsEUL-OVA/Cubs formulation elicited robust immune responses through the enhancement of phagocytic activity in DCs and macrophages, thereby enhancing antigen presentation efficiency (Feng et al., 2021; Feng et al., 2022). Furthermore, Feng et al. (Feng et al., 2020) ingeniously encased OVA and EU polysaccharides within long-circling nanoliposomes and conjugated them with an antibody targeting the DEC-205 receptor to develop a groundbreaking nanoliposome formulation known as anti-DEC-205-EUPS-OVA-LPSM. This formulation effectively served as a means of antigen delivery, thereby enhancing both cellular and humoral immune responses by stimulating the maturation of DCs. These studies lay the foundation for the development of novel adjuvant-antigen

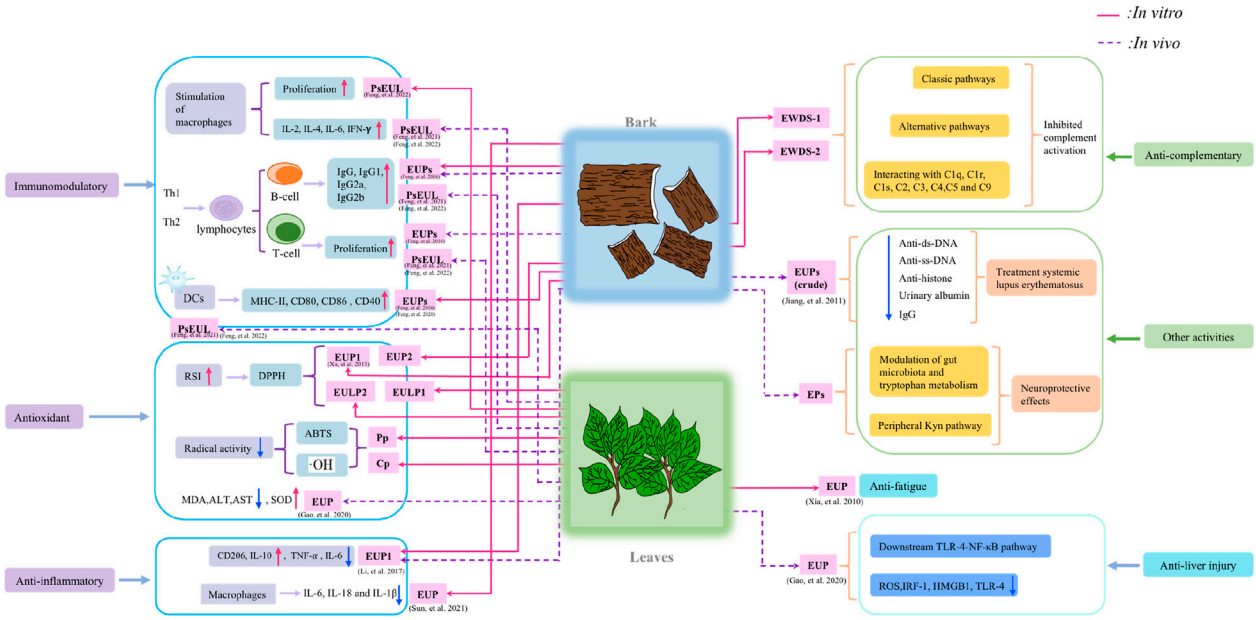

FIGURE 3
Biological activities of polysaccharides from *Eucommia ulmoides*.

delivery systems. These findings highlight the potential usefulness of EU polysaccharides as a natural active ingredient to enhance and regulate human immune function.

Collectively, the immunomodulatory activity of EU polysaccharides controls and regulates the balance of the immune response, promoting the release of immunoreactive substances and enhancing immune cell function. It is clear that EU polysaccharides show potential as novel immunomodulators with therapeutic potential for a broad spectrum of diseases. However, most current studies are limited to *in vitro* studies utilizing a limited selection of cell lines, necessitating further *in vivo* and clinical investigations to fully evaluate their therapeutic potential. For the study of macrophages and DCs, we focused on their quantitative changes and antigen presentation ability and ignored their specific roles in the immune response. Their mechanism of action in the specific immune response should be further explored in combined *in vitro* and *in vivo* experiments. Consistent focus is needed to understand how these factors interact in order to effectively change polysaccharides and achieve the best results.

5.2 Antioxidant activity

Oxidative stress refers to the detrimental impact resulting from the excessive generation of ROS in the human body, which is associated with the occurrence of a myriad of ailments, including cancer, diabetes mellitus, and atherosclerosis (Ismail Iid et al., 2020; Chen et al., 2021). Antioxidants can prevent and reduce damage to the body to a certain extent. The current study found that natural plants are valuable sources of novel antioxidants, and their main active components are polysaccharides. Usually, antioxidant activities are evaluated through the utilization of various free radical assays, including DPPH, hydroxyl, and ABTS radicals *in vitro* (Luan et al., 2021). For example, Hong et al. (Hong et al., 2013) found that EU polysaccharide extracts reversed IR-induced decreases in superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and glutathione reductase (GR) activities in the kidney in a dose-dependent manner. These results suggest that EU polysaccharides may play a protective role in rabbit kidney oxidative injury through their free radical scavenging activities. In addition. Liu et al., (2020a) obtained EU leaf polysaccharides by UAE. Through meticulous power tests, the DPPH test, OH test and ABTS test, it was found that EU polysaccharides had notable DPPH, OH and ABTS radical scavenging activities. Notably, the 50% inhibitory concentration (IC_{50}) values of EU polysaccharides for scavenging DPPH radicals in Cp (crude) and Pp (pure) were found to be 0.005 mg/mL and 0.011 mg/mL, respectively. additionally. Xu et al. (2015) used UAE of polysaccharides from EU bark and leaves. The findings revealed that ultrasonic treatment improved the yield and selectivity of polysaccharide antioxidants. Furthermore, the antioxidant activity of leaves proved to be significantly higher than that of the bark. EU polysaccharides can act as potential antioxidants, and there is a strong correlation between antioxidant activity and the content of bioactive compounds. Although various studies have proven the antioxidant actions of EU polysaccharides *in vitro*, there have been few *in vivo* studies, which need further investigation. The potential molecular mechanism of the antioxidant effect of EU polysaccharides is unclear, and it is worthwhile to explore the underlying mechanisms responsible for this effect.

5.3 Anti-inflammatory activity

Inflammation is a crucial defensive mechanism of the body's immune system, triggered by inflammatory agents or physical

TABLE 3 Summary of pharmacological activities of polysaccharides from *Eucommia ulmoides* and their applications or prospects.

Pharmacological activities	Name	Source	Types	Models/ Testing subjects	Doses/ duration	Effects/ mechanisms	Applications/ Prospects	Reference
Neuroprotective effects	EPs	*E. ulmoides* bark	*In vivo*	OD-induced obesity-related symptoms mice model	400 mg/kg	*Escherichia coli*, LPS, peripheral Kyn pathway, QA and glutamic acid↓	Mitigate obesogenic diet-induced cognitive and social dysfunction	Sun et al. (2022b)
A beneficial effect on systemic lupus erythematosus	EUPs(crude)	*E. ulmoides* bark	*In vivo*	Systemic lupus erythematosus-like syndrome induced by *campylobacter jejuni* in BALB/c mice model	15, 30 mg/kg/day	Anti-ds-DNA, anti-ss-DNA, anti-histone antibodies levels, IgG production, urinary albumin↓	New agent for the treatment of autoimmune disease	Jiang et al. (2011)
Antioxidant activity	EUP1	*E. ulmoides* bark	*In vitro*	The 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical	0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1.0, and1.2 mg/mL	The scavenging activity was 56.3%, RSI↑	Preparing nutritional health foods	Xu et al. (2015)
	EUP2	*E. ulmoides* bark	*In vitro*	The 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical	0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1.0, and1.2 mg/mL	The scavenging activity was 70.9%, RSI↑	Preparing nutritional health foods	Xu et al. (2015)
	EULP1	*E. ulmoides* leaves	*In vitro*	The 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical	0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1.0, and1.2 mg/mL	The scavenging activity was 89.6%, RSI↑	Preparing nutritional health foods	Xu et al. (2015)
	EULP2	*E. ulmoides* leaves	*In vitro*	The 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical	0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1.0, and1.2 mg/mL	The scavenging activity was 96.7%, RSI↑	Preparing nutritional health foods	Xu et al. (2015)
	Pp	*E. ulmoides* leaves	*In vitro*	DPPH radical, OH radical, ABTS radical	0.04 mg/mL to 0.18 mg/mL	Scavenging activity↑	Be applied as potential antioxidant	Liu et al. (2020b)
	Cp(crude)	*E. ulmoides* leaves	*In vitro*	DPPH radical, OH radical, ABTS radical	0.04 mg/mL to 0.18 mg/mL	Scavenging activity↑	Be applied as potential antioxidant	Liu et al. (2020b)
	EULP	*E. ulmoides* leaves	*In vitro*	DPPH radical	0.1–2.0 mg/mL	DPPH radical scavenging index (0.87–1.22) ↑	Act as a favorable antioxidant in functional food	Xu et al. (2018)
	EUP	*E. ulmoides* leaves	*In vitro*	Liver I/R model in rats	320 mg/kg, 160 mg/kg, 80 mg/kg for 10 days	SOD↑ ALT, AST and MDA↓	May be a promising drug in liver surgery to prevent HIRI	Gao et al. (2020)
Immunomodulatory effects	EUPS	*E. ulmoides* bark	*In vitro*	Mice splenocyte cell, mice bone marrow cells	100 μL for 68 h	MHC I/II, CD80, CD40, CD86, lymphocyte proliferation, IL-4 and IFN-γ↑	A strong immunostimulant, potential adjuvant for vaccine design	Feng et al. (2016)
	EUPS	*E. ulmoides* bark	*In vitro*	DC cells	5, 10, 20, 40, 80, 160, 320, 640, 1280 μg/mL for a further 24 h	IgG, IgG isotypes levels and maturation of DCs↑	Immune-enhancing agent and antigen delivery system	Feng et al. (2020a)
	PsEUL	*E. ulmoides* leaves	*In vitro*	RAW264.7	200 μg/mL for a further 24 h	Macrophages proliferation and phagocytosis↑	-	Feng et al. (2021a); Feng et al. (2022b)
	EUPS	*E. ulmoides* bark	*In vitro*	Females ICR mice	100 μg for 14 days	Splenocyte proliferation, NK cell and CTL cytotoxicity↑	Immune-enhancing agent and antigen delivery system	Feng et al. (2020a)

(Continued on following page)

TABLE 3 (*Continued*) Summary of pharmacological activities of polysaccharides from *Eucommia ulmoides* and their applications or prospects.

Pharmacological activities	Name	Source	Types	Models/ Testing subjects	Doses/ duration	Effects/ mechanisms	Applications/ Prospects	Reference
	EUPS	*E. ulmoides* bark	*In vitro*	Female ICR mice	0.5 mg for 14 days	IgG, IgG1, IgG2a, IgG2b and T cell proliferation↑	A strong immunostimulant, potential adjuvant for vaccine design	Feng et al. (2016)
	PsEUL	*E. ulmoides* leaves	*In vivo*	Female ICR mice	500 μg/mL for 7 days	IgG, IgG1, IgG2a, IgG2b, IL-2, IL-4, IL-6, IFN-γ, T cells and DCs ↑	Developing natural polysaccharide-conjugated antigen nano delivery systems	Feng et al. (2021a); Feng et al. (2022)
Anti-inflammatory activity	EUP1	*E. ulmoides* bark	*In vitro*	Raw 264.7 cells	10 μg/mL, 25 μg/mL,50 μg/mL for 18 h	IL-10↑ IL-1β, TNF-α and IL-6↓	A valuable candidate for further development into an anti-septic therapeutic agent	Li et al. (2017)
	EUP	*E. ulmoides* bark	*In vitro*	RAW 264.7 cell	10,50,100,200 μg/mL for 1,3,5 days	BMP-6, Arg-1 and TGF-β ↑ IL-6, IL-18 and IL-1β, M1 polarized macrophages↓	Treatment for osteoarthritis	Sun et al. (2021)
	EUP-Sr	*E. ulmoides* bark	*In vitro*	RAW 264.7	10, 50, 100, 500 μg/mL for 1 and 3 days	Osteogenesis and inhibits osteoclastogenesis↑	Create a positive prodegenerative environment for skeleton tissue engineering	Deng et al. (2019)
	EUP1	*E. ulmoides* bark	*In vivo*	LPS-induced sepsis model in mice	10 mg/kg	CD206, IL-10↑ TNF-α↓	A valuable candidate for further development into an anti-septic therapeutic agent	Li et al. (2017)
Anti-complementary activity	EWDS-1	*E. ulmoides* bark	*In vitro*	Sheep erythrocytes, Guinea pigs, Rabbit erythrocytes, NHS	100,150,200 μL	Classical pathway, alternative pathway and spontaneous activation of NHS↓	Promising benefits in treatment of the complement associated diseases	Zhu et al. (2008)
	EWDS-2	*E. ulmoides* bark	*In vitro*	NHS, Guinea pigs, Sheep erythrocytes, Rabbit erythrocytes	100,150,200 μL	Classical pathway, alternative pathway and spontaneous activation of NHS↓	Be valuable for the treatment of diseases associated with the excessive activation of the complement system	Zhu et al. (2009)
Anti-fatigue	EUP	*E. ulmoides* leaves	*In vitro*	Gastrocnemius was isola-ted from toad	0.01, 0.05, 0.25 mg/mL	The maximum contraction time of toad muscle and the muscular fatigue↓	Develop a new medicine for the preventing and remedying of myocardial ischemia reperfusion induced injury	Xia and Piao (2010)
Anti-liver injury	EUP	*E. ulmoides* leaves	*In vivo*	Liver I/R model rat serum	320 mg/kg, 160 mg/kg, 80 mg/kg for 10 days	IRF-1, HMGB1↑ TNF-α, IL-1β, ROS, TLR-4, MyD88, P-p65, P-IKB-αproteins and TLR-4-NFκB pathway↓	May be a promising drug in liver surgery to prevent HIRI	Gao et al. (2020)

↑: improve or promote.
↓: inhibit or reduce.
-: information was not available.

damage to the tissue. According to existing research results, the aetiology of numerous ailments stems from inflammatory processes, such as obesity, hypertension and cancer (Fang et al., 2020; Li and Huang, 2021). Among natural products, polysaccharides have immense potential as anti-inflammatory drugs. In recent years, the safety and anti-inflammatory activity of plant polysaccharides have attracted increasing attention from researchers worldwide (Hou et al., 2021). It was found that EU polysaccharides had anti-inflammatory activity. For instance. Sun et al. (2021) found that EU polysaccharides had a positive effect on osteoarthritis. *In vitro*, inhibition of the expression of inflammation-related genes, such as IL-6, IL-18, and IL-1β, was observed following exposure to EU polysaccharides, while the expression of bone and chondrogenic genes, such as BMP-6, Arg-1, and TGF-β, was enhanced. *In vivo*, the destruction of articular cartilage is reduced. Additionally, Li et al. (2017) reported that the polysaccharide EUP1 from EU bark promoted the expression of CD206, a pivotal proinflammatory cytokine, and IL-10 in Raw 264.7 cells. These results indicate that EUP1 can effectively suppress the expression of major inflammatory factors, reduce lung injury, and augment the survival rate of animals. Furthermore. Deng et al. (2019) found that EUP-Sr could enhance the expression of osteoblast factors in RAW 264.7 cells. Consequently, these results indicate that EU polysaccharides exert an anti-inflammatory effect by inhibiting the expression of various proinflammatory cytokines. However, evidence of anti-inflammatory activity is still scarce, and in-depth *in vivo* studies and clinical trials are needed. The precise mechanism underlying the anti-inflammatory action requires further investigation. Current research on the anti-inflammatory effects of EU polysaccharides focuses on bark extracts, with no studies on leaf extracts. More research on leaf extracts is needed to support the future application of EU polysaccharides. We believe that EU polysaccharides may have potential as natural drug candidates in suppressing inflammatory responses in the future and make more contributions to human health.

5.4 Anti-complementary activity

The complement system is a complex biochemical reaction system and constitutes a crucial component of the innate immune system, which is the cornerstone of the body's defence network (Samuelsen et al., 1999). Therefore, we need to pay more attention to the protection and repair of the complement system to safeguard our health. Recently, medicinal plants have been extensively studied for the extraction of polysaccharides, which exhibit remarkable efficacy and minimal toxicity. These polysaccharides have proven to be invaluable as raw materials for the development of novel natural complement inhibitors for the treatment of associated diseases caused by overactivation of complement, which has attracted increasing attention (Xia et al., 2019; Huo et al., 2020; Xia et al., 2020). Zhu et al. (2008), Zhu et al. (2009) extracted polysaccharides (EWDS-1 and EWDS-2) from the EU Cortex by the hot water method. The CH50 and AP50 values of EWDS-1 were 203 ± 20 μg/mL and 45 ± 8 μg/mL, respectively. The CH50 and AP50 values obtained for EWDS-2 were 282 ± 11 μg/mL and 144 ± 17 μg/mL, respectively. Preliminary mechanistic studies showed that EWDS-1 and EWDS-2 effectively suppressed complement activation through their interactions with C1q, C1r, C1s, C2, C3, C4, C5, and C9, thereby providing a crucial regulatory mechanism for both the classical and alternative pathways. Therefore, EWDS-1 and EWDS-2 have potential value in treating diseases linked to overactivation of the complement system. However, to date, there are few reports on the anti-complementary effect of EU polysaccharides, and the structure-activity relationship remains unclear, so it cannot be confirmed that only EWDS-1 and EWDS-2 have this activity. Experiments on EU polysaccharides' anti-complementary activity is needed soon to show their potential in treating diseases from complement overactivation.

5.5 Hepatoprotective activity

Damage to the liver can result in cirrhosis, fibrosis, steatohepatitis, and even cancer. A plethora of studies have elucidated that polysaccharides derived from plants have low toxicity and outstanding effects on liver protection (Yuan et al., 2019; Qu et al., 2020). EU polysaccharides are no exception. Gao et al. (Gao et al., 2020) found that EU polysaccharides can effectively alleviate liver injury in the context of HIRI. Ischaemic liver tissue and serum were collected for the detection of biochemical indices and pathological damage to liver tissue. The data showed that the serum concentrations of aspartate aminotransferase, alanine aminotransferase, tumor necrosis factor-α, and IL-1β were significantly decreased after EU polysaccharide pretreatment, the liver malondialdehyde level was markedly decreased, while the enzymatic activity of superoxide dismutase was significantly increased, and a remarkable reduction in the liver necrotic area was observed. The specific mechanism involves alleviating hepatic injury by reducing reactive oxygen species (ROS) levels and inhibiting the activation of the TLR-4-NF-κB pathway. Clinically, serum markers such as alanine transaminase (ALT) and aspartate transaminase (AST) serve as valuable indices for the assessment of hepatoprotective potential (Feng et al., 2020). According to existing studies, EU polysaccharides significantly reduce ALT and AST levels, and EU polysaccharides exhibit a hepatoprotective effect on hepatic ischaemia–reperfusion injury by improving antioxidant capacity and attenuating oxidative stress injury. Therefore, EU polysaccharides may be assumed to be an effective medicine for liver injury. However, as the results are based on only a few scholars and have not been tested in a broader clinical population, further studies are needed to confirm its safety and efficacy.

5.6 Other activities

Other biological activities have also been identified in EU polysaccharides. In the first instance. Xia and Piao (2010) found that polysaccharides from EU leaves can improve the muscle function of toads and have anti-fatigue properties. In comparison to that in the control group, the time needed for maximum muscle contraction in the polysaccharide group significantly decreased ($P< 0.05$), indicating that muscle fatigue was alleviated ($P< 0.05$; $P< 0.01$). Second. Jiang et al. (2011) found that EU polysaccharides

demonstrated a favourable influence on CJ-S131-induced systemic lupus erythematosus syndrome in BALB/c mice. Treatment with EU polysaccharides (from *Eucommiae cortex*) reduced immunoglobulin deposition and albuminuria, protecting the kidneys from glomerular damage. Increases in serum autoantibodies and total IgG levels were also effectively suppressed. EU polysaccharides have the potential to be novel drugs for the treatment of autoimmune disorders. Moreover, Sun et al. (Sun et al., 2022) found that EU polysaccharides inhibited subsequent neuroinflammation by inhibiting the expansion of *Escherichia coli*, regulating the intestinal microbiome and tryptophan metabolism, and reducing the concentration of lipopolysaccharides (LPS) in colon contents and serum. In addition, oral EU polysaccharides inhibit peripheral Kyn pathways and rescue glutamate-induced neuroexcitatory toxicity. These findings suggest that EU polysaccharides have significant neuroprotective potential. Overall, previous research has demonstrated that EU polysaccharides have various biological activities, which was consistent with other natural polysaccharides. Exploring the structure-activity relationship of EU polysaccharides in greater depth will advance the development of potential health products and clinical drugs. Moreover, many the biological effects of EU polysaccharides need to be further studied to elucidate possible structure-activity.

6 Structure-bioactivity relationship

The chemical structures of EU polysaccharides are the basis of their pharmacological activities. Detailed correlation between structures and activities of EU polysaccharides obtained is of great importance for providing theoretical basis and future directions for exploring the treatment of diseases with EU polysaccharides. However, with these inadequate studies on polysaccharides derived from EU, the current understanding of the structure-activity relationship of EU polysaccharides is still lacking and somewhat inconsistent.

Currently, it is widely recognized that molecular weight and monosaccharide composition are two of the most important factors affecting the biological activities of polysaccharides (Mo et al., 2022). Liu et al. (2023b) believed that differences in antioxidant activity among EUP1, EUP2 and EUP3 purified from EU leaves might result from the differences among molecular weight, branching degree, monosaccharide composition and connection position of monosaccharide residues. When the molecular weights of these three components were within a certain range, the higher the molecular weight was, the lower the scavenging capacity of DPPH and OH radical would be. Obviously, EUP1 with the largest molecular weight (1.51×10^5 Da) of the three had the weakest scavenging ability. Nevertheless, studies have found that the correlation between molecular weight and bioactivities of polysaccharides is not always straightforward (Chen H. et al., 2021). Zhu et al. (2008), Zhu et al. (2009) extracted and isolated from stem barks of EU, obtaining two homogeneous polysaccharides EWDS-1 and EWDS-2, with molecular weights of 2000 and 1000–2000 kDa, respectively. The CH50 (282 ± 11 μg/mL) and AP50 (144 ± 17 μg/mL) of EWDS-2 were significantly higher than EWDS-1 with the CH50 and AP50 values of 203 ± 20 μg/mL and 45 ± 8 μg/mL, suggesting that EWDS-2 with smaller molecular weight exhibited weaker anticomplement activity. In addition to molecular weight, it would be of interest to investigate regarding the relationships between monosaccharide composition and activity of polysaccharides, because different monosaccharide compositions of polysaccharides may contribute to different bioactivities. In the same study by Liu et al. (2023a) mentioned above, EUP1 exhibited the weakest free radical scavenging ability. It was found that the mole ratio of Rha 3.66 mol% in EUP1 was much lower than that in EUP2 (7.92 mol%) and EUP3 (18.67 mol%). In agreement with the literature, therefore, Liu et al. believed that Rha was the most important factor related to the free radical scavenging ability of polysaccharides.

In general, biological activities of EU polysaccharides can be also affected by their other structural features such as branching, glycosidic bond and chain conformation. Studies on a detailed structure–activity relationship in EU polysaccharides still needs further investigation. Thus, there is an urgent need to conduct substantial numbers of studies for comprehensive understanding on the structure–activity relationship in EU polysaccharides, which will facilitate the development of EU food supplements and medications.

7 Discussion

7.1 Applications

EU polysaccharides are rich in biological activities and have broad application prospects. Currently, EU polysaccharides are not only recognized for their ability to prevent and treat diseases, but also widely used in animal husbandry (as veterinary pharmaceutical biologics, livestock immune adjuvants, feed additives, etc.) and food industry (as dietary supplements, nutrition enhancers, etc.). The relevant patent information is shown in Table 4. The application status and future development of EU polysaccharides are shown in Figure 4 (made with assets from Freepik.com). Meanwhile, Figure 5 details the current status of EU related invention patents, including the annual global application and publication of patents for EU and its polysaccharides. In addition, we also inquired about the application for the approval of EU products in the State Administration for Market Regulation, as detailed in Table 5.

7.1.1 Field of medicine

With the deepening of research on plant polysaccharides, a large number of plant polysaccharides have been developed and applied in pharmaceutical industry, and become a hot spot in the study of natural active ingredients (Elango et al., 2023). EU polysaccharides are no exception, in the pharmaceutical industry, EU polysaccharides are utilized in traditional Chinese medicine preparations due to their biological activities. Studies have found that strontium plays an important role in bone formation, and it can promote the proliferation of osteoblasts and pluripotent stem cells and improve bone metabolism (Liu et al., 2023). However, free strontium ions are toxic *in vivo*, and the application of strontium is difficult to develop. The strontium complex of EU polysaccharides can avoid the toxicity of free strontium ions. At the same time, the prepared EU polysaccharides strontium complex also enables the

TABLE 4 Application of *Eucommia ulmoides* polysaccharides related patents.

NO	Application	Name of the invention	Main composition	Effect	Publish number (A)
1	Daily necessities	The invention relates to a toothpaste with the effect of whitening, removing stains and improving tooth sensitivity and a preparation method	Polysaccharides of *E. ulmoides*	Restore enamel health	CN113599285
2	Biomedicine	*E. ulmoides* polysaccharide strontium complex and its preparation method and application	Polysaccharides of *E. ulmoides*	Promote cell proliferation	CN110604742
3	Health product	A composition that modulates human microbiota and immune function	Polysaccharides of *E. ulmoides*	Regulates human microbiota and immune function	CN102813113
4	Silver nanoparticles	Synthesis method of *E. ulmoides* polysaccharides silver nanoparticles, synthesized *E. ulmoides* polysaccharides silver nanoparticles and their applications	Polysaccharides of *E. ulmoides* bark and leaves	Hemostasis, antibacterial and anti-inflammatory	CN113527769
5	Nanomaterials	Method of preparing selenium nanoparticles using *E. ulmoides* polysaccharide and the prepared selenium nanoparticles	Polysaccharides of *E. ulmoides* bark or leaves	Stabilize and enhance the biological activity of selenium nanoparticles	CN113456831
6	Veterinary medicine	A soluble granule for improving pig immunity and a preparation method	Polysaccharides of *E. ulmoides* leaves	Immune regulation	CN110812389
7	Feed additive	An additive that increases disease resistance to promote growth in pigs	Polysaccharides of *E. ulmoides* leaves	Promotes the proliferation of beneficial microorganisms in the animal gut, Immune regulation	CN105707437
8	Functional food	*E. ulmoides* leaves polysaccharides with antitumor activity, extraction and isolation methods, and their application in the preparation of supplements for antitumor drugs	Polysaccharides of *E. ulmoides* leaves	Anti-tumor	CN114989323
9	Traditional Chinese medicine	The invention relates to a preparation method of *Physalis calyxseuf* ructus extract for treating wound healing and scald	Polysaccharides of *E. ulmoides* leaves	Immune regulation, antioxidant	CN114159508
10	Biological products for veterinary use	African swine fever virus vaccine strains and vaccines containing vaccine strains	Polysaccharides of *E. ulmoides* bark	Anti-virus	CN110393798
11	Food	The invention relates to a *Elaeagnus sarmentosa* compound fruit and vegetable juice and a preparation method	Polysaccharides of *E. ulmoides* bark	Immune regulation	CN108967760
12	Clinical implant surgery	An implant containing a polysaccharide coating that can bind growth factors and a preparation method	Polysaccharides of *E. ulmoides* bark	Binding growth factors	CN108478298
13	Traditional Chinese medicine	A *E. ulmoides* targeting agent for colon cancer and a preparation method	Polysaccharides of *E. ulmoides* bark	Anti-tumor	CN104434950
14	Immune adjuvants for livestock and poultry	Chinese medicine Morus alba and *E. ulmoides* polysaccharides immunopotentiator and its application	Polysaccharides of *E. ulmoides* bark	Immune regulation	CN103768594

effective release of strontium in the body, plays the role of strontium in reducing inflammation and osteocyte proliferation, and can be a good auxiliary treatment of osteoarthritis. In addition, the above polysaccharides strontium complex can change the immune environment of bone, promote the proliferation and differentiation of bone repair cells, and play an important role in bone repair. In addition, EU polysaccharides can also be used as anti-tumor drugs and active ingredients. There are *in vivo* anti-tumor tests that show, *Eucommiae folium* polysaccharide can effectively inhibit the growth of solid tumor in mice, protect immune organs and liver tissues of mice, induce tumor cell apoptosis, block tumor cells in S phase, prevent their further proliferation, destroy mitochondrial membrane potential of cells, and induce tumor cell apoptosis through mitochondrial pathway in a certain dose dependent manner. Therefore, polysaccharides from *Eucommiae folium* can be used in the preparation of supplements for antitumor drugs. It is worth mentioning that EU polysaccharides can also be used to prepare a targeted preparation for the treatment of colon cancer. This EU targeted preparation contains EU polysaccharides, which can evenly distribute the drug release throughout the colon, increase the drug concentration in the diseased part of the colon, reduce the dose, reduce the stimulation of the gastrointestinal tract and the toxic side effects caused by the drug absorption, so as to exert the best curative effect and facilitate the treatment. Because polysaccharides from EU can be used as a sugar source of colon flora and can be specifically degraded, it is an ideal choice for the treatment of colon cancer. Furthermore, polysaccharides from *Eucommiae folium* have been

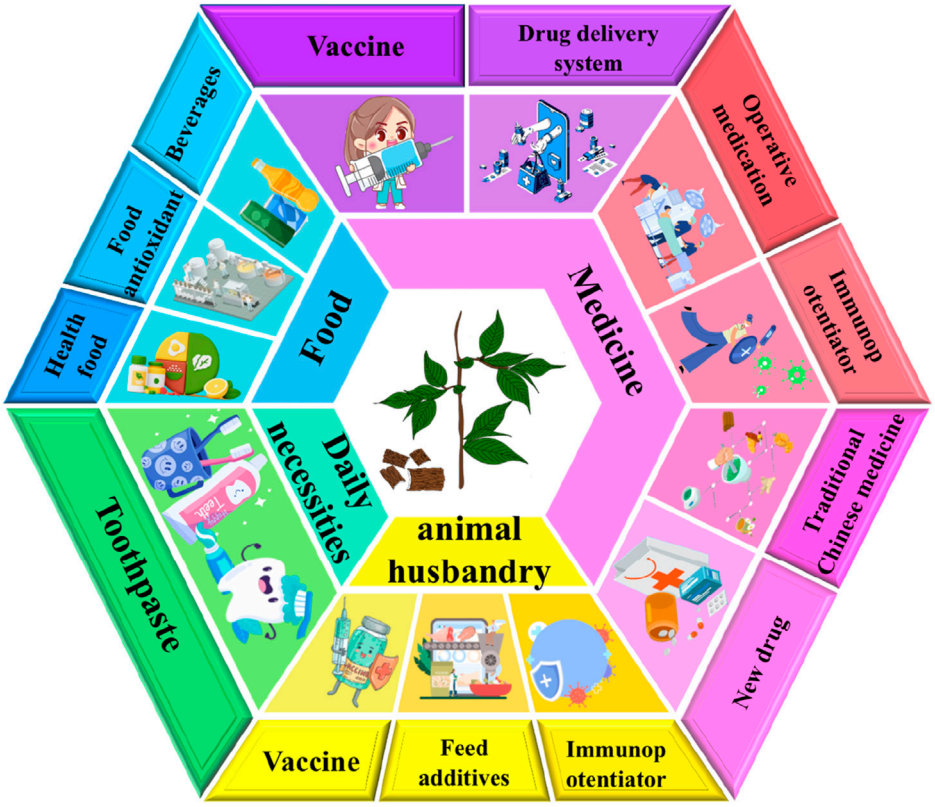

FIGURE 4
Current application and future development trend of *Eucommia ulmoides* polysaccharides.

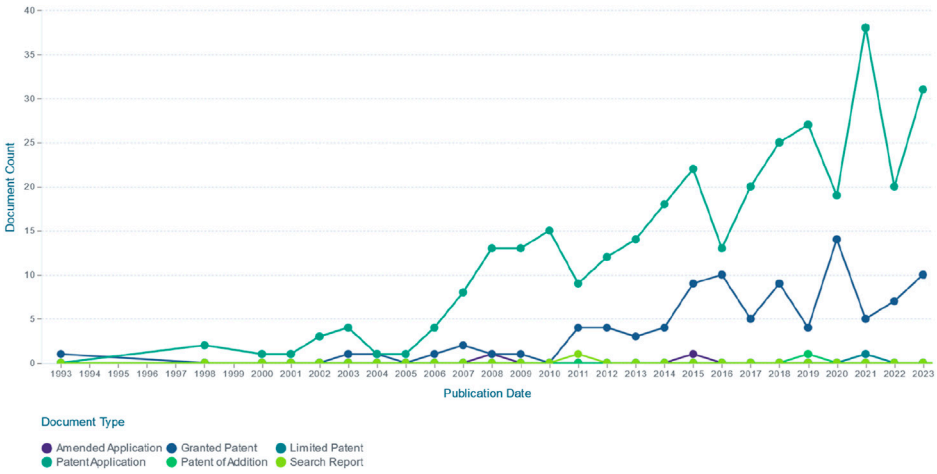

FIGURE 5
Final dataset of the number of *Eucommia ulmoides* polysaccharide-related patents per year globally up to 2023. (Source: http://www.lens.org).

used as a paste for wound healing and scalding due to their immunomodulatory activity. Polysaccharides extract from *Eucommiae folium* can enhance specific immunity, activate T and B lymphocytes, promote the proliferation of beneficial bacteria, and have the ability to remove hydroxyl radical and superoxide anion radical, promote cell growth, and have good therapeutic effect on wound healing and scalding.

7.1.2 Field of functional food

With the progress of science and technology and the development of society, environmental pollution has intensified, air pollution, water pollution and food safety threaten every family, and at the same time, modern people have a fast pace of life, great work pressure and irregular diet, resulting in more and more sub-health people, so functional food has been favored by more and

TABLE 5 Applications in health foods related to *Eucommia ulmoides*.

Approval number	Name	Category	Function
G20050865	Meidakang Brand Sheep Kidney Hardy Rubbertree Chinese Boxthorn Oral Liquid	Health Food	Relieve Physical Fatigue
G20110543	Tianyuankang Brand American Ginseng Mongolian Milkvetch Hardy Rubbertree Capsule	Health Food	Relieve Physical Fatigue
G20140918	Renyibao Brand Ginseng Hardy Rubbertree Solomon's Seal Oral Liquid	Health Food	Relieve Physical Fatigue
G20200030	Jimei Brand Bishop's Hat Hardy Rubbertree Tablet	Health Food	Relieve Physical Fatigue
G20200462	Jishengyuan Brand Hardy Rubbertree Jiaogulan European Dogbane Capsule	Health Food	Boost Immunity
G20100508	Jinqi Brand Roseroot Stonecrop Chinese Boxthorn Hardy Rubbertree Tea	Health Food	Boost Immunity
G20100185	Guojianglong Brand Cherokee Rose Hardy Rubbertree Vinum	Health Food	Boost Immunity
G20120373	Jijia Brand Hardy Rubbertree Sea Buckthorn Capsule	Health Food	Boost Immunity, Relieve Physical Fatigue
G20130563	Wudangzixiao Brand Hardy Rubbertree Solomon's Seal Vinum	Health Food	Boost Immunity, Relieve Physical Fatigue
G20110321	Huiyuannonggu Brand Hardy Rubbertree Beverages	Health Food	Boost Immunity, Relieve Physical Fatigue
G20100749	Baibang Brand European Dogbane Hardy Rubbertree Kudzu Danshen Safflower Flos Sophorae Immaturus Granules	Health Food	Reduce Blood Press
G20100436	Jianerma Brand Kudzu Hardy Rubbertree European Dogbane Tea	Health Food	Reduce Blood Press
G20041320	Zhengbenjingfang Brand Hardy Rubbertree Kudzu Capsule	Health Food	Reduce Blood Press
G20100054	Meiluo Brand Gastrodia Semen Ziziphi Spinosae Water Plantain Hardy Rubbertree Capsule	Health Food	Reduce Blood Press, Improve Sleep
G20120245	Fuzhen Hardy Rubbertree Squirrel's Foot Fern Bishop's Hat Capsule	Health Food	Increases Bone Mineral Density
G20140620	Maojitang Brand Squirrel's Foot Fern Hardy Rubbertree Extractive Capsule	Health Food	Increases Bone Mineral Density
G20120435	Jiuzhang Brand Hardy Rubbertree Honeysuckle Reishi Shell-broken Spore Powder Chewable Tablets	Health Food	Resistance to Chemical Liver Injury
G20120100	Linlanhua Brand Dendrobium Kudzu Hardy Rubbertree Capsule	Health Food	Resistance to Chemical Liver Injury, Boost Immunity
(1997) No. 072	Lijian Brand Hardy Rubbertree Beverages	Health Food	Anti-fatigue
G20040051	Heshou Brand Hardy Rubbertree Tea	Health Food	Anti-fatigue
G20141164	Wansongtang Brand Ginkgo Hardy Rubbertree San Qi Tea	Health Food	Reduce Blood Lipid
G20050086	Hongheyuan Brand Hardy Rubbertree Capsule	Health Food	Boost Immunity, Improve Sleep

more people (Yu et al., 2024). At present, some health food containing EU polysaccharides has been developed. For example, a compound fruit and vegetable juice not only have good taste, clear color, long-term storage without discoloration, but also is rich in nutrition, can enhance human immunity, prevent various diseases, and help solve human sub-health problems. It contains EU polysaccharides can enhance the body's immunity, and play a role in lowering blood sugar, anti-oxidation and anti-liver fibrosis. At the same time, it can improve the taste of fruit and vegetable juice and make it easier for consumers to accept. In addition, EU is added to oral liquids, beverages, or alcohol. Because the addition of EU makes these products have the effect of relieving body fatigue and enhancing immunity. It is worth mentioning that *Eucommiae folium* tea has also been developed as a healthcare product, which can enhance immunity, anti-fatigue, and assist in lowering blood pressure and blood lipids. Of course, in addition to drinking products, EU extract is also added to some chewable tablets and capsules to develop health products with the effects of strengthening immunity, anti-fatigue, anti-chemical liver damage, increasing bone density, improving sleep, etc. It can be seen that EU and its polysaccharides have great potential in the development of functional food and can bring good commercial value.

7.1.3 Other fields

With the deepening of EU research, EU polysaccharides have also been used in animal husbandry. First, EU polysaccharides can be used as vaccine adjuvants. The patented research shows that the vaccine containing vaccine strain prepared with the venom and polysaccharides of EU can prevent and control African swine fever, and has high popularization and application value. Another kind of animal immune adjuvant containing EU polysaccharides can

significantly stimulate the proliferation of chicken lymphocytes, increase the titer of serum antibodies, and enhance cellular and humoral immunity of chicks *in vitro*. It can be seen that EU polysaccharides have broad application prospects in the field of vaccine adjuvant. Secondly, EU polysaccharides can also be used as feed additives to promote the growth of pigs and improve the disease resistance of piglets. Or it can be added into veterinary drug granules to comprehensively adjust and play the immune function and adaptability of pig's own body, so as to significantly improve the disease resistance of pig, improve the health status of pig, play the role of disease prevention and treatment, reduce the morbidity and mortality of pig, and help improve the breeding efficiency and breeding quality. In addition, EU polysaccharides also have relevant applications in the field of daily necessities. A kind of toothpaste has the effect of whitening and removing stains and improving the sensitivity of teeth. The polysaccharide of EU is added to the toothpaste, which promotes the diphosphoglyceric acid to further play its role, plays a promoting role in restoring the health of tooth enamel and plays a whitening effect. Therefore, we believe that in the future, EU polysaccharides can be added to more daily necessities, such as creams, masks, etc., to enhance the whitening effect of products.

7.2 Resource utilization

EU is a unique tree species in China. This species has been employed as a medicine for the treatment of various diseases for a long time, and its medicinally functional plant part is mainly bark. However, harvesting the bark for medicinal use requires long growing periods, the tree itself is easily damaged during the harvesting process, and processing is relatively complex compared to that of the leaves, resulting in a waste of resources to a certain extent. There have been studies on replacing EU bark with leaves in some applications. According to the literature, the current research on EU polysaccharides is mainly focused on the bark and leaves of EU. In this research work, we explored whether polysaccharides from EU leaves could be used as a direct substitute for polysaccharides from EU bark. The results suggest that both EU bark polysaccharides and EU leaf polysaccharides have immunomodulatory, antioxidant and anti-inflammatory effects. In terms of immunomodulatory activity, EU leaf polysaccharides could play an immunomodulatory role by inducing DC maturation, promoting lymphocyte proliferation, and stimulating macrophages to produce NO, TNF-α, IL-6 and other immunomodulatory mediators, while EU bark polysaccharides could play an immunomodulatory role by stimulating B lymphocytes and DC cells. Further comparison of antioxidant activity showed that EU leaf polysaccharides exhibited a notable scavenging effect on DPPH, ABTS and hydroxyl radicals, while EU bark polysaccharides only had a notable scavenging effect on DPPH radicals. In addition, EU leaf polysaccharides have anti-fatigue and hepatoprotective pharmacological activities, and EU bark polysaccharides have anti-complementary and neuroprotective effects. Based on current applications, EU leaf polysaccharides and EU bark polysaccharides have been used in the pharmaceutical and food industries due to their immunomodulatory activities, and EU leaf polysaccharides have also been used in pharmaceuticals due to their antioxidant effects. All things considered, EU leaf polysaccharides may replace EU bark polysaccharides in immune regulation and antioxidant applications and be used in the pharmaceutical and food industries. These researches are important for promoting the conservation and sustainable use of EU plant resources. It is expected that soon, EU leaf polysaccharides may be a suitable substitute for EU bark polysaccharides and will be widely used in nutritional foods and natural medicine to promote human health.

7.3 Limitations of the current research

Despite the research of EU polysaccharides has come a long way, currently many continuing challenges and limitations for researchers still remain ahead to facilitate practical application.

Firstly, although studies have shown that EU polysaccharides exhibited diverse bioactivities, their underlying molecular mechanisms with inherent complexity remain elusive. At present, it is sobering to note that studies on these bioactivities of EU polysaccharides are still in the early stage. What is more, few data currently exist on the safety and efficacy of EU polysaccharides in clinical trials. Therefore, further studies are needed for application of molecular approaches to further explore the underlying mechanisms by using "omics", such as genomics, proteomics and metabolomics.

Secondly, more detailed advanced structural features of EU polysaccharides were not very well known nor well studied. Further adequate investigation of EU polysaccharides should be considered on glycosidic linkage types, glycosidic linkage sequence, glycosidic linkage configuration, branch point position and conformation, which can be fully characterized by various classical approaches, such as FT-IR, GC–MS, NMR, X-ray diffraction, AFM, and methylation analysis.

Thirdly, deep insights into structural and functional properties of EU polysaccharides remain lacking, which could hinder further development and novel biological and pharmacological applications. Different medicinal parts may have different functions, thus to easily observe more commonalities and differences among EU polysaccharides researchers could do more work on comparing the structure features and activities of polysaccharides derived from more other EU medicinal parts, such as roots, fruits and flower. These results will be helpful to further elucidate the relationship between the structure and function of EU polysaccharides in the future.

Fourthly, in spite of good performance by EU polysaccharides in biological activities and broad application prospects, there are relatively scarce studies of EU polysaccharides in cosmetics. More efforts should be exerted toward great potential application not only in the areas of biomedicine and food industries but also in the areas of cosmetics. Therefore, there is an urgent need to develop a green sustainable standardized extraction and purification techniques that are economical, efficient and environment-friendly for high-quality EU polysaccharide products. So far, however, preparation of EU polysaccharides is restricted to laboratories only. As a result, preparation process for EU polysaccharides needs to be further optimized to offer scalable and reproducible manufacturing processes for commercial use.

In summary, EU has been widely used in food, health products and cosmetics, and EU polysaccharides have great potential for a

wide range of industrial, biomedical and clinical applications with broad application prospects. Although there is still a long way to go to reach this goal, it is believed that in the coming years the product containing EU polysaccharides will assume a place in the health product market.

8 Conclusion and future prospectives

In this review, recent research progress in extraction, purification, structure, various pharmacological activities, structure-activity relationship and applications of polysaccharides derived from two different medicinal parts (bark and leaves) of EU was systematically summarized. Additionally, resource utilization and the limitations of the current research were also discussed. EU is a precious medicinal plant widely distributed in China. Its bark and leaves can both be used as medicinal remedies nourishing liver and kidney and strengthening muscles and bones since ancient times. To date, multiple extraction and purification methods have been used obtaining a total of about 12 and nine polysaccharides from EU bark and leaves, respectively. These EU polysaccharides have various important biological activities such as neuroprotective, antioxidant, immunomodulatory, anti-inflammatory, anti-complementary, anti-fatigue and anti-liver injury bioactivities and a beneficial effect on systemic lupus erythematosus. Such bioactivities prompted EU polysaccharides to have further diverse applications in medicine, functional foods, and animal husbandry, showing significant commercial potential and broad application prospects currently and in the future. These researches also suggest that EU leaf polysaccharides with broader immunomodulatory, antioxidant, and pharmacological effects could potentially replace EU bark polysaccharides in pharmaceutical and food industries. Such substitution would potentially promote the sustainable use of EU plant resources and enhance human health through their application in nutritional foods and natural medicine.

Author contributions

YS: Writing–review and editing, Supervision, Project administration, Funding acquisition. YZ: Writing–original draft, Project administration, Investigation, Data curation. MS: Writing–original draft, Visualization, Investigation. WG: Writing–original draft, Visualization, Investigation. YH: Writing–original draft, Investigation, Data curation. YW: Writing–original draft, Investigation, Data curation. BY: Writing–review and editing, Supervision, Conceptualization. HK: Writing–review and editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This work was supported by the Chief Scientist of Qi-Huang Project of National Traditional Chinese Medicine Inheritance and Innovation "One Hundred Million" Talent Project (Grant Number: [2021] No. 7), The Seventh Batch of National Famous Old Traditional Chinese Medicine Experts Experience Heritage Construction Program of National Administration of TCM (Grant Number: [2022] No. 76), Heilongjiang Touyan Innovation Team Program (Grant Number: [2019] No. 5), The Assisted Project by Heilong Jiang Postdoctoral Funds for Scientific Research Initiation (Grant Number: LBH-Q21045), The Special Project by Heilongjiang Administration of TCM for Research of Popularization of Classic Traditional Chinese Medicine (Grant Number: ZYW 2022-066), Fund from Heilongjiang University of Chinese Medicine (Grant Number: 2018RCQ05).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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frontiers | Frontiers in Pharmacology

TYPE Original Research
PUBLISHED 22 August 2024
DOI 10.3389/fphar.2024.1457780

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OPEN ACCESS

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RECEIVED 01 July 2024
ACCEPTED 12 August 2024
PUBLISHED 22 August 2024

CITATION
Chen J, Yang X, He Z, Chen W, Zhao Y, Li J, Zong Y and Du R (2024) *Cynomorium songaricum* Rupr. flavonoids improve cyclophosphamide-induced reproductive function damage by regulating the testosterone synthesis pathway.
Front. Pharmacol. 15:1457780.
doi: 10.3389/fphar.2024.1457780

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Cynomorium songaricum Rupr. flavonoids improve cyclophosphamide-induced reproductive function damage by regulating the testosterone synthesis pathway

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Introduction: The prevalence of male infertility has been increasing globally, necessitating the search for safe and nontoxic active compounds to alleviate reproductive dysfunction. Although the precise mechanism remains unknown, *Cynomorium songaricum* Rupr. (CS) extract has protective effects on the reproductive system. The effect of C. songaricum Rupr. flavonoids (CSF) on reproductive injury and testicular mesenchymal stem cell viability in male mice and TM3 cells was investigated.

Methods: We explored the possible association between these effects and the testosterone (T) synthesis pathway. Mice were administered cyclophosphamide to induce reproductive damage, followed by CSF administration. Body mass and organ index were recorded. Pathological changes in T and the epididymis were observed using hematoxylin-eosin staining. ELISA measured the serum levels of T, luteinizing hormone (LH), gonadotropin-releasing hormone (GnRH), follicle-stimulating hormone (FSH), and estradiol (E_2) in mice. Fructose and zinc ion levels in the seminal plasma were measured. TM3 cells were treated with Bisphenol A (BPA) and different concentrations of CSF, followed by proliferative evaluations using the CCK-8 assay and T and LH level assessments using ELISA. Furthermore, the expression of steroidogenic enzyme genes and proteins was investigated using western blotting and RT-PCR.

Results: CSF exhibited a notable reduction in reproductive damage and improved pathological changes in testicular and epididymal tissues. CSF group demonstrated substantially higher levels of seminal plasma fructose and zinc ions; markedly elevated serum levels of T, LH, GnRH, and FSH; and lower levels of E2 than those of the model group. Intracellular T content and secretion of T and LH increase with CSF while effectively mitigating BPA-induced damage to TM3 cells. CSF group exhibited substantially higher gene and protein expression of steroidogenic enzymes than those of the model group, both *in vivo* and *in vitro*. CSF ameliorates reproductive impairment by enhancing the expression of pivotal enzymes involved in synthesizing T.

Discussion: CSF ameliorates cyclophosphamide-induced reproductive impairment and bisphenol A-induced TM3 cell damage in mice by regulating sex hormone levels in the Hypothalamic-Pituitary-Gonadal Axis (HPG axis) and upregulating the expression of steroidogenic enzymes. Therefore, CS is a potential treatment for male reproductive impairment.

KEYWORDS

Cynomorium songaricum **Rupr., total flavonoids, reproductive function damage, testosterone synthesis pathway, testosterone (androgen)**

Introduction

Male infertility is becoming increasingly prevalent worldwide. Potential causes include environmental factors, endocrine abnormalities, immune system dysfunction, and genetic factors (Li et al., 2020; Kirsten et al., 2020). Approximately 46% of male patients with infertility are affected by oligospermia, which is characterized by reduced sperm count and viability (Zhang et al., 2020). Research indicates that in China, sperm quality declines by 1% annually, whereas Western countries experience a decline of 2.6% in sperm density and 0.3% in sperm viability rates per year (Chyra-Jach et al., 2018; Yang et al., 2020). In light of the growing demand for fertility and the escalating incidence of infertility, it is imperative to explore safe and non-toxic active compounds for the treatment of reproductive impairment.

Testosterone, a vital hormone responsible for male secondary sexual characteristics, is primarily secreted by testicular interstitial cells, with 95% of its production regulated by the HPG axis (Wang et al., 2022). Within this axis, the hypothalamus produces GnRH, which stimulates the pituitary gland to secrete FSH and LH. These hormones stimulate the testes, initiate spermatogenesis, and trigger T secretion. The feedback loop is completed as T acts on the hypothalamus and pituitary gland through negative feedback, regulating endocrine hormones and maintaining normal function (Zhang et al., 2021). Several studies have shown that oxidative stress may contribute to male infertility, and increased levels of ROS can affect both spermatogenesis and the HPG axis (Shokoohi et al., 2022).

The steroidogenic enzyme pathway is the main pathway for T synthesis. StAR is a key factor in testicular interstitial cells that promotes cholesterol transport from the outer mitochondrial membrane to the inner membrane. Cholesterol passes through the outer mitochondrial membrane into the inner mitochondrial membrane under the regulation of STAR and is cleaved by CYP11A1 to pregnenolone, which is converted into progesterone by 3β-HSD, and progesterone is converted into dehydroepiandrosterone by CYP17A1 in the endoplasmic reticulum and finally metabolised to testosterone by 17β-HSD (Aghazadeh et al., 2015; Dolatkhah et al., 2022). Alterations in the expression of key enzymes can directly affect T synthesis. T plays a vital role in maintaining the normal viability of spermatozoa, promoting sperm development and maturation, and facilitating the proper functioning of the reproductive system. Collectively, these factors contribute to the essential foundation of male fertility (Witherspoon and Flannigan, 2022).

The impairment of reproductive function is a notable adverse effect associated with cyclophosphamide (CP), a nonspecific cytotoxic drug commonly used in tumor therapy (Chen et al., 2017). Research suggests that CP can cause substantial damage to the testes and increase the risk of oligospermia or azoospermia, ultimately leading to infertility. Consequently, the creation of an animal model for studying spermatogenic disorders using CP has become a widely adopted approach for exploring the underlying mechanisms and assessing potential treatment interventions. This method offers the advantages of rapid onset, short duration, and enhanced stability (Wang et al., 2019).

In Chinese medicine, *Cynomorium songaricum* Rupr (CS) is primarily used to treat impotence and renal yang deficits (Nickrent et al., 2005; Tao et al., 1999). Contemporary pharmacological studies have identified terpenoids, organic acids, and flavonoids as the active chemical constituents of CS extracts (Wei et al., 2019; Jiang et al., 2001). These constituents exert various pharmacological effects, including fertility enhancement, immune system stimulation, antiviral properties, and nervous system protection (Tian et al., 2019; Xue et al., 2018; Wang et al., 2017). The aqueous extract of CS has been observed to substantially increase sperm count and viability while reducing the number of abnormal spermatozoa (Abd el-Rahman et al., 1999). Furthermore, the CS extract has been shown to directly affect seminiferous tubules, leading to an increase in the wet weight of animal testes and increase testosterone levels (Abd el-Magied et al., 2001). However, there is a lack of published studies on the specific mechanisms through which CS improves reproductive dysfunction, highlighting the need for further research in this area.

We investigated the protective effects of CS flavonoids (CSF) in male mice with impaired reproductive function. Additionally, we examined the mechanism through which CSF ameliorates reproductive dysfunction and stimulates the proliferation of TM3 cells. The main constituents of the CSF were identified using LC-MS/MS analysis (Figure 1), laying the groundwork for the future development and exploration of CS.

Materials and methods

Preparation and separation of CSF

The dried fleshy stems of CS were collected from the Altay region in the Xinjiang Autonomous Region. Professor Zhang Hui from the Changchun University of Traditional Chinese Medicine identified this plant. To extract its components, a feed-to-liquid ratio of 1:30 was used with 50% ethanol refluxed three times at 100°C. The resulting extracts were evaporated, concentrated, and freeze-dried. The extracts were further enriched using an AB-8 macroporous adsorbent resin to obtain CSF (91.3% enriched flavonoid-subfraction).

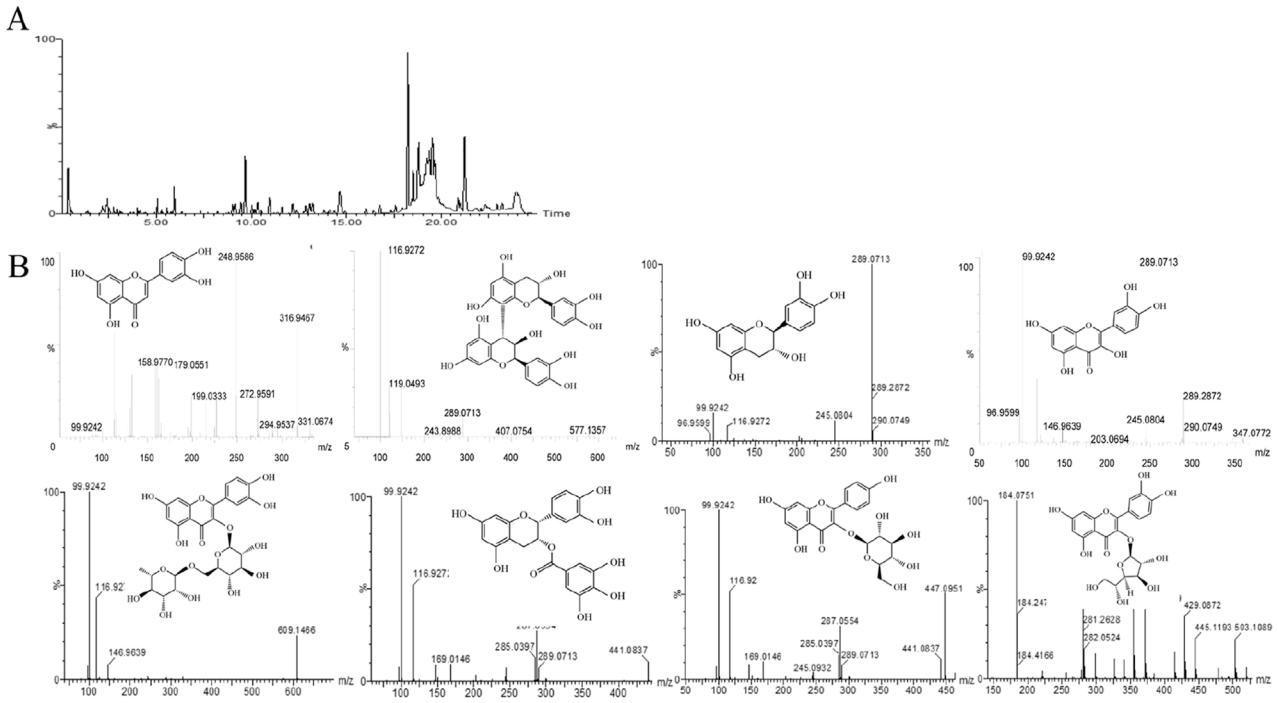

FIGURE 1
Main components of CSF. **(A)** Total ion flow diagram (negative spectrum) of CSF using LC-MS/MS **(B)** Secondary mass spectra and chemical structure of CSF screened for chemical constituents. CSF, *Cynomorium songaricum* Rupr. flavonoid.

TABLE 1 Gradient elution conditions.

Time (min)	Flow rate mL/min	Acetonitrile (A) (%)	H_2O (B) (%)
0	0.4 mL/min	5	95
2	0.4 mL/min	20	80
5	0.4 mL/min	40	60
15	0.4 mL/min	60	40
18	0.4 mL/min	90	10
23	0.4 mL/min	95	5
25	0.4 mL/min	5	95

LC-MS/MS

Liquid phase conditions:The separation was carried out on an ACQUITY UPLC®BEH C18 column (2.10 mm*50 mm, 1.7 μm). Mobile phase: A: 0.01% formic acid-acetonitrile, B: 0.01% formic acid-water, injection volume 2 μL. Gradient elution conditions Table 1.

Mass spectrometry conditions: Through the ESI ion source, using positive, negative ions, sensitivity analysis mode (ESI+, ESI-), MSEcontinuum mode scanning, scanning time of 0.5 s, low-energy ionisation collision energy of 6 V; high-energy collision energy of 10–30 V, cone hole voltage of 30 V; scanning range 40–1500 Da.

Sample treatment: Flavonoid extracts were dissolved in methanol by mass spectrometry, filtered through a 0.22 μm filter membrane and injected into the sample.

Animals

Six groups, each consisting of ten Kunming mice, were randomly selected to participate in the experiment. All animal-related techniques and procedures were authorized by the Jilin Agricultural University Animal Research Committee (ethical code: 20210915001).

1. Group 1 (BG): This group served as the blank control group. Gastric gavage of equal volume of saline daily for 28 days.
2. Group 2 (MG): Mice in this group were assigned to the cyclophosphamide model group. They received intraperitoneal injections of cyclophosphamide (60 mg/kg) for five consecutive days.

TABLE 2 Primer information.

Primers	Sequence (5′ to 3′)	Length (bp)
GAPDH-F	AACTTTGGCATTGTGGAAGG	20
GAPDH-R	ACACATTGGGGGTAGGAACA	20
StAR-F	CTGCTAGACCAGCCCATGGAC	21
StAR-R	TGATTTCCTTGACATTTGGGTTCC	24
CYP11A1-F	CGATGACCTATTCCGCTTTGC	21
CYP11A1-R	TGTGGAACATCTGGTAGACGGC	22
CYP17A1-F	TGGGCACTGCATCACGATAA	20
CYP17A1-R	GCTCCGAAGGGCAAATAACT	20
3β-HSD-F	AGAAGTGACAGGCCCAAACT	20
3β-HSD-R	ACATGGATCTCAGGGCACAA	20

3. Group 3 (FL): In this group, the mice were modeled as the total flavonoid low-dose group. They were administered cyclophosphamide as described for the MG group, dissolve flavonoids (45 mg/kg) in physiological saline and administer gastric gavage continuously for 28 days.
4. Group 4 (FM): Mice in this group were assigned to the total medium-dose group. They were administered cyclophosphamide as described for the MG group, dissolve flavonoids (90 mg/kg) in physiological saline and administer gastric gavage continuously for 28 days.
5. Group 5 (FH): Mice in this group were assigned to the total flavonoids high-dose group. They were administered cyclophosphamide as described for the MG group, dissolve flavonoids (180 mg/kg) in physiological saline and administer gastric gavage continuously for 28 days.
6. Group 6 (PG): Mice in this group were assigned to the clomiphene citrate-positive group. They were administered cyclophosphamide according to the MG group, clomiphene citrate (7.5 mg/kg) in physiological saline and administer gastric gavage continuously for 28 days (Pavin et al., 2018; Watcho et al., 2019).

After the last oral administration, mice were euthanized by intraperitoneal injection of sodium pentobarbital. The testicular and epididymal tissues were weighed for subsequent analysis, and blood samples were collected. One testis was used for histological examination, whereas the other was used for RT-PCR and WB assays.

Calculation of organ indices and sperm density

The testicular index (TI) was calculated using Equation 1: (Predes Fde et al., 2016)

$$TI = (\text{wet weight of testis}/\text{body weight of mice}) \times 100\% \quad (1)$$

Similarly, The epididymal index (EI) was calculated using Equation 2: (Takagi-Morishita et al., 2022)

$$EI = (\text{wet weight of epididymis}/\text{body weight of mice}) \times 100 \quad (2)$$

The tail of the left epididymis of mice was taken, rinsed with preheated saline at 37°C, placed in saline at 37°C, and sufficiently cut with scissors, and incubated for 10 min in a 37°C water bath to allow sperm to fully swim out, 10 μL of sperm suspension was added to the sperm counting plate, and the concentration of spermatozoa was observed and recorded under a microscope.

Hematoxylin-eosin (HE) assay

HE staining was performed as previously described (He et al., 2021). Fresh testicular and epididymal tissues were fixed in 4% paraformaldehyde. After the tissues were appropriately labeled and processed through gradient ethanol dehydration, xylene clearing, paraffin embedding, sectioning, and staining, images of the stained samples were examined and recorded.

Serum hormone measurement

The experimental animals were subjected to blood collection from the eyeballs, serum was separated by centrifugation at 3,000 rpm for 3 min and stored at −80 °C. Serum hormone levels were determined using enzyme-linked immunoassay kits (Shanghai Preferred Biotechnology) T, LH, GnRH, FSH, and E2 (Wang et al., 2017).

Semen plasma assay

Blood was collected from the eyeballs after the last dose, and semen was dissected from the seminal vesicle glands, and seminal plasma obtained by centrifugation after liquefaction (Huang et al., 2023; Kerns et al., 2018). Kits (Nanjing Jiancheng Bioengineering Research Institute) were used to measure fructose and ionic zinc levels in seminal plasma.

Cell culture

Mouse testicular mesenchymal stromal cells (TM3) were provided by the Wuhan Punosai Life Science and Technology Co. Cell culture conditions: 89% Ham's F12 nutrient medium +10% FBS +1% P/S, 37°C 5% CO_2.

Cell viability

The growth of TM3 cells was measured using the CCK-8 assay. TM3 cells were exposed to different concentrations of CSF (12.5, 25, and 50 μg/mL), while 200 μmoL/L BPA served as the MG (Zhang et al., 2022).

Cellular secretion of T and LH levels

TM3 cells were co-cultured with 200 μmoL/L BPA for 4 h. The drug group was then cultured for 24 h with CSF concentrations of

TABLE 3 Chemical composition of CSF.

No	Component name	Neutral mass (Da)	Observed (m/z)	Observed RT (min)	Adducts	Formula
1	Luteolin	286.04774	331.0674	0.36	$+HCOO^-$, -H	$C_{15}H_{10}O_6$
2	Proanthocyanidins	578.14243	577.1357	2.15	−H	$C_{30}H_{26}O_{13}$
3; 4	(−)-Catechin/(+)-Catechin hydrate	290.07904	289.0713	2.86/2.4	−H	$C_{15}H_{14}O_6$
5	Quercetin	302.04265	347.0772	2.88	−H, $+HCOO^-$	$C_{15}H_{10}O_7$
6	Rutin	610.15338	609.1466	3.47	−H	$C_{27}H_{30}O_{16}$
7	(−)-Epicatechin gallate	442.09	441.0837	3.62	−H	$C_{22}H_{18}O_{10}$
8	Astragalin	448.10056	447.0942	3.63	−H	$C_{21}H_{20}O_{11}$
9	Isoquercitrin	464.09548	503.1089	22.39	+K	$C_{21}H_{20}O_{12}$

CSF, *Cynomorium songaricum* Rupr. flavonoid.

12.5, 25, and 50 μg/mL. Cells and cell supernatants were separated, and the levels of LH, intracellular T and secreted T in the supernatants were determined by an enzyme immunoassay kit (Shanghai Preferred Biotechnology).

RT-PCR analysis

Total RNA was isolated from TM3 cells exposed to 200 μmoL/L BPA, TM3 cells treated with 12.5, 25 and 50 μg/mL CSF, and mouse testis tissue using the Ultrapure RNA Extraction Kit (TaKaRa). RT-PCR was performed to assess the relative expression of each gene. The primer information is shown in Table 2.

WB analysis

Proteins were extracted and their concentrations were measured from TM3 cells exposed to 200 μmol/L BPA, TM3 cells treated with 12.5, 25, and 50 μg/mL CSF, and mouse testicular tissue using a total protein extraction kit and a protein concentration determination kit (Bestbio). Protein blot analysis was conducted, and image processing was performed using PictureJ software.

Statistical analyses

Statistical analyses were performed using the SPSS software (version 17.0). The results are represented as mean ± SD. Group comparisons were made using ANOVA, and a *p*-value of less than 0.05 was considered statistically significant.

Results

The main components of CSF were determined by LC-MS/MS.

The primary chemicals present in CS were identified using an LC-MS/MS system, consistent with previous reports (Zhang et al., 2012). Table 3 shows the primary constituents of CSF determined using LC-MS/MS. The negative spectrum of the total ion flow diagram is represented in Figures 1A, B shows the structural equations and secondary mass spectra.

Effects of CSF on body mass, T, epididymal index, and sperm count in mice with reproductive impairment

After 28 days of modeling and therapy, the MG group exhibited significantly lower body mass, testicular and epididymal indices, and sperm count than those of the BG group. Compared with the MG group, the body masses of mice in the FM and FH groups were significantly higher (Figure 2A). All the other dosing groups displayed increased testicular and epididymal indices, as depicted in Figures 2B, C. Furthermore, all other dosing groups exhibited significantly higher sperm counts than that in the MG group (Figure 2D).

CSF regulates serum hormone levels and seminal plasma energy substance content

The ability to regulate hormone levels along the HPG axis. Compared with the BG group, the MG group showed significantly lower serum levels of T, LH, GnRH, and FSH ($P < 0.01$) (Figures 3A–D). Conversely, serum E2 levels were significantly higher in the MG group than in the BG group but were significantly lower in all other dosing groups compared with those in the MG group (Figure 3E). Furthermore, seminal fructose and zinc levels were significantly higher ($P < 0.05$; $P < 0.01$) (Figures 3F, G) in all the other dosing groups than those in the MG group. These findings indicate that CSF can enhance the quality of mouse spermatozoa by elevating the concentrations of fructose and zinc in seminal plasma.

CSF improves testicular and epididymal histopathology

In the BG group, the testes exhibited a well-covered plasma membrane, a high number of seminiferous tubules, and an interstitium consisting of loose connective tissue between the tubules. However, in the MG testes, a lower number of spermatogonia, spermatocytes, and spermatozoa was observed compared with those in the BG group, indicating an impaired maturation process of spermatogonia. Notably, the thickness of the spermatogonial cell layer in the MG group was significantly decreased ($P < 0.001$) (Figure 4C) (Kerns et al., 2018). A few seminiferous tubules

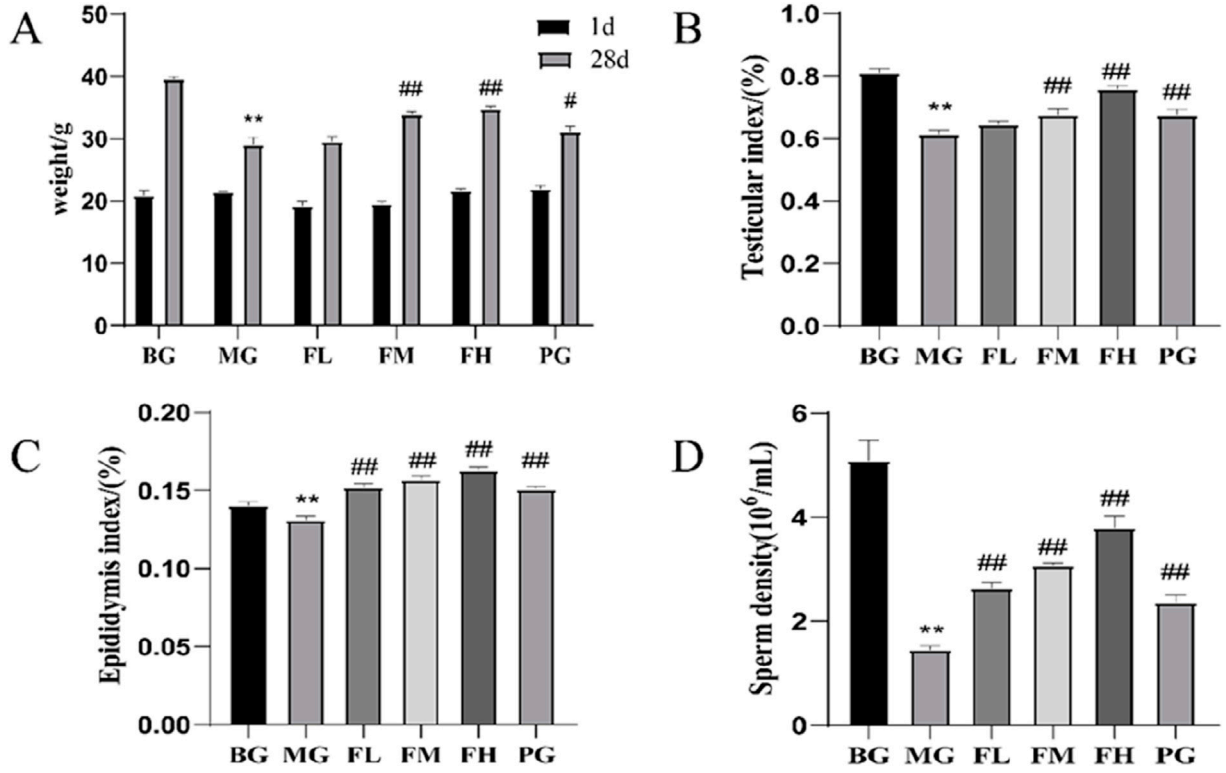

FIGURE 2
CSF improves **(A)** body mass, **(B)** testicular index, **(C)** epididymal index, and **(D)** sperm count in mice with cyclophosphamide-induced reproductive impairment. $^{**}P < 0.01$ vs. BG; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. MG, n = 10. BG, blank group; CSF, *Cynomorium songaricum* Rupr. flavonoid; FL, low-dose flavonoid group; FM, medium-dose flavonoid group; FH, high-dose flavonoid group; MG, model group; PG, clomiphene citrate-positive group.

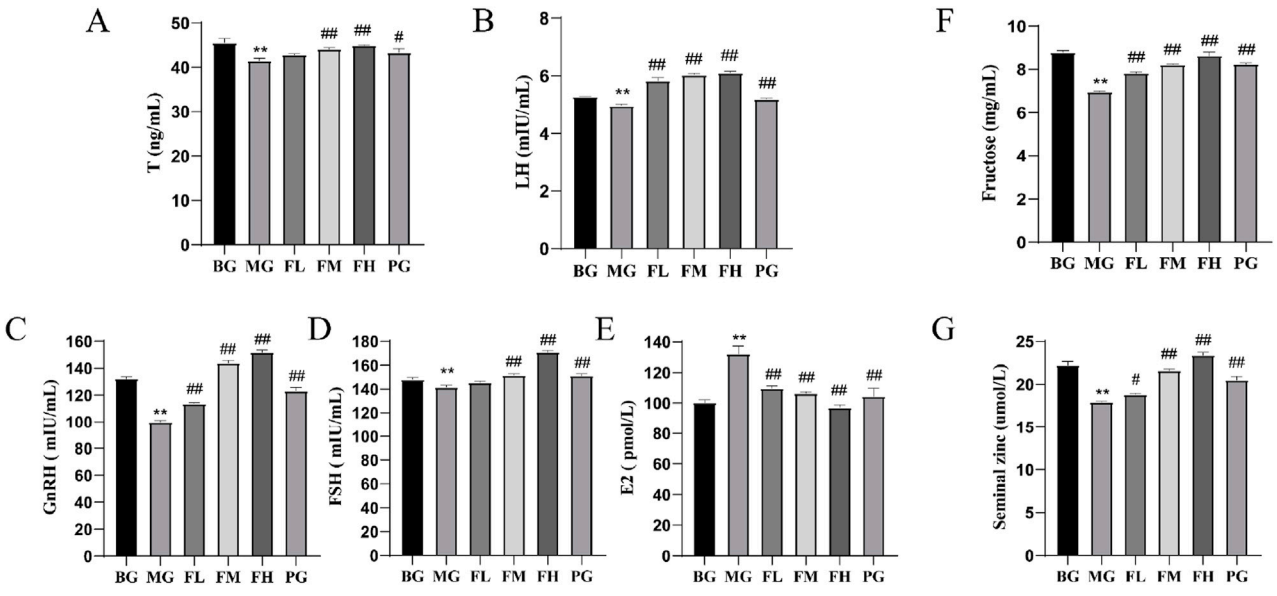

FIGURE 3
Effects of CSF on sex hormone levels and seminal plasma energetics in mice. **(A)** T, **(B)** LH, **(C)** GnRH, **(D)** FSH, **(E)** E2, **(F)** fructose, and **(G)** zinc ions. $^{**}P < 0.01$ vs. BG; $^{\#}P < 0.05$, $^{\#}P < 0.05$ vs. 0.05; $^{\#\#}P < 0.01$ vs. MG, n = 3. BG, blank group; CSF, *Cynomorium songaricum* Rupr. flavonoid; FL, low-dose flavonoid group; FM, medium-dose flavonoid group; FH, high-dose flavonoid group; LH, luteinizing hormone; MG, model group; PG, clomiphene citrate-positive group; T, testosterone.

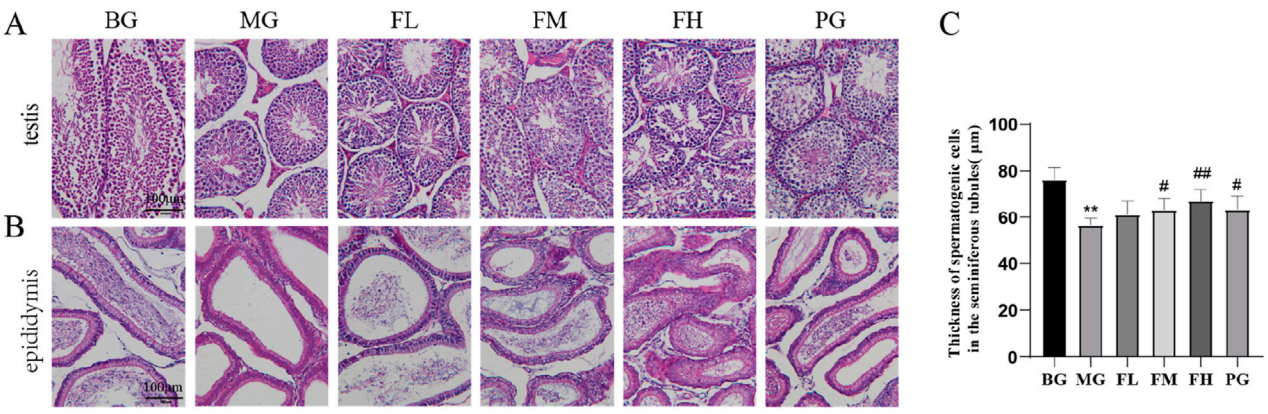

FIGURE 4
Effect of CSF on testicular and epididymal tissues. Sections of **(A)** testis and **(B)** epididymis from each group of mice were analyzed using HE staining. **(C)** Measurement of spermatogenic cell layer thickness. $^{**}P < 0.01$ vs. BG; $\#P < 0.05$, $\#\#P < 0.01$ vs. MG, n = 10. BG, blank group; CSF, *Cynomorium songaricum* Rupr. flavonoid; FL, low-dose flavonoid group; FM, medium-dose flavonoid group; FH, high-dose flavonoid group; MG, model group; PG, clomiphene citrate-positive group.

exhibit a small number of disorganized spermatocytes, and in other tubules, no spermatozoa were observed, effectively reproducing animal models (Kerns et al., 2018). Overall, spermatogenesis was significantly reduced in the testes.

In the groups administered with total flavonoids, the content of spermatogonial cells in the testis and the distribution of spermatozoa showed varying degrees of improvement among the different dose groups. The FM and FH groups were more effective in enhancing the spermatogenic capacity of the testicular spermatogonia. Furthermore, the thickness of spermatogonial cells in the testes improved in the PG group, although the degree of improvement was not as pronounced as that in the FH group (Figure 4A).

A well-arranged distribution of epididymal ducts containing numerous spermatozoa was observed in the epididymis of the BG group. However, the MG group displayed a significant decrease in the number of spermatozoa distributed in the lumen of the epididymal ducts compared with that in the BG group. In contrast, the total flavonoids in the groups exhibited a significant improvement in the number of spermatozoa distributed in the epididymal ducts compared with that in the MG group (Figure 4B).

Assessment of steroidogenic enzyme-related genes and protein expression in testicular tissues

CSF administration resulted in the upregulation of steroidogenic enzyme-related genes and proteins, including StAR, CYP11A1, CYP17A1, and 3β-HSD, in mouse testicular tissues. Compared with the BG group, the MG group exhibited a significant reduction in the expression of these genes and proteins. However, the FL, FM, FH, and PG groups demonstrated a significant increase in the expression of steroidogenic enzyme-related genes and proteins in their testicular tissues compared with that in the MG group (Figures 5A–C).

CSF promotes TM3 cell viability and BPA modeling concentrations

The proliferation of TM3 cells was assessed using CCK-8, revealing that cell viability reached its peak at a concentration of 50 μg/mL CSF. However, cell viability started to decline as the concentration increased (Figure 6A). Additionally, the inhibitory effect of BPA on TM3 cells was evaluated, demonstrating that the cell survival rate was 53.57% ± 2.08% after a 4-h exposure to 200 μmoL/L BPA (Figure 6B). Hence, a 4-h incubation period with 200 μmoL/L BPA was selected for subsequent experiments. Comparatively, in the presence of BPA-induced injury, CSF concentrations of 12.5, 25, and 50 μg/mL demonstrated a significant dose-dependent increase in the viability of TM3 cells when compared with the MG ($P < 0.05$; $P < 0.01$) (Figure 6C). Therefore, CSF concentrations of 12.5, 25, and 50 μg/mL were chosen for further trials.

CSF alleviates BPA inhibition of T and LH secretion by TM3 cells

After 4 h of stimulation with 200 μmol/L BPA, the intracellular T level in the MG was significantly lower than that in the BG ($P < 0.01$) (Figure 7A). However, after 24 h of pharmacological protection with varying concentrations of CSF, the intracellular T content showed a dose-dependent increase in each dose group, with significantly higher levels than those in the MG ($P < 0.01$). The higher the CSF concentration within the safe dosage range, the higher the intracellular T content (Figures 7B, C). Luteinizing hormone (LH) is a glycoprotein gonadotropin secreted by adenopituitary cells, which contributes to the synthesis and release of T from TM3 cells. Furthermore, upon stimulation with 200 μmol/L BPA for 4 h, the secretion of T by TM3 cells in the MG was significantly lower than that observed in the BG ($P < 0.01$) (Figures 7B, C). However, in the presence of CSF within a safe dose range, increasing concentrations of the drug corresponded with higher cellular secretion levels of T. These results indicate that CSF exhibits a certain degree of improvement in alleviating the inhibition of TM3 cells secretion of both T and LH by BPA.

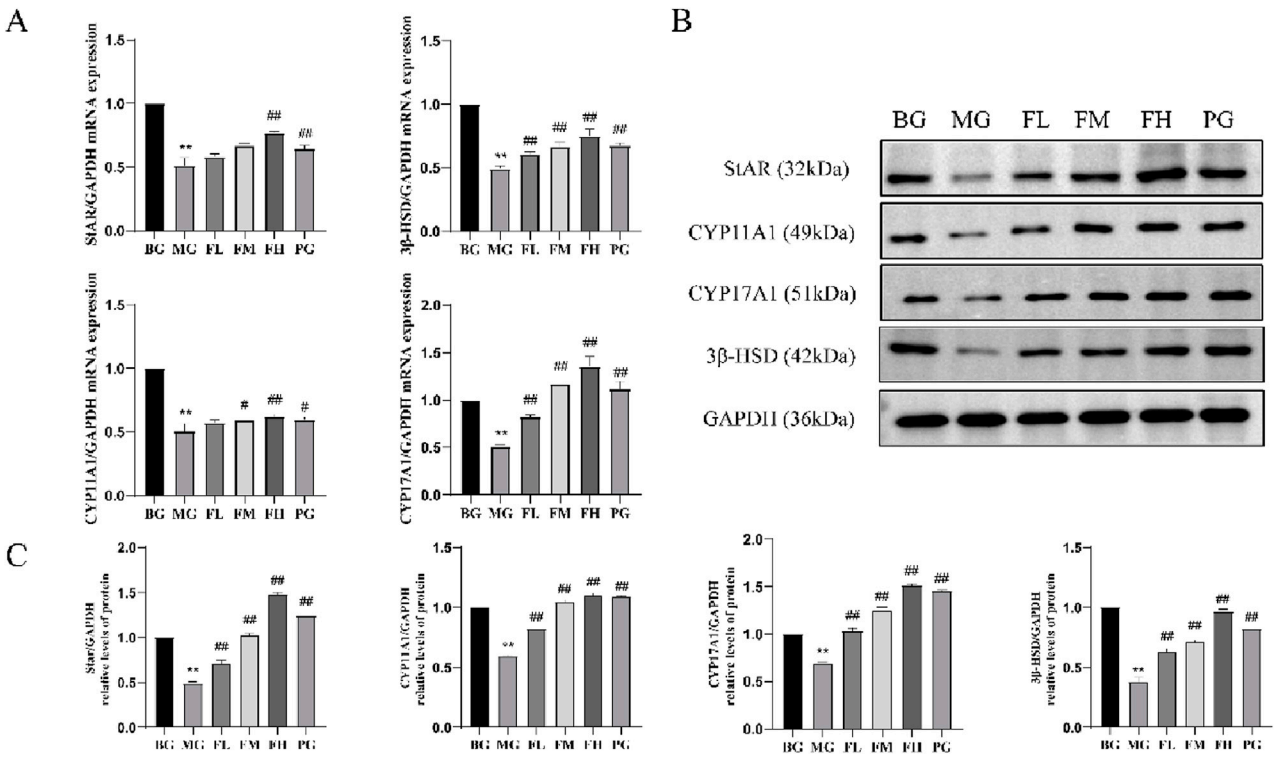

FIGURE 5
Effect of CSF on testicular tissue steroidogenic enzyme-related gene and protein expression. **(A)** CSF promotes testicular tissue steroidogenic enzyme mRNA expression, **(B,C)** CSF promotes testicular tissue steroidogenic enzyme protein expression. *$P < 0.05$, **$P < 0.01$ vs. control; #$P < 0.05$, ##$P < 0.01$ vs. model. BG, blank group; CSF, *Cynomorium songaricum* Rupr. flavonoid; FL, low-dose flavonoid group; FM, medium-dose flavonoid group; FH, high-dose flavonoid group; MG, model group; PG, clomiphene citrate-positive group.

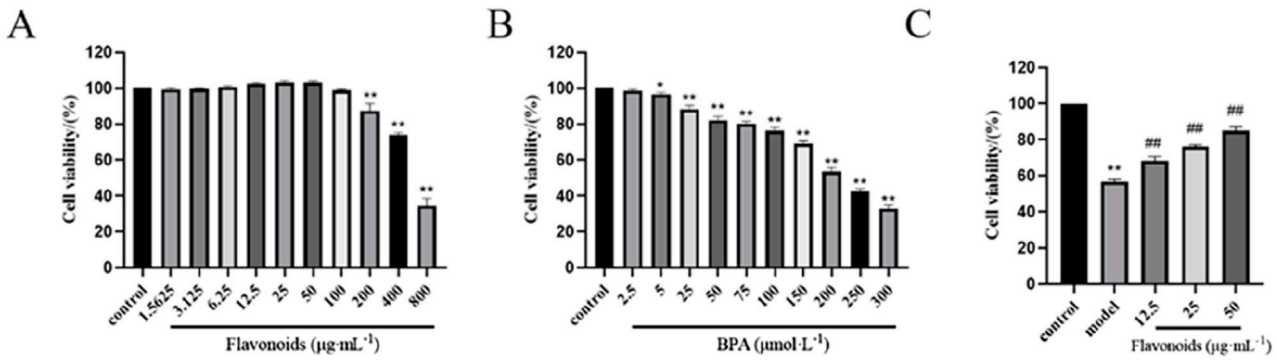

FIGURE 6
Effects of CSF and BPA on TM3 cell viability *in vitro*. **(A)** CSF promoted the proliferation of TM3 cells (selection of concentrations 12.5, 25, and 50 μg/mL). **(B)** BPA effectively inhibited the proliferation of TM3 cells (selected concentration 200 μmoL/L). **(C)** CSF improved BPA inhibited TM3 cell viability. *$P < 0.05$, **$P < 0.01$ vs. control; #$P < 0.05$, ##$P < 0.01$ vs. model, n = 8. CSF, *Cynomorium songaricum* Rupr. flavonoid.

Assessment of steroidogenic enzyme-related genes and protein expression in TM3 cells

Administration of CSF effectively reversed the BPA-induced decrease in the expression of steroidogenic enzyme genes and proteins (Figure 8). These findings suggested that BPA treatment negatively affected the T production pathway in TM3 cells, leading to decreased steroidogenic enzyme expression. However, administration of CSF counteracted this effect and restored the expression of steroidogenic enzymes.

Discussion

Studies have indicated that the CS extract can be used to treat spermatorrhea, premature ejaculation, and erectile dysfunction (Gu et al., 2018; Umamaheswari et al., 2022). CS has been reported to

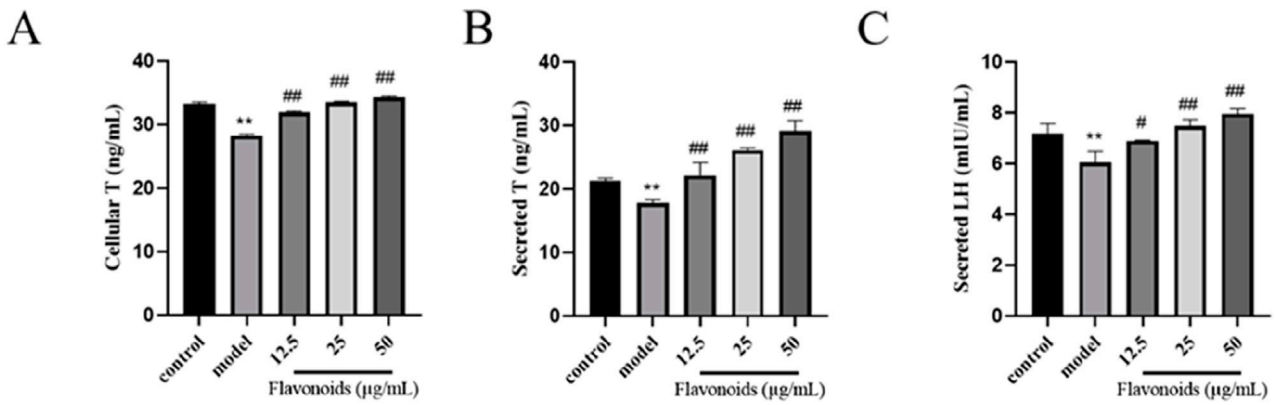

FIGURE 7
Effect of CSF on T and LH secretion in TM3 cells. ELISA for **(A)** intracellular T levels, **(B)** secreted T levels, and **(C)** secreted LH levels in TM3 cells. $^{**}P < 0.01$ vs. BG; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. MG, n = 3. BG, blank group; CSF, *Cynomorium songaricum* Rupr. flavonoid; LH, luteinizing hormone; MG, model group; T, testosterone.

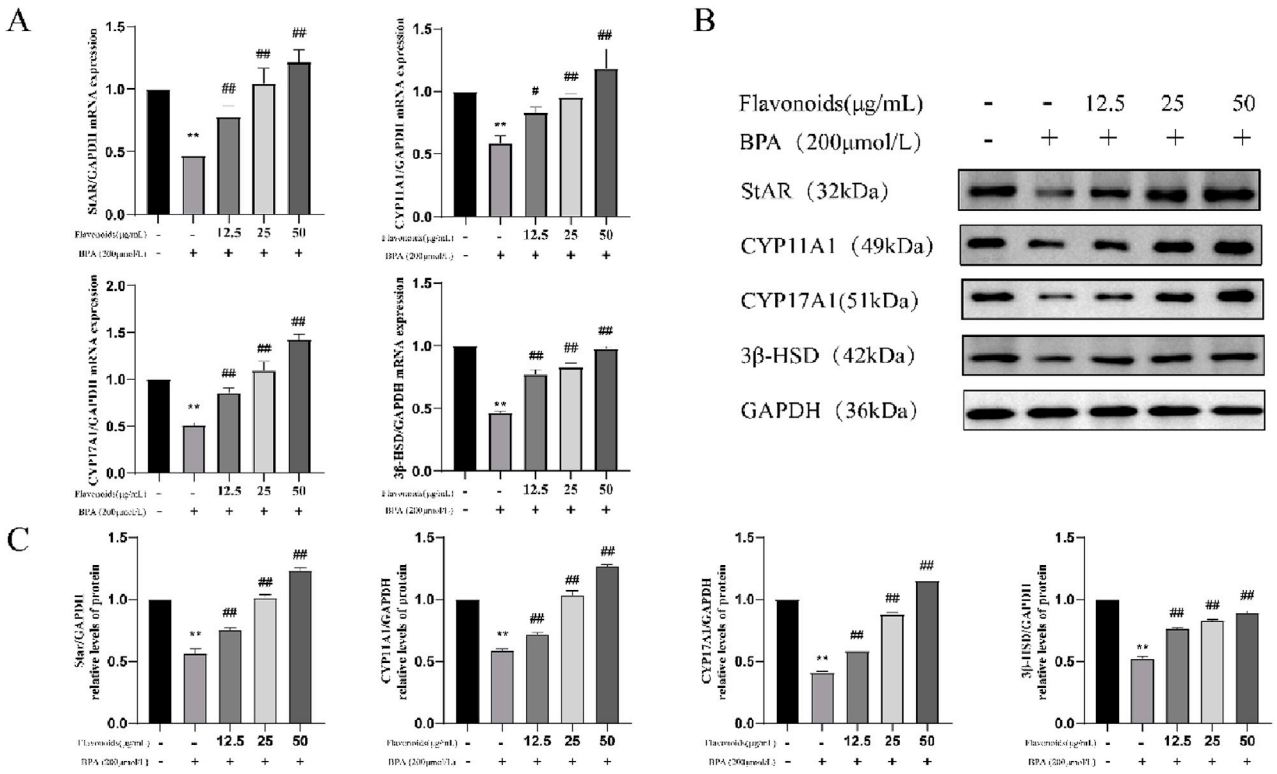

FIGURE 8
Effect of CSF on the expression of TM3 cell steroidogenic enzyme-related genes and proteins. **(A)** CSF promotes TM3 cell steroidogenic enzyme mRNA expression, **(B,C)** CSF promotes TM3 cell steroidogenic enzyme protein expression. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. model. CSF, *Cynomorium songaricum* Rupr. flavonoid.

elevate serum T levels, facilitate sexual maturation in animals, and enhance male sexual behavior in rats (Cui et al., 2013). Cyclophosphamide, an alkylating agent commonly used in cancer treatment, is known to inflict severe damage to testicular tissues, leading to oligospermia and infertility (Ghobadi et al., 2017). Studies have demonstrated that cyclophosphamide substantially reduces sperm density and viability, increases sperm malformation rate, and disrupts reproductive hormone levels (Namoju et al., 2021). In this study, we used a mouse model of reproductive dysfunction by administering cyclophosphamide to investigate the protective effects of CSF against cyclophosphamide-induced testicular tissue damage.

Objective markers of reproductive function include pathological changes in the testes and epididymis (Wei et al., 2022). Previous studies have shown that cyclophosphamide causes thinning of the spermatogenic epithelium, reduced epithelial layers, disordered arrangements, and decreased sperm populations in the testicular

spermatogenic tubules of rats (Zhang et al., 2013). These results were consistent with our observations. Our MG exhibited successful modeling characterized by a reduced content of components at various levels in spermatogenic cells and observed obstacles in the development and spermatogenic cell maturation processes. In the CSF group, the distribution of spermatozoa and the number of spermatogenic cells in the testes demonstrated varying degrees of improvement, thus enhancing the spermatogenic potential of testicular cells.

The hypothalamic-pituitary-gonadal (HPG) axis plays an integral role in the regulation of human and animal reproduction (Xie et al., 2022), and the process of spermatogenesis in males is highly driven by the HPG axis (Khodamoradi et al., 2020). The regulation of the HPG axis is necessary for the maintenance of normal testicular function in males, including testosterone production and male fertility. The hypothalamus secretes GnRH to stimulate the pituitary gland to synthesize LH and FSH, which in turn maintains the production of T and spermatogenesis in the gonads (Corradi et al., 2016). These hormones maintain proper reproductive function and support physiological homeostasis through feedback regulatory mechanisms. In our mouse model, cyclophosphamide-induced reproductive dysfunction led to significantly elevated E2 levels and significantly reduced serum levels of T, LH, GnRH, and FSH. Conversely, the CSF-administered group exhibited significantly lower E2 levels but significantly higher levels of T, LH, GnRH, and FSH. Clomiphene citrate promotes the secretion of male gonadal hormones and increases serum testosterone concentration, thus clomiphene citrate-positive group had significantly higher T levels. These findings suggest that the CSF can maintain sex hormone levels by regulating the gonadal axis.

T, a crucial sex hormone in male animals, is primarily synthesized and secreted by the testicular mesenchymal cells (Lai et al., 2005). Steroidogenic enzymes play a substantial role in this process by affecting T synthesis and, subsequently, reproductive function (Ge et al., 2021). Our study demonstrated that CSF administration upregulated the mRNA and protein expression levels of steroidogenic enzymes both *in vivo* and *in vitro*, suggesting its potential to mitigate reproductive impairment by modulating the T synthesis pathway. Natural products exhibit various activities and therapeutic effects against various diseases. Single-molecule natural products, such as flavonoids, alkaloids, polysaccharides, and terpenoids, have a wide range of biological activities, many of which have been observed to enhance male sterility by improving testicular function and semen quality (Li et al., 2024). Quercetin ameliorated the toxic effects of fenitrothion in rats via the steroidogenic enzyme pathway and the oxidative stress pathway (Saber et al., 2016). Escin can improve sperm quality in male patients with varicocele by reducing oxidative stress in the testicular tissue, alleviating inflammatory reactions, and promoting tension and contraction after venous wall injury, making it a safe and effective drug (Fang et al., 2010). Epigallocatechin-3-O-gallate [EGCG] has excellent antioxidant activity, and studies have shown that it substantially reduces testicular lesions, sperm malformations, and spermatogenic cell apoptosis (Ge et al., 2015). Yu et al. (2010) Significant and dose-dependent antioxidant and anti-fatigue effects of Cynomorium songaricum flavonoids (rutin, catechin and isoquercitrin) on swimming rats were observed during 10 days of swimming exercise. Experimental results suggest that flavonoid supplementation could not only reduce free radical formation and scavenge free radicals, but also enhance endurance exercise performance by reducing muscle fatigue. Additionally, several studies have shown that certain traditional Chinese herbs, such as *Epimedium brevican, Cuscuta australis, Cistanche deserticola*, and Huangqi-Guizhi-Wuwutang, have promising effects on promoting reproduction (Wang et al., 2023; Ozegbe and Omirinde, 2012; Gu et al., 2016; Zhao et al., 2024). Combining these herbs with CS may further enhance their reproductive-promoting properties via synergistic effects.

Cynomorium songaricum is a traditional kidney tonifying and yang enhancing medication commonly used to treat erectile dysfunction, premature ejaculation, and nocturnal emissions. Modern pharmacological studies have shown that *C. songaricum* can increase plasma testosterone levels, promote animal sexual maturity, and enhance animal sexual behavior in young male rats (Yan et al., 1991; Wang et al., 2003). Abd el-Magied et al. (2001) found that *C. songaricum* can significantly improve testicular mass and serum testosterone levels in immature rats. These research results are consistent with the findings of this study. Gu et al. (2021) further analyzed the hormone levels in rats and found that *C. songaricum* can significantly increase testosterone levels and regulate FSH and LH secretion levels in oligoasthenozoospermia rats, leading to the normalization of the negative feedback regulation system of sex hormones in oligoasthenozoospermia rats. This is consistent with our research findings. In addition, Yang et al. (2010) studied that *C. songaricum* has a significant effect on sperm parameters and testicular GDNF expression in rats. The results indicate that traditional Chinese medicine extracts can significantly increase the expression of GDNF at the mRNA and protein levels. Cao et al. (2016) found that *C. songaricum* can significantly increase the sperm count, sperm motility, and serum testosterone levels in oligozoospermia and asthenozoospermia rats. The improvement mechanism may be: 1. By inducing GDNF expression in Sertoli cells of the testes, it promotes the proliferation of undifferentiated spermatogonia, thereby promoting the process of spermatogenesis and increasing the number of sperm in the tail of the epididymis; By promoting testosterone secretion and increasing serum testosterone levels, sperm motility can be improved. Therefore, the mechanism of the reproductive promoting effect of *C. songaricum* needs further research.

Conclusion

Our study showed that CSF ameliorates reproductive impairment by enhancing the expression of pivotal enzymes involved in synthesizing T. The results show that CSF ameliorates cyclophosphamide-induced reproductive impairment and bisphenol A-induced TM3 cell damage in mice by regulating sex hormone levels in the HPG axis and upregulating the expression of steroidogenic enzymes. This study expands our knowledge of the potential mechanisms by which CS protects male fertility, thereby

establishing a theoretical foundation for its potential development as a functional food or pharmaceutical drug.

Study limitations and suggestions

Due to budget and equipment restrictions, we were unable to explore the impact of individual CSF components on enhancing T production and protecting male fertility. Therefore, we recommend investigating this in future studies.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

Ethics statement

The animal study was approved by Ethics Committee of Jilin Agricultural University. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

JC: Conceptualization, Writing–original draft. XY: Validation, Writing–original draft. ZH: Validation, Writing–original draft. WC: Validation, Writing–original draft. YaZ: Writing–original draft. JL: Validation, Writing–original draft. YiZ: Conceptualization, Writing–review and editing. RD: Writing–review and editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. The Science and Technology Development Plan Project of Jilin Province provided support for this work [Nos. 20220304003YY, 20220304001YY, and 20220304002YY], along with the Xinjiang Aid Project [Nos. 20210032 and 20210031].

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2024.1457780/full#supplementary-material

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frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 22 August 2024
DOI 10.3389/fphar.2024.1440979

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RECEIVED 30 May 2024
ACCEPTED 05 August 2024
PUBLISHED 22 August 2024

CITATION
Li X-L, Sun Y-P, Wang M, Wang Z-B and Kuang H-X (2024) Alkaloids in *Chelidonium majus* L: a review of its phytochemistry, pharmacology and toxicology.
Front. Pharmacol. 15:1440979.
doi: 10.3389/fphar.2024.1440979

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Alkaloids in *Chelidonium majus* L: a review of its phytochemistry, pharmacology and toxicology

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Chelidonium majus L. (*C. majus*), commonly known as "Bai Qu Cai" in China, belongs to the genus *Chelidonium* of the Papaveraceae family. It has rich medicinal value, such as alleviating coughs, asthma, spasms and pain. Recent studies have demonstrated that *C. majus* is abundant in various alkaloids, which are the primary components of *C. majus* and have a range of pharmacological effects, including anti-microbial, anti-inflammatory, anti-viral, and anti-tumor effects. So far, 94 alkaloids have been isolated from *C. majus*, including benzophenanthridine, protoberberine, aporphine, protopine and other types of alkaloids. This paper aims to review the research progress in phytochemistry, pharmacology and toxicology of *C. majus* alkaloids, in order to provide a theoretical basis for the application of *C. majus* in the field of medicinal chemistry and to afford reference for further research and development efforts.

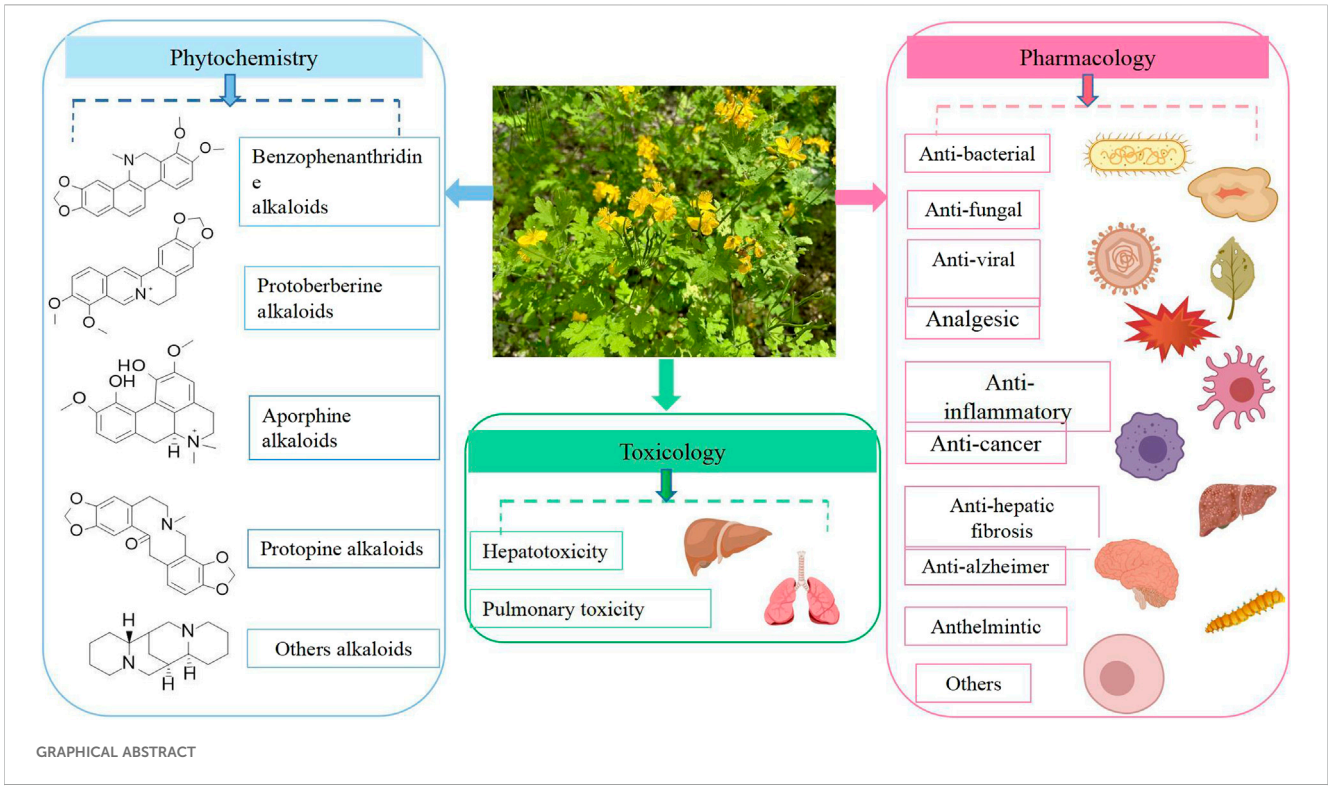
KEYWORDS

***Chelidonium majus* L., alkaloids, phytochemistry, pharmacological effect, toxicity**

1 Introduction

Chelidonium majus L., a traditional medicinal plant from the *Chelidonium* genus of the Papaveraceae family, is a perennial herb extensively distributed in Europe, Asia, and Africa (Figure 1). It has been widely used as a traditional Chinese ethnic medicine for centuries and was first documented in the "Herbal for Relief of Famines." Known by various names in folklore, such as great celandine, swallow-wort, rock poppy, bai qu cai, tuhuanglian (土黄连) and dunchangcao (断肠草), it mostly thrives on hillsides, valley forest edges, grasslands, roadsides, and rock crevices (Wei et al., 2009; Gilca et al., 2010). According to the online records of China's flora (http://www.cn-flora.ac.cn/index.html), the height of *C. majus* is approximately 30–60 (−100) cm. The stems are erect, multi-branched, and the branches are frequently covered with small hairs and may exude yellow latex when broken. The leaf blade is obovate-oblong or broadly obovate, 8–20 cm long. They are pinnatisect and divided into 2–4 pairs of lobes with irregularly parted or lobed crenate margins, appearing glaucous abaxially and green adaxially. Additionally, the blade is sparsely pubescent abaxially and glabrous adaxially. Flower buds are oval, with a diameter of 5–8 mm. The sepals are also oval

Abbreviations: *C. majus, Chelidonium majus L.;* TCM, Traditional Chinese medicine; CHE, Chelerythrine; SNG, Sanguinarine; CHLD, Chelidonine; CHECL, Chelerythrine chloride; HHS, 8-hydroxydihydrosanguinarine; HHC, 8-hydroxydihydrochelerythrine.



and cymbiform, with a length of 5–8 mm. They may be glabrous or sparsely pubescent, and tend to be caducous. Petals are yellow, obovate, and entire, approximately 1 cm long. The capsule is narrowly terete at 2–5 cm × 2-3 mm, with a pedicel usually shorter than the fruit. The seeds are dark brown, ovoid, about 1 mm long or shorter, and have a shiny, alveolate appearance. The flowering and fruiting period is from April to September.

In traditional Chinese medicine (TCM), *C. majus* is classified as a heat-clearing herb (Gilca et al., 2010). The 2020 edition of the Pharmacopoeia of the People's Republic of China describes *C. majus* as bitter, cool, and toxic, returning to the lung and stomach meridians. The whole herb has the effect of relieving spasms and pain, coughs, and asthma. As extraction techniques have evolved and advanced, significant progress in research on *C. majus* has been made. Various active ingredients of *C. majus* have been isolated and purified, including alkaloids, flavonoids, saponins, volatile oils, vitamin C, and other components (Kwasniewski, 1958; Colombo and Bosisio, 1996; Bai and Zhang, 2009). Pharmacological studies have shown that its active components exhibit wide-ranging pharmacological activities, such as antibacterial, antifungal, anti-inflammatory, antiviral, and antitumor effects (Hong et al., 2022). Further studies have identified that the major active compounds of *C. majus* are isoquinoline alkaloids (Colombo and Bosisio, 1996), including benzophenanthridine, protoberberine, aporphine, protopine, and other types (Tomè and Colombo, 1995). As a hemicryptophyte, the concentration of alkaloids in *C. majus* continues to accumulate with changes in light (Tomè and Colombo, 1995). These alkaloids are found in concentrations ranging from 0.27% to 2.25% in aerial parts and 3%–4% in the roots (Maji and Banerji, 2015), with varying alkaloid content across different plant organs. Compared to the aerial parts and underground parts, the total content of alkaloids in the leaves is lower, while the content in latex is 32 times higher than in leaves and 9 times higher than in roots (Tomè and Colombo, 1995). These findings suggest that the alkaloid content in plant organs is influenced by the number of laticifers where they are stored (Zielińska et al., 2018). Despite extensive literature reviews, we have not yet found any article that provides a comprehensive and detailed review of the alkaloids of *C. majus*. Therefore, this paper focuses on the alkaloids of *C. majus,* reviewing research progress in phytochemistry, pharmacological effects, and toxicology. The aim is to provide a reference for the application of *C. majus* in medicinal drugs, which is extremely significant for the further advancement of traditional ethnic medicine.

2 Methodology

To comprehensively understand the research status of *C. majus*, we conducted a thorough literature search using various electronic databases, including Web of Science, PubMed, Google Scholar, and China National Knowledge Infrastructure (CNKI). Additionally, we referred to other literature sources, such as Pharmacopoeia of the People's Republic of China, to obtain relevant information about the alkaloids in *C. majus*. This article exclusively utilizes Chinese and English texts. The keywords employed were *C. majus* L., alkaloids, phytochemistry, pharmacological effects, and toxicity. As of May 2024, a total of 915 relevant literature sources were retrieved. To ensure the accuracy and relevance of the review, we conducted screening based on the title, abstract, and full text of the article. Duplicate articles, conference abstracts, and unavailable articles have been excluded.

10.3389/fphar.2024.1440979

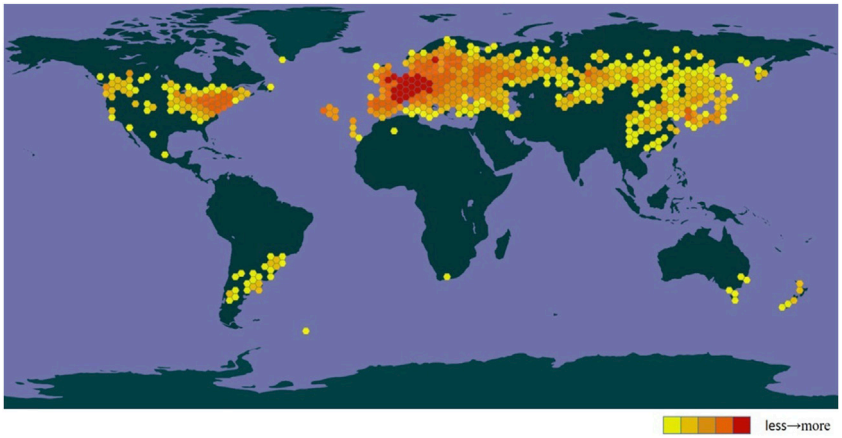


FIGURE 1
The global distributions of *Chelidonium majus* L. (https://www.gbif.org/species).

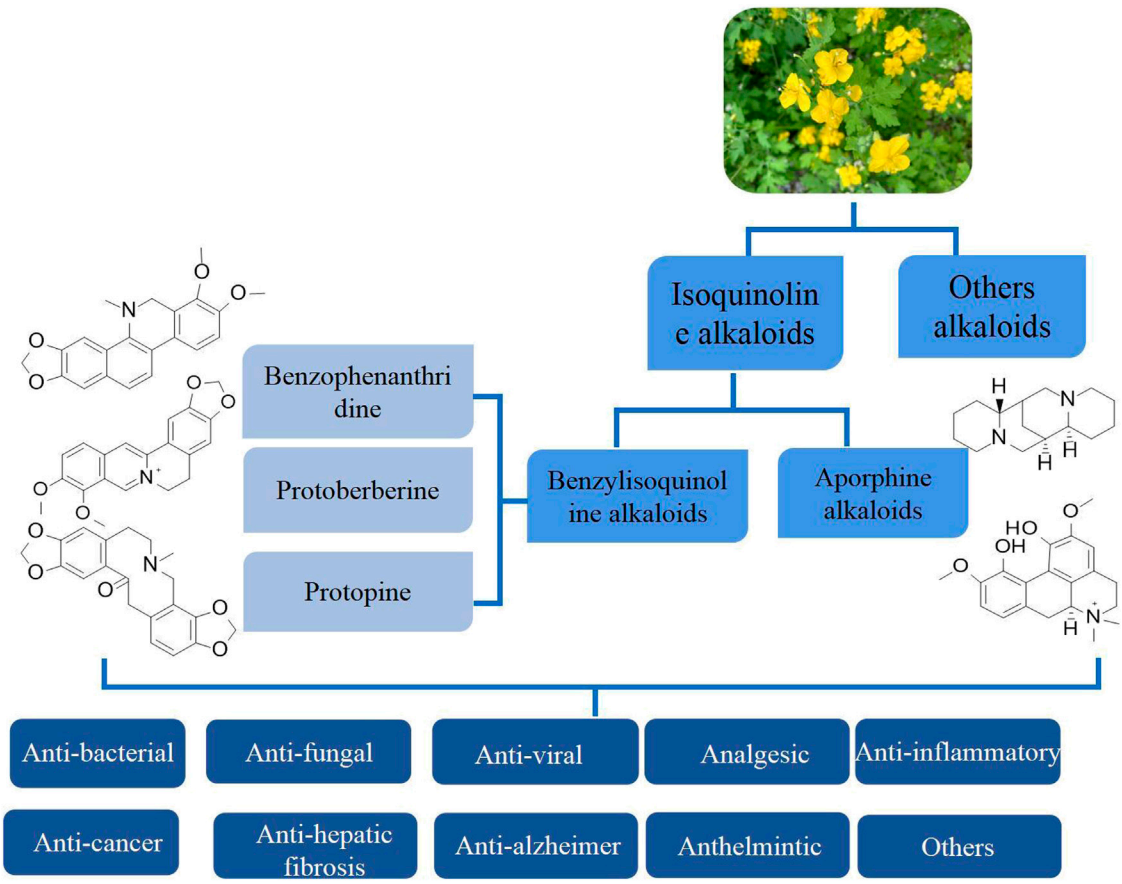


FIGURE 2
The alkaloids groups of alkaloids and pharmacological effects of *Chelidonium majus* L.

Additionally, articles with research purposes not relevant to the topic of this review, as well as non-English and non-Chinese articles, have also been excluded. Finally, 166 eligible articles were included.

3 Phytochemistry

The chemical composition of *C. majus* is complex, with isoquinoline alkaloids being recognized as the main active

TABLE 1 Benzophenanthridine alkaloids compounds isolated from *Chelidonium majus* L.

No.	Compound name	Formula	Part of the plant	Ref.
Dihydrobenzophen-anthridine alkaloids				
1	Dihydrochelerythrine	$C_{21}H_{19}NO_4$	Whole plant	Kadan et al. (1990), Oechslin et al. (1991)
2	Dihydrosanguinarine	$C_{20}H_{15}NO_4$	Whole plant	Slavík and Slavíková (1977), Kadan et al. (1990)
3	Dihydrochelirubine	$C_{21}H_{17}NO_5$	Root	Tanahashi and Zenk (1990), Táborská et al. (1994)
4	Dihydrochelilutine	$C_{22}H_{21}NO_5$	Aerial part	Hanaoka et al. (1991), Deng et al. (2016)
5	8-Hydroxydihydrosanguinarine	$C_{20}H_{15}NO_5$	Aerial part	Zuo et al. (2008)
6	8-Hydroxydihydrochelerythrine	$C_{21}H_{19}NO_5$	Aerial part	Zuo et al. (2008)
7	6-Methoxydihydrochelerythine/Angoline	$C_{22}H_{21}NO_5$	Whole plant	Zhou et al. (1989)
8	6-Methoxydihydrosanguinarine	$C_{21}H_{17}NO_5$	Whole plant	Zhou et al. (1989)
9	8-Acetonyldihydrochelerythrine	$C_{24}H_{23}NO_5$	Whole plant	Kadan et al. (1990)
10	8-Acetonyldihydrosanguinarine	$C_{23}H_{19}NO_5$	Whole plant	Kadan et al. (1990)
11	Methyl-2'-(7,8-dihydrosanguinarine-8-yl) acetate	$C_{23}H_{19}NO_6$		Park et al. (2011)
12	Dihydromacarpine	$C_{22}H_{19}NO_6$	Aerial part	Tanahashi and Zenk (1990), Deng et al. (2016)
13	Spallidamine	$C_{18}H_{19}NO_4$	Aerial part	Kim et al. (2015a)
14	6-Ketenesanguinarine	$C_{23}H_{17}NO_5$	Aerial part	Zhang et al. (2014)
15	(1′R,6 R) -1-(Dihydrochelerythrine-6-yl) ethanol	$C_{23}H_{24}NO_5$	Aerial part	Deng et al. (2017)
16	(1′S,6 S) -1-(Dihydrochelerythrine-6-yl) ethanol	$C_{23}H_{24}NO_5$	Aerial part	Deng et al. (2017)
17	(1′R,6 R) -1-(Dihydrosanguinarine-6-yl) ethanol	$C_{22}H_{20}NO_5$	Aerial part	Deng et al. (2017)
18	(1′S,6 S) -1-(Dihydrosanguinarine-6-yl) ethanol	$C_{22}H_{20}NO_5$	Aerial part	Deng et al. (2017)
19	(1′S,6 R) -1-(Dihydrochelerythrine-6-yl) ethanol	$C_{23}H_{24}NO_5$	Aerial part	Deng et al. (2017)
20	(1′R,6 S) -1-(Dihydrochelerythrine-6-yl) ethanol	$C_{23}H_{24}NO_5$	Aerial part	Deng et al. (2017)
21	(1′S,6 R) -1-(Dihydrosanguinarine-6-yl) ethanol	$C_{22}H_{20}NO_5$	Aerial part	Deng et al. (2017)
22	(1′R,6 S) -1-(Dihydrosanguinarine-6-yl) ethanol	$C_{22}H_{20}NO_5$	Aerial part	Deng et al. (2017)
23	(6 S) -Ethyl 2-(dihydrosanguinarine-6-yl) acetate	$C_{24}H_{22}NO_6$	Aerial part	Deng et al. (2017)
24	(6 R) -Ethyl 2-(dihydrosanguinarine-6-yl) acetate	$C_{24}H_{22}NO_6$	Aerial part	Deng et al. (2017)
25	(6 R) -Ethyl-dihydrosanguinarine-6-carboxylate	$C_{23}H_{20}NO_6$	Aerial part	Deng et al. (2017)
26	(6 S) -Ethyl dihydrosanguinarine-6-carboxylate	$C_{23}H_{20}NO_6$	Aerial part	Deng et al. (2017)
27	Oxychelerythrine	$C_{21}H_{17}NO_5$	Root	Táborská et al. (1994)
28	Oxysanguinarine	$C_{20}H_{13}NO_5$	Whole plant	Kadan et al. (1990), Táborská et al. (1994)
29	N-Demethyloxysanguinarine	$C_{19}H_{11}NO_5$		Chang et al. (2003), Wei et al. (2009)
30	N-dimethyl-9,10-dihydroxysanguinarine	$C_{19}H_{13}NO_5$	Root	Wei et al. (2009), Kopyt'ko et al. (2005)
31	Oxynitidine	$C_{21}H_{17}NO_5$		Maji and Banerji (2015)
32	Dihydronitidine	$C_{21}H_{19}NO_4$	Root	Maji and Banerji (2015), Kopyt'ko et al. (2005)
Hexahydrobenzophenthridinealkaloids				
33	Chelidonine	$C_{20}H_{19}NO_5$	Whole plant, latex	Bugatti et al. (1987), Zhao et al. (2020), Warowicka et al. (2021)
34	Isochelidonine	$C_{20}H_{19}NO_5$	Aerial part	Rosa and Vincenzo (1992)
35	Oxychleidonine	$C_{20}H_{17}NO_6$	Root	Kaczmarek and Malek (1959)

(Continued on following page)

TABLE 1 (*Continued*) Benzophenanthridine alkaloids compounds isolated from *Chelidonium majus* L.

No.	Compound name	Formula	Part of the plant	Ref.
36	Methoxychelidonine	$C_{21}H_{21}NO_6$	Root	Kaczmarek and Malek (1959)
37	Norchelidonine	$C_{19}H_{17}NO_5$	Whole plant	Kadan et al. (1992)
38	Chelamine	$C_{20}H_{19}NO_6$	Whole plant	Slavík and Slavíková (1977), Táborská et al. (1994)
39	Chelamidine	$C_{21}H_{23}NO_6$	Whole plant	Slavík and Slavíková (1977), Táborská et al. (1994)
40	(+)-Homochelidonine	$C_{21}H_{23}NO_5$	Whole plant	Kadan et al. (1990)
41	10-Hydroxyhomochelidonine	$C_{21}H_{24}NO_6$	Root	Slavík and Slavíková (1977)
42	10-Hydroxychelidonine	$C_{20}H_{19}NO_6$	Root	Slavík and Slavíková (1977)
Dimindihydrobenz-ophenthridine alkaloids				
43	Chelidimerine	$C_{43}H_{32}N_2O_9$	Whole plant	Tin-wa et al. (1972b), Kadan et al. (1990)
44	Chelerythridimerine	$C_{45}H_{40}N_2O_9$		MacLean et al. (1969), Wei et al. (2009)
45	Sanguidimerine	$C_{45}H_{40}N_2O_9$		Tin-wa et al. (1972a), Wei et al. (2009)
46	Rhoeadine	$C_{21}H_{21}NO_6$	Root	Pfeifer et al. (1965), Yang et al. (2024b)
Benzophenanthrine quaternary amine alkaloids				
47	Chelerythrine	$C_{21}H_{18}NO_4^+$	Whole plant, latex	Bugatti et al. (1987), Warowicka et al. (2019), Zhao et al. (2020)
48	Sanguinarine	$C_{20}H_{14}NO_4^+$	Whole plant, latex	Kaczmarek and Malek (1959), Warowicka et al. (2019), Zhao et al. (2020)
49	Chelirubine	$C_{21}H_{16}NO_5^+$	Root	Kaczmarek and Malek (1959)
50	Chelilutine	$C_{22}H_{21}NO_5$	Root	Slavík and Slavíková (1977), Táborská et al. (1994)
51	Demethylchelerythrine	$C_{20}H_{15}NO_4$	Aerial part	Zhang et al. (2014)
52	Demethylsanguinarine	$C_{19}H_{11}NO_4$	Aerial part	Zhang et al. (2014)
53	Didehydrochelidonine	$C_{20}H_{20}NO_5$		Maji and Banerji (2015)
54	Nitidine	$C_{21}H_{18}NO_4^+$		Maji and Banerji (2015)
55	Macarpine	$C_{22}H_{18}NO_6^+$	Root	Táborská et al. (1994), Maji and Banerji (2015)

ingredients. In addition, some scholars have reported that *C. majus* also contains flavonoids, triterpenoids, volatile oils, and other components. Isoquinoline alkaloids are a class of alkaloids derived from phenylalanine or tyrosine, which are abundant in quantity and complex in structure, providing a rich material basis for the pharmacological effects of *C. majus*. At present, 94 alkaloids have been isolated and identified from *C. majus*, which can be categorized into benzophenanthridines, protoberberines, aporphines, protopines, and other alkaloids based on their carbon skeletons (Figure 2) (Gerenčer et al., 2006; Wei et al., 2009). Among them, the three main alkaloid groups, including benzophenanthridines, protoberberines, and protopines, belong to benzylisoquinoline alkaloids, and aporphines belong to isoquinoline alkaloids, which are considered to be the active ingredients of *C. majus* and exhibit significant pharmacological activity (Tuzimski and Petruczynik, 2023; Zwerger et al., 2024). The concentrations of these alkaloids differ according to the plant parts and growth conditions, but they generally have high medicinal value. This section provides information on the types, molecular formulas, plant parts, and references of these alkaloids isolated from *C. majus*.

3.1 Benzophenanthridine alkaloids

Benzophenanthridine alkaloids are classified as isoquinoline alkaloids, characterized by a tetracyclic structural motif, and represent an important category of nitrogen-containing small molecules (Bisai et al., 2019). These alkaloids are a common in the Papaveraceae family and are the most abundant and important components in *C. majus*. Currently, 55 alkaloids (1–55) have been identified and extracted from *C. majus*. The skeletal structure consists of one isoquinoline nucleus and two benzene rings. The benzophenanthridine alkaloids isolated and identified from *C. majus* can be further divided into four structural types according to the degree of unsaturated skeleton: dihydrobenzophenthridine, hexahydrobenzophenthridine, dimindihydrobenzophenthridine and benzophenanthrine quaternary amine (Table 1; Figure 3) (Wei et al., 2009; Bisai et al., 2019; Laines-Hidalgo et al., 2022). These alkaloids are structurally variable due to their N-atom content and have been reported to have a wide range of pharmacological activities (Wei et al., 2021), with significant anti-inflammatory, analgesic, and antitumor activities (Han et al., 2016).

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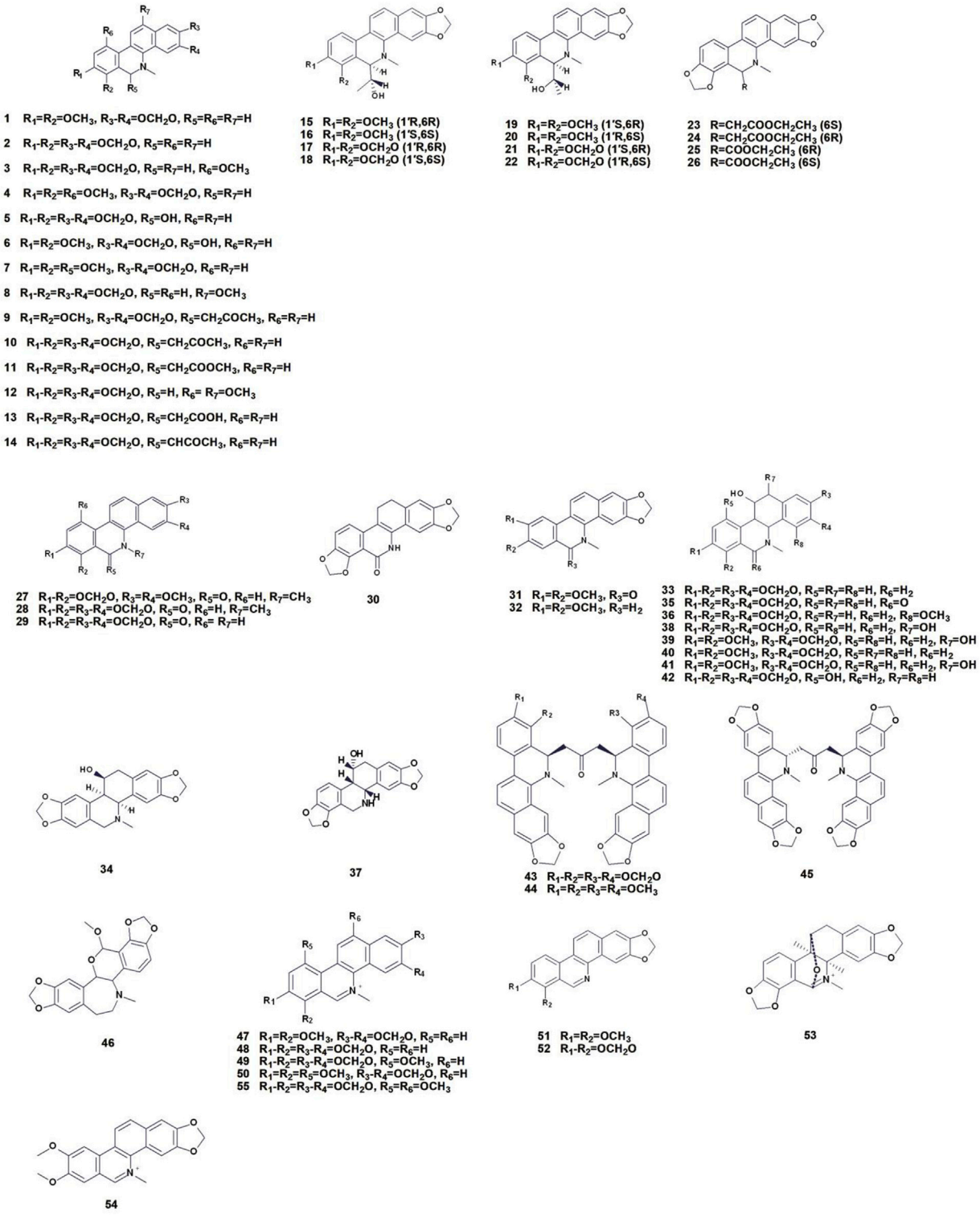

FIGURE 3
Chemical structures of benzophenanthridine alkaloids isolated from *Chelidonium majus* L. (The numbers in Figure 3 refer to the numbers of alkaloids present in Table 1).

3.2 Protoberberine alkaloids

Protoberberine alkaloids are widely distributed and represent one of the largest categories of isoquinoline alkaloids. They are synthesized in plants through a series of complex enzymatic reactions using tyrosine as a substrate (Liu et al., 2023). This type of alkaloid is composed of two fused isoquinoline rings, primarily in the form of hydrochloride.

TABLE 2 Protoberberine alkaloids compounds isolated from *Chelidonium majus* L.

No.	Compound name	Formula	Part of the plant	Ref.
56	Berberine	$C_{20}H_{18}NO_4^+$	Whole plant, latex	Bugatti et al. (1987), Zhao et al. (2020), Warowicka et al. (2021)
57	Berberrubine	$C_{19}H_{16}NO_4^+$	Whole plant	Yang et al. (2017), Yang et al. (2024b)
58	Coptisine	$C_{19}H_{14}NO_4^+$	Whole plant, latex	Sárközi et al. (2006), Zhao et al. (2020), Warowicka et al. (2021)
59	Jatrorrhizine	$C_{20}H_{20}NO_4^+$	Whole plant	Yang et al. (2017), Yang et al. (2024b)
60	Columbamine	$C_{20}H_{20}NO_4^+$	Whole plant	Yang et al. (2017), Yang et al. (2024b)
61	Corysamine	$C_{20}H_{16}NO_4^+$	Whole plant	Golkiewicz and Gadzikowska (1999), Zhao et al. (2020)
62	Dihydroberberine	$C_{20}H_{19}NO_4$	Root	Kopyt'ko et al. (2005)
63	Dihydrocoptisine	$C_{19}H_{15}NO_4$	Root	Kopyt'ko et al. (2005)
64	8-Oxycoptisine	$C_{19}H_{13}NO_5$	Whole plant	Zhou et al. (1989)
65	Tetrahydroberberine/Canadine	$C_{20}H_{21}NO_4$	Aerial part	Ruizhi et al. (1985), Bozhadze et al. (2013)
66	Stylopine/Tetrahydrocoptisine	$C_{19}H_{17}NO_4$	Whole plant	Kadan et al. (1990), Sárközi et al. (2006)
67	13,14-Dihydrocoptisine	$C_{19}H_{16}NO_4^+$	Whole plant	Paulsen et al. (2015), Yang et al. (2024b)
68	Tetrahydrocoptisine N-oxide	$C_{19}H_{18}NO_6$	Aerial part	Huang et al. (2019)
69	7 R,14 S-cis-tetrahydrocoptisine N-oxide	$C_{19}H_{17}NO_5$	Aerial part	Le et al. (2021)
70	7 R,14 R-trans-tetrahydrocoptisine N-oxide	$C_{19}H_{17}NO_5$	Aerial part	Le et al. (2021)
71	(S)-N-Methylstylopine	$C_{20}H_{20}NO_4^+$	Whole plant	Zhao et al. (2020)
72	Scoulerine	$C_{19}H_{21}NO_4$	Root, leaf	Schrittwieser et al. (2011), Yahyazadeh et al. (2017)
73	Cheilanthifoline	$C_{19}H_{19}NO_4$	Root, leaf	Yahyazadeh et al. (2017)
74	Menisperine	$C_{21}H_{26}NO_4^+$	Root	Tomita and Kikuchi (1955), Yang et al. (2024b)
75	Worenine	$C_{20}H_{16}NO_4^+$	Root	Zhang et al. (2011a), Yang et al. (2024b)
76	Sanguilutine	$C_{23}H_{24}NO_5^+$	Root	Táborská et al. (1994)

Protoberberine alkaloids are abundantly present in nature, with *C. majus* containing a relatively high content of these compounds. Currently, 21 protoberberine compounds (56–76) have been isolated from this plant (Table 2; Figure 4). In clinical practice, this class of alkaloids demonstrates a range of biological activities, including antimicrobial and anti-inflammatory properties. These alkaloids exhibit various beneficial effects in the field of medicine.

3.3 Aporphine alkaloids

Aporphine alkaloids are natural compounds that are widely distributed in nature and have important biological activities. This group of alkaloids belongs to isoquinoline alkaloids, which is an important type of natural alkaloids (Lin et al., 2020). These alkaloids are composed of four fused hexagonal rings, formed by connecting the C-2 position of the benzyl part of the benzylisoquinoline and the C-8 position of the isoquinoline part and eliminating one hydrogen molecule. A total of 6 species of aporphine alkaloids have been isolated and identified (Table 3; Figure 5). Aporphine alkaloids exhibit various pharmacological effects, including antioxidant, antiviral, and antitumor activities.

3.4 Protopine alkaloids

Protopine alkaloids are a class of isoquinoline alkaloids with ten-membered nitrogen heterocycles formed by protoberberine alkaloids through N- methylation and ring splitting in the biosynthetic pathway. The most noticeable characteristic of these compounds is C-14 carbonylation. These natural isoquinoline alkaloids possess a fundamental three-ring structure, comprising two benzene rings (A and C rings) and one ten-membered nitrogen heterocyclic ring (B ring) (Paul and Maurer, 2003). Currently, 4 species of protopine alkaloids have been isolated from *C. majus* (83–86) (Table 3; Figure 5).

3.5 Other alkaloids

In addition to the above four groups of alkaloids, other alkaloids have been reported. For example, Kaczmarek and Malek isolated sparteine by paper chromatography (Kaczmarek and Malek, 1959); Kadan G et al. isolated (−)-turkiyenine from the dried whole plant (Kadan et al., 1990); Yuan et al. obtained noroxyhydrastinine, indole-3-carboxaldehyde, and flazin (Yuan et al., 2022); Kim et al. identified N-trans-feruloyltyramine and arnottianamide (Kim et al., 2015); and Huang et al. extracted chelidoniumine from the aerial part of *C. majus*. Although the

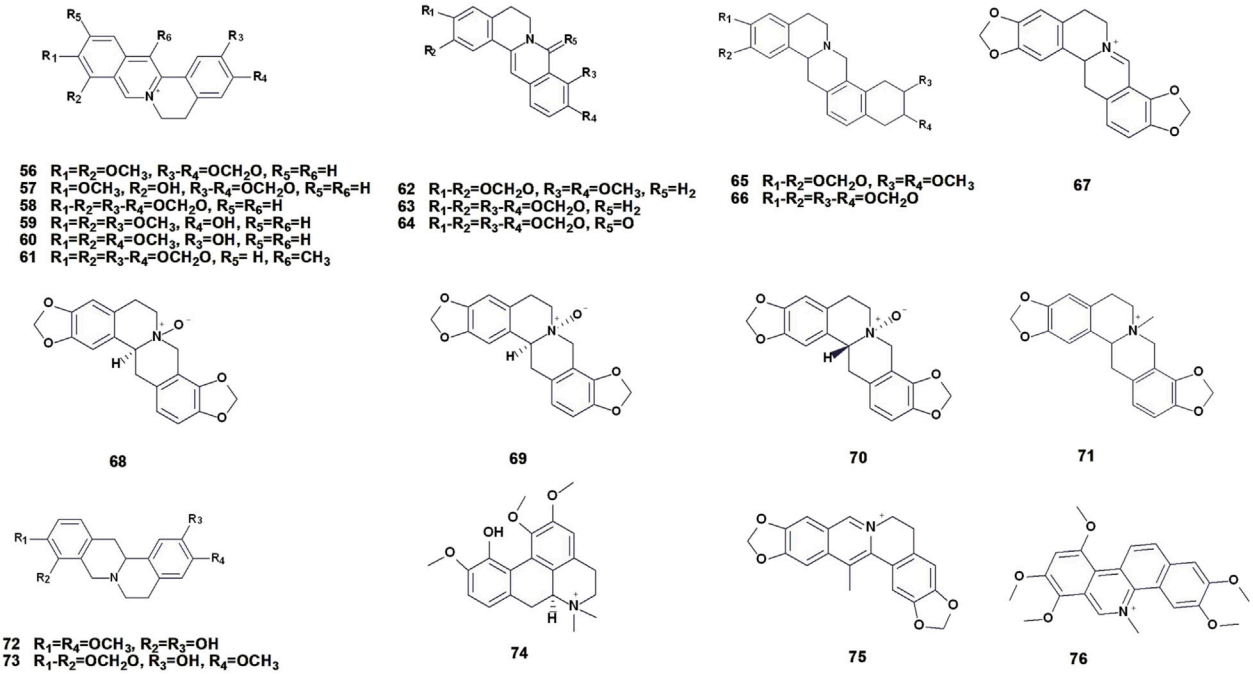

FIGURE 4
Chemical structures of protoberberine alkaloids isolated from *Chelidonium majus* L. (The numbers in Figure 4 refer to the numbers of alkaloids present in Table 2).

concentrations of these alkaloids are low, they may have unique pharmacological activities that merit further research and development (Table 3; Figure 5).

4 Pharmacology

To date, *C. majus* has demonstrated a diverse range of pharmacological effects, with its aerial parts and roots being rich in various alkaloids. This paper explores the alkaloids of *C. majus*, which are widely found in numerous plants and significantly impact human health. As research on *C. majus* deepens, its alkaloid components have been found to interact with multiple biological targets, exerting therapeutic effects on various diseases. These include antibacterial, antifungal, anti-tumor, anti-inflammatory, analgesic, expectorant, anti-cough, anti-asthma, and anti-liver fibrosis activities.

4.1 Anti-bacterial effect

Studies conducted in the past few years have shown that the alkaloids in *C. majus* have broad-spectrum antibacterial effects. Chelerythrine (CHE), extracted from *C. majus*, exhibits a strong antibacterial effect on *Streptococcus mutans*, the main caries-causing bacterium in the oral cavity. It effectively reduces the adhesion ability of *S. mutans*, suggesting its potential use as a preventative treatment for dental caries (Chen et al., 2011). *Staphylococcus aureus* (*S. aureus*) and *methicillin-resistant S. aureus* (MRSA) are common clinical pathogens. 8-hydroxydihydrosanguinarine (HHS) and 8-hydroxydihydrochelerythrine (HHC), solated from *C. majus*, have shown significant inhibition against MRSA strains, with minimal inhibitory concentrations/minimal bactericidal concentrations (MIC/MBC) of MRSA strains ranging from 0.49–15.63/1.95–62.50 μg/mL (Zuo et al., 2008). Sanguinarine (SNG) disrupts the cytoplasmic membrane, causing cell lysis, and is effective against MRSA, with MIC values between 3.12 μg/mL and 1.56 μg/mL, and the activity range was found to be between 3.12 μg/mL and 6.25 μg/mL (Obiang-Obounou et al., 2011). Remarkably, a study confirmed that SNG, CHE, and their derivatives exhibit robust antibacterial effects against *S. aureus*, *Escherichia coli* (*E. coli*), and *Aeromonas hydrophila* (Miao et al., 2011). Another study evaluated the antimicrobial potential of the major alkaloids in *C. majus* and found that *C. majus* was most effective against *Pseudomonas aeruginosa* (MIC of 1.9 mg/L), while SNG showed effectiveness against *S. aureus* (MIC of 1.9 mg/L) (Zielińska et al., 2019). Moreover, chelidonine (CHLD), SNG, and CHE also demonstrated inhibitory effects on *E. coli* (Móricz et al., 2015). These antibacterial effects have been summarized in Table 4.

4.2 Anti-fungal effect

The alkaloids and derivatives in *C. majus* exhibit significant antifungal activity, and the mechanism of action has gradually been revealed. This provides innovative insights and approaches for the development of new antifungal agents and the control of agricultural diseases. CHE induces the accumulation of reactive oxygen species (ROS) by increasing the intracellular calcium concentration in mycelium, thus inhibiting the growth of *Candida albicans* mycelium, with MIC values ranging from 2 to 16 μg/mL (Gong

TABLE 3 Aporphine, protopine and other alkaloids compounds isolated from *Chelidonium majus* L.

No.	Compound name	Formula	Part of the plant	Ref.
Aporphine alkaloids				
77	Magnoflorine	$C_{20}H_{24}NO_4^+$	Root	Slavík and Slavíková (1977)
78	Magnocurarine	$C_{19}H_{24}NO_3^+$	Whole plant	Yang et al. (2017), Yang et al. (2024b)
79	Corydine	$C_{20}H_{23}NO_4$	Root	Shafiee and Jafarabadi (1998)
80	Isocorydine	$C_{20}H_{23}NO_4$	Root	Jeong and Lim (2017), Yang et al. (2024b)
81	Norcorydine	$C_{19}H_{21}NO_4$	Root	Shafiee and Jafarabadi (1998)
82	Corytuberine	$C_{19}H_{21}NO_4$	Aerial part	Táborská et al. (1994)
Protopine alkaloids				
83	Protopine	$C_{20}H_{19}NO_5$	Whole plant	Kadan et al. (1990), Kim et al. (1999)
84	Cryptopine	$C_{21}H_{23}NO_5$	Whole plant	Seger et al. (2004), Zhao et al. (2020)
85	α-Allocryptopine	$C_{21}H_{23}NO_5$	Root	Kaczmarek and Malek (1959), Marek et al. (1998)
86	β-Allocryptopine	$C_{21}H_{23}NO_5$	Root	Kadan et al. (1990), Marek et al. (1998)
Others alkaloids				
87	Sparteine	$C_{15}H_{26}N_2$	Seed, Root, Aerial part	Kaczmarek and Malek (1959), Kopyt'ko et al. (2005)
88	(−)-Turkiyenine	$C_{20}H_{15}NO_6$	Whole plant	Kadan et al. (1990)
89	Noroxyhydrastinine	$C_{10}H_9NO_3$	Whole plant	Yuan et al. (2022)
90	Indole-3-carboxaldehyde	C_9H_7NO	Whole plant	Yuan et al. (2022)
91	Flazin	$C_{17}H_{12}N_2O_4$	Whole plant	Yuan et al. (2022)
92	Arnottianamide	$C_{21}H_{19}NO_6$	Aerial part	Kim et al. (2015a)
93	N-Trans-feruloyltyramine	$C_{18}H_{19}NO_4$	Aerial part	Kim et al. (2015a)
94	Chelidoniumine	$C_{20}H_{15}NO_6$	Aerial part	Huang et al. (2019)

et al., 2019). Furthermore, Wei QH's experiment studied the *in vitro* antifungal activity of CHE against five rice pathogenic fungi. The results indicated that the EC_{50} values for *Ustilaginoidea virens* and *Cochliobolus miyabeanus* were 6.53×10^{-3} mg/mL and 5.62×10^{-3} mg/mL, respectively (Wei et al., 2020). These antifungal effects have been summarized in Table 4.

4.3 Anti-viral effect

The yellow latex of *C. majus* is widely used in folk medicine to treat human papillomavirus (HPV) because of its antiviral properties. Recent studies have found that its antiviral effect is mainly attributed to the alkaloid and protein components contained in the latex, which can target different stages of the virus replication cycle, effectively reducing HPV infection, and suppressing the expression of the viral oncogenes (E6, E7) at the mRNA and protein levels (Musidlak et al., 2022). Moreover, CHE can directly target the gB and gD glycoproteins on the surface of HSV-1, thereby inhibiting HSV-1 infection by preventing the binding of the virus to cells (Hu et al., 2023). In addition to its effect on human viruses, *C. majus* can also be employed for the prevention and control of agricultural plant virus es. Three alkaloids isolated from *C. majus* have shown **activity against** Tobacco mosaic virus (TMV). CHE and CHLD significantly inhibit TMV, while SNG moderately reduces TMV infection, thereby mitigating virus-induced damage in plants (Guo et al., 2021).

4.4 Analgesic effect

The alkaloids in *C. majus* exhibit significant analgesic effects on inflammatory pain, cancer-related pain, peripheral neuralgia and other types of pain, demonstrating high clinical application value. At present, *C. majus* is used as the main ingredient in clinical analgesic drugs. For example, Weitongshu capsules alleviate pain associated with gastric ulcers, and the compound Chinese medicine Tongan injection treats cancer pain caused by radiotherapy and chemotherapy or non-radiotherapy and chemotherapy. The analgesic mechanism of *C. majus* extract is different from that of morphine, indicating that it does not act as a narcotic analgesic and is devoid of side effects such as addiction, showing peripheral analgesic effects that are highly valued in clinical application (Li et al., 2013). CHE is one of the primary constituents of *C. majus*, and progress has been made in understanding its analgesic mechanism. It mitigates the occurrence of neuropathic pain by inhibiting the activation of PKC and spinal cord astrocytes (Chen et al., 2014). In addition, for chronic pain such as functional abdominal pain, a

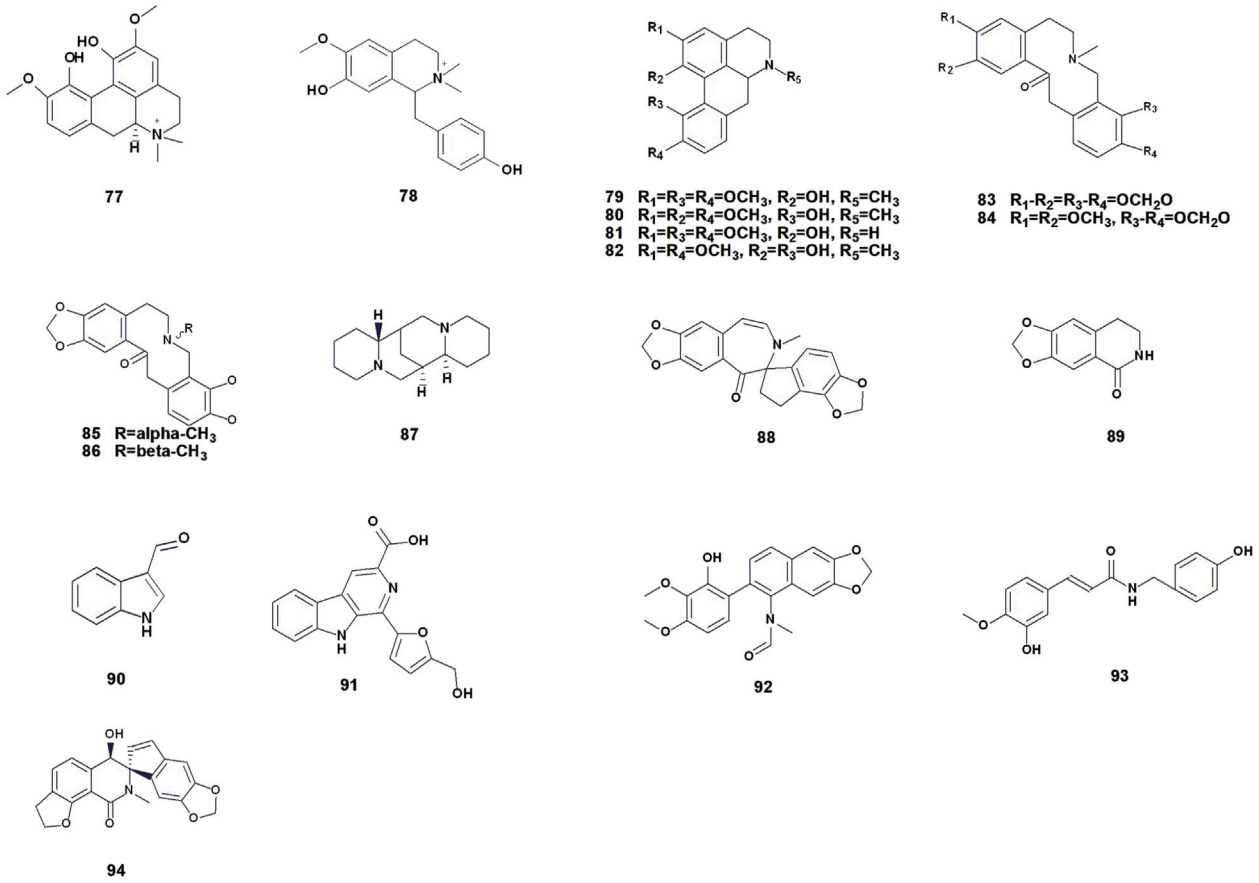

FIGURE 5
Chemical structures of aporphine, protopine and other alkaloids isolated from *Chelidonium majus* L. (The numbers in Figure 5 refer to the numbers of alkaloids present in Table 3).

combination of network pharmacology and molecular docking technology revealed that alkaloids in *C. majus* mainly induce central analgesia through a network mode of multiple target interventions, targeting SRC, AKT1, EGFR, CASP3, and MAPK3, resulting in anti-functional abdominal pain effects (Zhang et al., 2023).

4.5 Anti-inflammatory effect

Inflammation is common in clinical practice, making the study of anti-inflammatory drugs essential. CHE can inhibit the production of PGE2 by modulating COX-2, a crucial enzyme in the response to inflammation, thus exerting an anti-inflammatory effect (Niu et al., 2011). The tumor necrosis factor (TNF)-induced nuclear factor-kappa B (NF-κB) signaling pathway has been discovered in some types of inflammation, among which the TNF-α/NF-κB pathway is a well-studied typical inflammatory signaling pathway and the core of coordinating inflammatory immune response. CHE was found to mitigate the inflammatory response of lipopolysaccharide (LPS)-induced serum levels of TNF-α and NO production in mouse models of endotoxin shock (Li et al., 2012). Another study found that CHE protects against LPS-induced acute lung injury, inhibits the production of inflammatory factors such as TNF-α, IL-6, and IL-1β, and reduces pulmonary edema and neutrophil infiltration. The mechanism may be related to the inhibition of NF-κB activation and interference with the nuclear translocation of Nrf2 protein (Fan et al., 2018). CHE also significantly reduces the gastric ulcer index, inhibits NO concentration, IL-6 and TNF-α levels in serum and gastric mucosa of mice with gastric ulcers, while markedly attenuating the overexpression of NF-κB in the gastric mucosa to exert anti-inflammatory activity (Li et al., 2014). CHE is used to treat mice with acetic acid-induced ulcerative colitis, an inflammatory bowel illness, by blocking the generation of NO and TNF-α inflammatory cytokines (Xu et al., 2014; Wu et al., 2022). It has also been found that CHE can promote apoptosis and autophagy in rheumatoid arthritis by influencing the expression of genes related to autophagy and apoptosis (including Bax, Bcl-2, PARP, and ULK1) and the AMPK/mTOR/ULK-1 signaling pathway, thus inhibiting rheumatoid arthritis *in vivo* and *in vitro* (Cai et al., 2022). In addition, it regulates key signaling pathways in SARS-CoV-2 infection (including Nrf2, NF-κB, and p38 MAPK activity) to prevent excessive inflammatory immune responses (Valipour et al., 2021).

Several researchers have also isolated 6-acetonyl-5,6-dihydrosanguinarine (ADS) from *C. majus* and discovered that

TABLE 4 The anti-bacterial and anti-fungal effects of alkaloids from *Chelidonium majus* L.

Compounds	Models	Positive control	Results	Ref.
Anti-bacterial				
Chelerythrine	*Streptococcus* mutans		Exhibited inhibitory activity	Chen et al. (2011)
	Pseudomonas aeruginosa		MIC = 1.9 mg/L	Zielińska et al. (2019)
	S. aureus, *E. coli*, *Aeromonas* hydrophila	Penicillin sodium Ceftriaxone sodium	Exhibited inhibitory activity	Miao et al. (2011)
Sanguinarine	*S. aureus*, *E. coli*, *Aeromonas* hydrophila	Penicillin sodium Ceftriaxone sodium	Exhibited inhibitory activity	Miao et al. (2011)
Sanguinarine	*S. aureus*		MIC = 1.9 mg/L	Zielińska et al. (2019)
Sanguinarine	MRSA	Ampicillin Ciprofloxacin	MIC = 3.12–6.25 μg/mL	Obiang-Obounou et al. (2011)
Sanguinarine	*E. coli*		Exhibited inhibitory activity	Móricz et al. (2015)
Chelidonine	*E. coli*		Exhibited inhibitory activity	Móricz et al. (2015)
HHS	MRSA	Vancomycin	MIC/MBC = 0.49–15.63 μg/mL	Zuo et al. (2008)
HHC	MRSA	Vancomycin	MIC/MBC = 1.95–62.50 μg/mL	Zuo et al. (2008)
Anti-fungal				
Chelerythrine	*Candida* albicans	Penicillin sodium	MIC = 2–16 μg/mL	Gong et al. (2019)
	Ustilaginoidea virens		EC_{50} = 6.53 × 10^{-3} mg/mL	Wei et al. (2020)
	Cochliobolus miyabeanus		EC_{50} = 5.62 × 10^{-3} mg/mL	Wei et al. (2020)

ADS can induce the production of the inflammatory cytokines TNF-α, IL-6, and IL-8 by macrophages and dendritic cells. These inflammatory cytokines are important for the inflammatory response, and their excessive production often leads to the aggravation of inflammation and the development of diseases. ADS can trigger the release of pro-inflammatory cytokines through the ROS-JNK/ERK-NF-κB signaling pathway, thus inhibiting the occurrence of the inflammatory response (Kim et al., 2013).

Furthermore, CHLD exhibits significant anti-inflammatory actions, inhibiting LPS-induced inflammatory responses *in vitro* and *in vivo* by blocking the TLR4/NF-κB signaling pathway in RAW264.7 macrophages (Liao et al., 2018). It has been experimentally demonstrated that inhibiting TNF-induced NF-κB activation and modulating NF-κB regulatory gene products display anti-inflammatory, anti-proliferative, pro-apoptotic, and anti-invasion effects. Therefore, CHLD can alleviate the inflammatory response in HCT 116 human colon cancer cells (Zhang et al., 2018). CHLD inhibits IL-1β-mediated inflammation by modulating the NF-κB pathway *in vitro*, thereby preventing cartilage degeneration and synovial inflammation in rats with osteoarthritis (Li et al., 2023). In particular, CHLD also suppresses the production of IL-4, IL-17, eotaxin-2, and Ovalbumin-specific IgE through the STAT6 and FOXP3 pathways and can be used to treat airway inflammation (Kim et al., 2015). CHLD can prevent inflammatory damage in porcine small intestinal epithelial cell line IPEC-J2 cells by significantly reducing pro-inflammatory factors and promoting IL-10 expression (Lin et al., 2024).

In addition to the above two alkaloids, there may be other ingredients in *C. majus* with anti-inflammatory activities. These components might influence the inflammatory response through different mechanisms, thereby synergistically enhancing the anti-inflammatory effects of *C. majus*. The anti-inflammatory actions of these alkaloids were examined in macrophage RAW264.7 cells to ascertain their inhibitory impact on the production of NO caused by LPS. CHLD and HHS exhibited strong inhibitory activities on NO production in LPS-induced macrophage RAW 264.7 cells (Park et al., 2011). The impact of five alkaloids on the secretion of IL-1β, IL-8, and TNF-α in human polymorphonuclear leukocytes (neutrophils) was determined. It was found that berberine, CHLD, and CHE notably reduced TNF-α secretion in a concentration-dependent manner, while SNG inhibited IL-1β secretion and coptisine slightly decreased TNF-α, IL-1β, and IL-8 secretion (Zielińska et al., 2020). In another study, stylopine was found to inhibit macrophage NO in a concentration-dependent manner by suppressing the expression of iNOS, COX-2, NO, and PGE2, which may be related to the anti-inflammatory activities of *C. majus* (Jang et al., 2004).

4.6 Anti-cancer effect

Cancer is currently the primary cause of disease-related death in humans, and both its incidence and mortality are rising globally (Chen et al., 2024). Therefore, research and treatment of cancer are particularly urgent. Researchers both domestically and overseas have carried out a significant number of *in vitro* and *in vivo* experiments on the anti-tumor effects of *C. majus* in recent

TABLE 5 The anti-cancer effects of alkaloids from *Chelidonium majus* L.

Compounds	Models	Results	*In Vivo*/*In Vitro*	Ref.
Chelidonine	HepG2 cells	Inhibited the proliferation	*In vitro*	Noureini and Wink (2009)
	MHCC97-H cells, LM3 cells, nude mice or BalB/c mice	Inhibited the process of EMT and enhances the antitumor effect of lenvatinib on HCC cells, IC_{50} = 7.72 ± 0.70 μmol/L and 6.34 ± 0.44 μmol/L, respectively	*In vivo* and *in vitro*	Hou et al. (2019)
	H1975 cells, nude mice	Inhibited cell growth *in vitro* and *in vivo*, IC_{50} = 2.58 ± 1.05 μmol/L	*In vivo* and *in vitro*	Xie et al. (2020)
	SGC-7901 cells	Induced mitotic slippage and apoptotic-like death, IC_{50} = 23.13 μmol/L		Qu et al. (2016)
	BALB/c mice, Renca C	Effectively inhibited tumor proliferation	*In vivo*	Pan et al. (2023)
	KB cells	Inhibited proliferation, invasion and promoted apoptosis	*In vitro*	Tao and Ran (2018)
	BxPC-3, MIA PaCa-2 cells	Induced apoptosis	*In vitro*	Jang et al. (2021)
	MCF-7 cells	Strongly suppressed cell growth	*In vitro*	Noureini and Esmaili (2014)
	MDA-MB-231 cells	Inhibited migration and invasion of cells	*In vitro*	Kim et al. (2015b)
	T98G cells	Through multipolar spindle assembly causing G2/M arrest in T98G cells	*In vitro*	Lee et al. (2019)
Chelerythrine chloride	SMMC-7721 cells	Inhibited tumor growth and induced apoptosis	*In vitro*	Zhang et al. (2011b)
	MHCC97-H cells	Inhibited cell growth, invasion and migration	*In vitro*	Cheng et al. (2019)
	HEK-293 and SW-839 cells	Inhibited cell proliferation and induced apoptosis	*In vitro*	Chen et al. (2016)
Chelerythrine	HepG2 cells	IC_{50} of 6,12,24 h was 12.98, 10.53, 11.21 μmol/L, respectively	*In vitro*	Han and Zhu (2016)
	HepG2 cells	Induced apoptosis	*In vitro*	Lin et al. (2022)
	C57BL/6 mice	Inhibited tumor growth	*In vivo*	Jin and Lu (2024)
	BGC823 cells	IC_{50} of 24, 48, 72 h was 2.87, 0.903, 0.468 μg/mL, respectively	*In vitro*	Zong and Liu (2006)
	HeLa cells	Induced apoptosis	*In vitro*	Yu et al. (2000)
	SKOV3 cells	Inhibited the proliferation, migration, invasion, and EMT	*In vitro*	Zhou et al. (2024)
	HL-60 cells	Induced apoptosis and necrosis, IC_{50} = 2.6 μmol/L	*In vitro*	Vrba et al. (2008)
	B16 cells	Inhibited proliferation and induced apoptosis	*In vitro*	Zhang et al. (2018a)
	5–8 F cells	Inhibited cell proliferation and induced apoptosis	*In vitro*	Chen et al. (2024)
	HCT-116, RKO cells	Inhibited cell growth and induced apoptosis	*In vitro*	Liu and Jiang (2019)
	Zebrafish, ACC2 cells	Inhibited cell growth and proliferation and induced apoptosis	*In vivo* and *in vitro*	Li et al. (2021a)
Sanguinarine	HeLa cells	Induced apoptosis	*In vitro*	Alakkal et al. (2022)
	SKOV3 cells	Inhibited cell viability, promoted cell apoptosis and suppressed cell migration and invasion	*In vitro*	Zhang et al. (2018b)

years. Studies have indicated that alkaloids extracted and isolated from *C. majus* have significant biological activities in anti-tumor treatment, inhibiting the proliferation, migration, and invasion of tumor cells through various mechanisms. These alkaloids play an anti-cancer role in cervical cancer, lung cancer, liver cancer, and other cancers by promoting apoptosis, altering the cell cycle, inducing autophagy, and activating mitochondrial apoptosis. These anti-cancer effects have been summarized in Table 5.

4.6.1 Liver cancer

Isoquinoline alkaloids in *C. majus* have anti-telomerase activity, a key target of therapeutic intervention in cancer cells. Experiments

have demonstrated that CHLD inhibits telomerase activity in HepG2 cells by down-regulating the expression of human telomerase reverse transcriptase and inducing apoptosis (Noureini and Wink, 2009; Noureini et al., 2017). CHLD inhibits the epithelial-mesenchymal transition (EMT) process, enhances the apoptotic effect of lenvatinib on HCC cells in nude mice, and reduces the *in vivo* growth of hepatocellular carcinoma tumors (Hou et al., 2019). Another study indicated that chelerythrine chloride (CHECL) significantly inhibits SMMC-7721 cell proliferation in a time-and dose-dependent manner by blocking the S-phase of SMMC-7721 cells, and activating the mitochondrial apoptosis pathway by regulating the expression of Bcl-2 family proteins, thus inducing apoptosis in SMMC-7721 cells (Zhang et al., 2011). CHECL also inhibits growth, invasion, and migration in the highly metastatic human hepatocellular carcinoma cell line MHCC97-H (Cheng et al., 2019). Additionally, CHE upregulates the relative expression of Bax and Caspase-3 proteins and mRNA, decreases the relative expression of Bcl-X_L protein and mRNA, prevents proliferation in HepG2 hepatoma cells, and ultimately induces apoptosis (Han and Zhu, 2016). Experiments by Lin's team showed that CHE exposure induces excess ROS generation and triggers oxidative stress and mitochondrial apoptosis pathways in HepG2 cells, ultimately causing apoptosis in HepG2 cells (Lin et al., 2022).

4.6.2 Lung cancer

Lung cancer (LC) is a serious health problem that can lead to significant morbidity and mortality. In the treatment of LC, CHE can reduce tumor growth in Lewis lung cancer transplanted mice by targeting the NF-κB/HIF-1α signaling pathway and down-regulating the expression levels of NF-κB and HIF-1α proteins, thereby achieving a therapeutic effect (Jin and Lu, 2024). Clinical findings indicate that the majority of non-small cell lung cancer (NSCLC) patients exhibit mutations in the epidermal growth factor receptor tyrosine kinase (EGFR), leading to increased EGFR activity and facilitating metastasis and progression of NSCLC (Jiang et al., 2022). Targeted therapy directed at mutant forms of EGFR, such as the small molecule inhibitor Gefitinib, has shown successful application in the treatment of NSCLC patients. Clinical data supports its efficacy in significantly prolonging patient survival; however, prolonged use often leads to acquired resistance in most individuals (Bai et al., 2019). Therefore, researchers found that CHLD can inhibit the growth of Gefitinib-resistant non-small cell LC cells by modulating the EGFR-AMPK signaling pathway. They also found that CHLD mediates apoptosis through the mitochondrial pathway by decreasing the expression of AKT and Bcl-2 and increasing the cleavage of Bax and Caspase-3 expression (Xie et al., 2020).

4.6.3 Gastric cancer

Gastric cancer is a malignant neoplasm originating in the upper lining of the stomach. CHE can effectively induce apoptosis in human gastric cancer (BGC 823) cells, and this apoptosis is cell cycle-dependent (Zong and Liu, 2006). In another study, CHECL was found to inhibit cell proliferation in a time - and dose-dependent manner, causing cell cycle arrest and, apoptosis in BGC-823 cells through the reduction of mitochondrial membrane potential, release of cytochrome c, activation of caspase-3, disruption of PARP, and dysregulation of BCL-2 family proteins (Zhang et al., 2012). Additionally, CHLD can induce SFC-7901 M phase arrest and mitotic slippage in human gastric cancer cells by down-regulating the expression of BubR1, Cdk1 and cyclin B1 proteins (Qu et al., 2016).

4.6.4 Renal cell carcinoma

Renal cell carcinoma is among the ten most prevalent cancers in humans. CHLD has been found to regulate the expression of proteins Smad3 and Smad7 in the TGF-β1/Smad pathway to inhibit tumor proliferation in tumor-bearing mice with renal cell carcinoma (Pan et al., 2023). Chen et al. (2016) also demonstrated that CHECL may induce apoptosis in renal cancer cells by inhibiting ERK activity.

4.6.5 Breast cancer

Breast cancer (BC) is a disease that threatens human life and health worldwide. According to TCM, BC belongs to the category of "ru yong" and "ru shi yong," mainly caused by the deficiency of zheng qi and the imbalance of yin and yang in the viscera (Liu et al., 2021). CHLD is highly cytotoxic to cancer cells and induces MCF-7 BC cell death by potently inhibiting telomerase activity and stimulating multiple mechanisms of cell death, including apoptosis, autophagy, and senescence (Noureini and Esmaili, 2014). In addition, CHLD demonstrates anti-migration and anti-invasion effects in MDA-MB-231 BC cells by preventing the formation of the integrin-linked kinase/PINCH/α-parvin complex (Kim et al., 2015). CHE can play an anti-BC role through the PI3K/AKT signaling pathway (Zhang et al., 2021). CHLD inhibits the mitosis of BC cells by inducing M-phase arrest and blocking the AKT/FOXO3/FOXM1 axis, thus exerting anti-BC effects (Li et al., 2024). Chelidonium Herba-Corydalis Rhizoma is one of the commonly used prescriptions for BC in TCM. It can suppress the expression of ERα, p-PI3K, p-Akt protein, and ESR1 mRNA, as well as inhibit the growth of MCF-7 cells (IC_{50} value: 693 μg/mL), suggesting that its anti-ER-positive BC effect may be connected to the modulation of ER and PI3K/Akt signaling pathways (Zou et al., 2023).

4.6.6 Cervical cancer

CHE has been proven to exert anti-tumor effects by inducing apoptosis in HeLa cells through activation of the P38/JNK signaling pathway (Yu et al., 2000). Chelerythrine hydrochloride inhibits the proliferation of cervical cancer HeLa cells by triggering mitochondrial apoptosis via the PI3K/BAD signaling pathway (Yang et al., 2020). Another isoquinoline alkaloid, CHLD, has also shown effectiveness in triggering apoptosis in HeLa cells, exerting anti-cancer effects by upregulating the expression of pro-apoptotic genes such as, p38 and p53 and downregulating the expression of anti-apoptotic genes including AKT, PI3K, JAK3, STAT3, E6, and E7 (Paul et al., 2012). Protoberberine alkaloids (coptisine, berberine, and their derivatives, like stylopine) isolated from *C. majus* have demonstrated anti-cervical cancer activity by interfering with reactive oxygen species production, intracellular caspase activation, and mitochondrial function (Warowicka et al., 2019). The main latex proteins in *C. majus*, along with berberine, 8-hydroxycheleritrine, and dihydroberberine, can reduce the *in vitro* activity of human

cervical cancer cells (both HPV-negative and HPV-positive) and synergistically play an anti-cancer role (Nawrot et al., 2021). SNG has long been considered an anti-tumor drug; studies have shown that it induces death in HeLa cells by activating apoptosis and ferroptosis (Alakkal et al., 2022).

4.6.7 Ovarian cancer

Ovarian cancer, often termed the "silent killer" due to its high mortality rate, shows promising response to SNG in epithelial ovarian cancer cells by controlling the CASC2-EIF4A3 axis, blocking NF-κB signaling, or the PI3K/AKT/mTOR pathway (Zhang et al., 2018). Meanwhile, research on CHE indicates its effectiveness in inhibiting proliferation, migration, and invasion of human Ovarian cancer SKOV3 cells, preventing or alleviating the occurrence of SKOV3 cell epithelial-mesenchymal transition, and suppressing tumor metastasis (Zhou et al., 2024).

4.6.8 Leukemia

CHE and dihydrochelerythrine have been shown to arrest the cell cycle of HL-60 cells in the G1 phase, alter cell cycle distribution, and activate the mitochondrial apoptosis pathway, inducing apoptosis and necrosis in human leukemia HL-60 cells (Vrba et al., 2008). Studies have also indicated that CHE and SNG induce dose-dependent DNA damage and increased cytotoxicity in primary mouse spleen cells and mouse Lymphpocytic Leukemia L1210 cells, while CHLD does not exhibit significant cytotoxic or DNA damaging effects on these cells but can completely inhibit the growth of L1210 cells (Kaminskyy et al., 2008). Moreover, the Havelek R team confirmed significant cytotoxicity of CHLD and homochelidonine, effectively inducing leukemia cell death (Havelek et al., 2016). SNG, berberine, and *C. majus* extracts have also exhibited significant cytotoxic and pro-apoptotic activities against hematopoietic cell lines HL-60, HL-60/MX1, HL-60/MX2, CCRF/CEM and CEM/C1, J45.01, and U266B, suggesting their potential utility in treating various types of leukemia (Och et al., 2019).

4.6.9 Melanoma

Melanoma, a type of skin cancer originating from the malignant transformation of melanocytes, is concerning due to its malignancy and treatment challenges. CHE inhibits the proliferation of B16 cells in a dose- and time-dependent manner significantly increases the early and late apoptosis rates of B16 cells, and upregulates the expression levels of Caspase-3 and Bax genes, while reducing the expression levels of Bcl-2 genes. It has been demonstrated that CHE suppresses the activation of the Wnt/β-catenin signaling pathway, thereby slowing B16 cell proliferation and promoting apoptosis (Zhang et al., 2018). Experimental data have shown that uveal melanoma cells undergo necrotic cell death and apoptosis when exposed to benzophenanthine alkaloids (SNG, CHE, and CHLD) (Kemény-Beke et al., 2006). Furthermore, benzphenanthridine alkaloids, including chelilutine, CHE, and SNG, exhibit a strong antiproliferative effect on malignant melanoma cells; these alkaloids induce apoptosis by reducing levels of anti-apoptotic proteins (Bcl-xL, Mcl-1, and xIAP), leading to decreased mitochondrial membrane potential and cleavage of caspase-3 and PARP (Hammerová et al., 2011).

4.6.10 Nasopharyngeal carcinoma

SNG, in combination with 5-fluorouracil, synergistically inhibits the growth of nasopharyngeal carcinoma grafts *in vivo*, inducing autophagy and apoptosis related to the PI3K/AKT/mTOR signaling pathway (Peng et al., 2022). SNG inhibits the growth of human nasopharyngeal carcinoma 5–8 F cells, induces autophagy, and suppresses proliferation by activating AMPK/mTOR signaling (Su et al., 2022). Experiments confirm that both SNG and CHE inhibit nasopharyngeal carcinoma cell proliferation and induce apoptosis by regulating the PI3K/AKT and MAPK signaling pathways (Chen et al., 2024).

4.6.11 Others

CHLD increases the growth of the human oral epithelioid cancer cell line KB in a time- and dose-dependent manner, and inhibits KB cells invasion in a dose-dependent manner. It upregulates Bax expression and decreases Bcl-2 expression, activating Caspase-3 and inducing KB cell apoptosis. This effect may be mediated through dual inhibition of Akt and MAPK signaling pathways, inhibiting Bcl-2 expression and promoting Caspase-3 expression, thereby suppressing cancer cell proliferation, fostering apoptosis, and impeding tumor growth and metastasis (Tao and Ran, 2018). Moreover, CHLD induces G2/M phase block in BxPC-3 and MIA PaCa-2 cells by downregulating CDK 1, and increases S-phase block induced by GADD 45a by upregulating p21 and p53, culminating in pancreatic cancer cell apoptosis through Caspase-3 cleavage (Jang et al., 2021). Dihydrosanguinarine exhibits inhibitory effects on K-Ras and TP53 mutant pancreatic cancer cell lines by bidirectionally modulating mut-p53/-Ras and WT-p53/-Ras proteins (Wu et al., 2019). CHLD induces apoptosis in human glioblastoma cells through G2/M phase arrest and Mcl-1 degradation (Lee et al., 2019). CHE triggers apoptosis by activating ROS-mediated mitochondrial dysfunction in colorectal cancer cells (Liu and Jiang, 2019). CHE effectively inhibits the growth and proliferation of adenoid cystic carcinoma cells and induces apoptosis by increasing ROS levels and upregulating NF-κB, p-JNK and p-p38 expression in cells (Li et al., 2021).

4.7 Anti-hepatic fibrosis effect

The mRNA expression of TGF-1, Smad3, Smad4, and the negative regulator Smad7 varied significantly following different doses of CHE in the mouse model of carbon tetrachloride-induced hepatic fibrosis, suggesting that CHE could inhibit the signaling of the TGF-β receptor complex from the cytoplasm to the nucleus. Additionally, CHE interfered with the expression of TGF-β1, Smad4, and Smad7 proteins, further confirming its inhibitory effect on hepatic fibrosis in mice, associated with the TGF-β/Smads signaling pathway (Li et al., 2018). After administering different doses of CHLD to rats with carbon tetrachloride-induced hepatic fibrosis, the protein phosphorylation levels of PI3K, Akt, mTOR, and mRNA expression levels of corresponding genes in their tissues were increased to varying degrees. This indicates that CHLD can regulate the mRNA and protein expression of genes related to the PI3K/Akt/mTOR pathway, affecting the expression of autophagy marker proteins LC3 and p62, preventing the activation of hepatic stellate cells, and inhibiting liver

fibrosis (Li et al., 2021). Using TGF-β1-activated rat hepatic stellate cells CFSC-8B as a model of hepatic fibrosis, it was observed that CHLD inhibited the proliferation of TGF-β1-activated hepatic stellate cells, further demonstrating its potential to reverse liver fibrosis (Li et al., 2019).

4.8 Anti-alzheimer effect

Alzheimer's disease (AD) is a progressive neurodegenerative disease with an insidious onset that causes neuronal damage in the brain, leading to memory loss, cognitive decline, and behavioral changes. Acetylcholinesterase plays a critical role in nerve function, as it is a serine protease produced by motor neurons and muscle junctions that hydrolyzes the neurotransmitter acetylcholine into acetic acid and choline, thereby terminating nerve impulses. Decreased levels of acetylcholine are a key factor in the onset of AD. Two compounds, HHC and HHS, isolated from *C. majus*, exhibit strong inhibitory activity against acetylcholinesterase. They slow down the breakdown of acetylcholine and increase its levels in the brain. Therefore, these compounds have been suggested as potential alternatives to anti-dementia drugs (Cho et al., 2006).

4.9 Anthelmintic effect

Chelidonium majus not only holds medicinal value, but also serves as a pesticide. Plant-derived alkaloid insecticides are crucial components of plant pesticides, showing significant repellent and insecticidal activities against numerous pests. CHLD has been found to possess substantial anthelmintic activity against Dactylogyrus intermedius, achieving a 100% anthelmintic effect at 0.9 mg L^{-1}. The EC_{50} value after 48 h of exposure (the concentration required to achieve a 50% deworming effect) was 0.48 mg L^{-1} (Yao et al., 2011). The insecticidal mechanisms of alkaloids in *C. majus* are diverse, contributing to larvicidal effects by disrupting enzyme activity, reducing food intake, affecting nutritional indexes, and downregulating mRNA expression of enzyme genes (Zou et al., 2017). In another study, alkaloids in *C. majus* induced resistance to dietary intake and larval mortality in *Lymantria dispar* by inhibiting food intake and digestive enzymes (Zou et al., 2019).

4.10 Other effects

In the isolated ileal spasmolytic test model of guinea pigs, CHLD and protopine, when their concentration reaches 1×10^{-5} g/mL, can significantly induce ileal relaxation in response to barium chloride stimulation, with relaxation rates reaching 68.8% and 54.8%, respectively (Hiller et al., 1998). The DPPH free radical scavenging rate was determined using the DPPH method, with ascorbic acid as a positive control to evaluate the antioxidative activity of CHE solid dispersion (SD). The results showed that different mass concentrations of CHE-PEG-SD exhibited certain DPPH radical scavenging ability (IC_{50} = 0.124 mg/mL), albeit weaker than ascorbic acid (IC_{50} = 0.041 mg/mL) (Wang et al., 2020). Traditional medicine traditions utilize natural small-molecule compounds from *C. majus* to treat various gynecological disorders, including coptisine, protopine, berberine, and dihydroberberine (Lans et al., 2018). Supplementation with the protoberberine-rich fraction from *C. majus* in the diet of rats with experimentally induced endometriosis has been shown to inhibit the recurrence of endometriosis (Warowicka et al., 2021).

5 Toxicology

The utilization of plants and herbs in traditional medicine has a long history, with many herbs used to treat various ailments. However, it's crucial to remember that not all herbs are harmless. *Chelidonium majus* was recorded as a toxic Chinese medicine in the Chinese Pharmacopoeia of the People's Republic of China (2020 edition). The alkaloids found in *C. majus*, including CHLD, SNG, berberine, coptisine, and CHE, either alone or in combination, possess potential toxicity (Benninger et al., 1999). Following evaluation using liver-targeted causality assessment methods, several cases of hepatotoxicity were found to be likely or highly likely related to *C. majus*. Therefore, the toxicity of *C. majus* to liver function has always been a topic of concern, with some components potentially causing liver damage through different mechanisms. The potential hepatotoxicity of *C. majus* has also been confirmed in several reports from European countries, defining this hepatotoxicity as a unique form of herbal-induced liver injury (HILI) due to idiosyncratic metabolic reactions (Pantano et al., 2017). Ciornolutchii et al. reported two case studies in which patients exhibited liver damage after using pharmaceutical preparations containing *C. majus*, characterized by severe hypertransaminasemia. Their literature review also identified multiple HILI cases related to *C. majus* preparations (Ciornolutchii et al., 2024). The metabolism of CHLD was studied in a human liver microsomal model, revealing two demethylated metabolites containing phenolic hydroxyl groups after incubation with liver microsomes. These hydroxyl groups are easily oxidized into quinone compounds, which can combine with glutathione to form quinone-sulfide, depleting glutathione in the liver and causing hepatotoxicity (Zhang et al., 2018).

Alkaloids, in *C. majus*, such as CHE, SNG, berberine and coptisine, have significant inhibitory effects on mitochondrial respiration in mice, suppressing liver respiration by inhibiting mitochondrial enzymes like NADH dehydrogenase or succinic acid (Barreto et al., 2003). Furthermore, SNG induces chromosome breaks and DNA damage in mouse bone marrow cells (Das et al., 2004). Additionally, CHLD, berberine, and SNG from *C. majus* distinctly block hERG potassium channels, delaying heart repolarization and prolonging QT intervals, potentially increasing the risk of death (Orvos et al., 2015). Chronic exposure to *C. majus* can also cause toxic effects in specific organs; CHE has been observed to induce a dose-dependent long-term toxicity in rat lung tissue, resulting in symptoms of pulmonary congestion and bloody ascites (Liu et al., 2019). Despite *C. majus* having abundant pharmacological effects, caution should be exercised to avoid excessive or prolonged use to prevent toxic reactions.

Chinese herbal medicines can be processed to reduce or eliminate drug toxicity and side effects, while also modifying

drug function and flavor, thereby improving therapeutic efficacy. Researchers have processed *C. majus* and discovered that the content of SNG and CHE in licorice products derived from *C. majus* is significantly lower than in raw products. The optimal processing method involves using 15% licorice, moistening for 3 h, and drying at 60°C for 12 h. This suggests that licorice processing technology has a certain detoxification effect (Xiao et al., 2021). In conclusion, although *C. majus* possesses numerous pharmacological effects and holds certain application value in the pharmaceutical field, its potential toxicity risks must also be given sufficient attention. A deeper understanding of its efficacy and safety through scientific research is essential, and it should be used cautiously, with a full understanding of its potential risks to prevent toxic reactions from excessive or long-term use.

6 Discussion

This plant has been revered for its antibacterial, antiviral, antitumor, anti-inflammatory, and other pharmacological effects for centuries. Alkaloids from C. majus, particularly benzophenanthridine and protoberberine alkaloids, show promising potential for application in anti-tumor therapy (Yang et al., 2024). Compounds such as CHE, CHLD, and SNG, known for their high content and notable anti-tumor activity, have been extensively studied. The alkaloids exert anti-tumor effects by promoting apoptosis, altering the cell cycle, inducing autophagy, and activating mitochondrial apoptosis. Even alkaloids present in small amounts, such as berberine (Mohammadlou et al., 2021), dihydrochelerythrine (Silva et al., 2018), 6-methoxydihydrosanguinarine (Wang et al., 2023), and berberrubine (He et al., 2023), demonstrate significant potential in tumor treatment. UkrainTM, a derivative of C. majus alkaloids comprising components like CHLD, CHE, SNG, protopine, and allocryptopine, induces apoptosis in cells and exerts toxic effects on cancer cells (Habermehl et al., 2006). However, the exact anti-tumor mechanisms of C. majus alkaloids remain unclear, necessitating further in-depth studies to elucidate their mechanisms of action.

Chelidonium majus alkaloids not only exhibit anti-tumor effects but also demonstrate significant antimicrobial effects. These alkaloid components are applied to human diseases and also play a role in preventing and controlling on plant diseases as well. Simultaneously, these alkaloids exert anti-inflammatory effects and treat various inflammations by regulating the immune system and various inflammation-related signaling pathways. As research on the pharmacological effects of C. majus alkaloids progresses, there is potential for further expanding their clinical applications. Analysis of other alkaloids with lower content has revealed that aporphine alkaloids, such as magnoflorine, may reduce blood glucose levels by promoting insulin release and stimulating insulin activity mechanisms, potentially improving postprandial hyperglycemia and demonstrating anti-diabetic effects (Patel and Mishra, 2011). Vennerstrom et al. discovered that protoberberine alkaloids showed potential antimalarial activity *in vitro* experiments (Vennerstrom and Klayman, 1988), while Xiang et al. explored the potential uses of these alkaloids in treating stomach diseases and providing gastric protective effects (Xiang et al., 2024). Berberine has also been noted to prevent and delay AD (Wang et al., 2024), while protopine may hold a potential role in asthma treatment (Yang et al., 2024). However, studies on these effects are limited and mostly at a preliminary stage. The results of pharmacological activity studies are mostly based on animal experiments, and the results of clinical studies may differ; further research is needed to verify their effects and mechanisms. Additionally, the synergistic effects between C. majus alkaloids and other drugs or ingredients cannot be overlooked and warrant further exploration.

The rich chemical composition and extensive biological activity of C. majus alkaloids provide valuable resources for the development of pharmaceutical drugs. However, there still exist limitations in the extraction process, content determination, and the relationship between structure and activity. Future research efforts should focus on optimizing extraction processes, improving the purity of bioactive alkaloids, delving into the relationship between structure and activity, elucidating the underlying mechanisms of action, and facilitating their practical application in the field of medicine.

7 Conclusions and perspectives

Chelidonium majus, a medicinal plant with a long history of application, has been extensively used in European countries as well as in China's TCM, highlighting its extremely high medicinal value. Alkaloids are the main active components of the plant, which have garnered attention from many scholars. These alkaloids are abundant in content and variety, but each alkaloid exhibits unique chemical structures and can act on multiple biological targets to play a therapeutic role in diseases. This makes *C. majus* a vast potential area for research and development in drug research.

To date, researchers both domestically and internationally have isolated and identified 94 alkaloids from *C. majus*. This review therefore summarizes the research findings on the phytochemistry, pharmacology, and toxicology of these alkaloids. Additionally, it systematically classifies these alkaloids according to their structural properties, providing a foundational basis for the phytochemical classification of *C. majus*.

Through further in-depth study of these alkaloids, more candidate compounds can be provided for drug research and development, expanding their potential applications in the pharmaceutical field. However, it is worth noting that these alkaloids also have some toxicity. Therefore, in the application of *C. majus* for drug development or treatment of diseases, it is necessary to further explore the toxic mechanisms of these alkaloids to optimize the extraction process, reduce toxicity, and improve efficacy, thereby laying a solid foundation for their broad application in the pharmaceutical field. Simultaneously, strengthening the quality control and safety evaluation of *C. majus* is also crucial for future research directions, to effectively manage potential risks during drug development and ensure the safety and effectiveness of drugs. In summary, this review provides a basis for understanding the current research status of alkaloid components in *C. majus*. As science and technology advance and research methods improve, more comprehensive studies on *C. majus* alkaloids are expected, aiming to develop clinically valuable drugs and provide a solid theoretical framework for the in-depth research and application of *C. majus*.

Author contributions

X-LL: Writing–original draft. Y-PS: Writing–original draft. MW: Writing–review and editing. Z-BW: Writing–review and editing. H-XK: Writing–review and editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. The work was supported by Heilongjiang Provincial Key Research Plan (Grant No. GA22B012), the Excellent Innovative Talents Support Program of Heilongjiang University of Chinese Medicine (No. 2018RCD03), the Heilongjiang Province Education Department project (No. 12511512), the Heilongjiang Postdoctoral Foundation (No LBH-Z10021), the Heilongjiang Touyan Innovation Team Program (Grant No: [2019] No. 5), the National Famous Old Traditional Chinese Medicine Experts Inheritance Studio Construction Program of National Administration of TCM (Grant Number: (2022) No. 75), the Seventh Batch of National Famous Old Traditional Chinese Medicine Experts Experience Heritage Construction Program of National Administration of TCM (Grant Number: (2022) No. 76).

Acknowledgments

We would like to thank Editage (http://www.editage.cn) for English language editing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 18 September 2024
DOI 10.3389/fphar.2024.1450733

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OPEN ACCESS

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RECEIVED 18 June 2024
ACCEPTED 29 July 2024
PUBLISHED 18 September 2024

CITATION
Kuang H, Kong L, Hou A, Yang A and Jiang H (2024) A review of the botany, metabolites, pharmacology, toxicity, industrial applications, and processing of Polygalae Radix: the "key medicine for nourishing life". *Front. Pharmacol.* 15:1450733. doi: 10.3389/fphar.2024.1450733

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A review of the botany, metabolites, pharmacology, toxicity, industrial applications, and processing of Polygalae Radix: the "key medicine for nourishing life"

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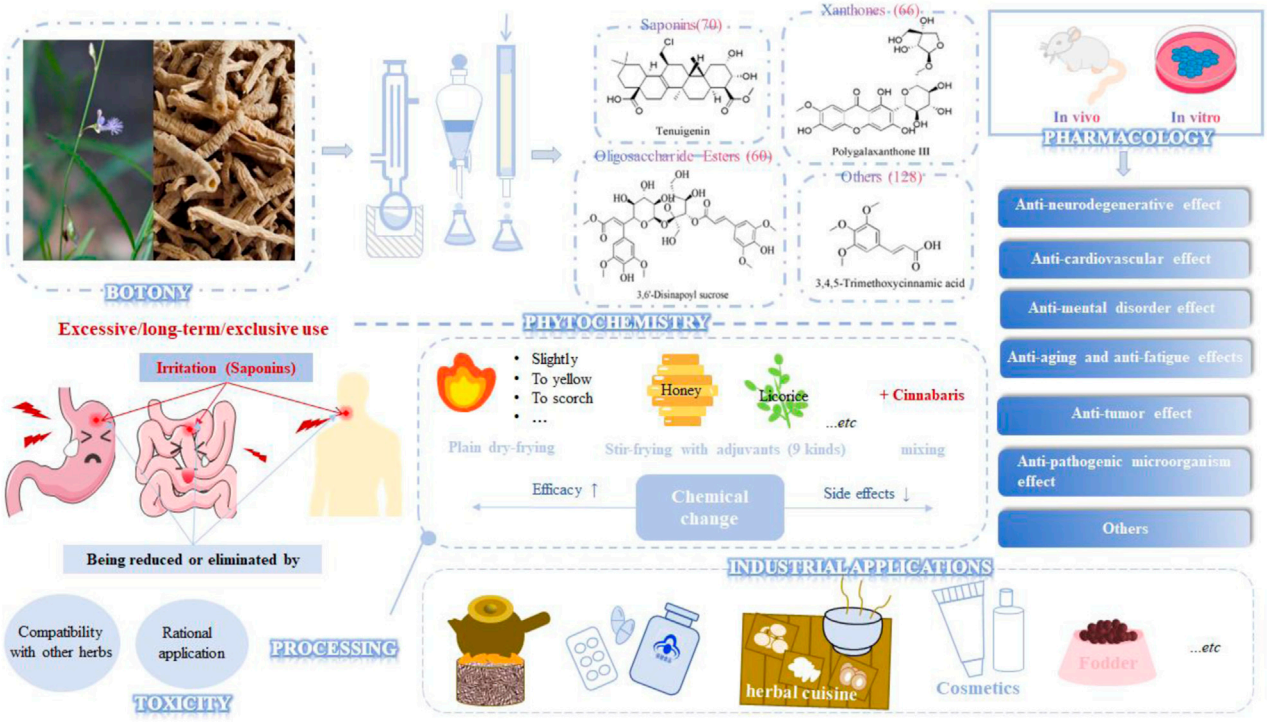
Polygalae radix (PR) is the dried root of *Polygala tenuifolia* Willd. and *Polygala sibirica* L. and enjoys the reputation as the "key medicine for nourishing life." In this study, information about "*Polygala tenuifolia* Willd.," "*Polygala sibirica* L.," and "Yuanzhi" was retrieved from scientific databases, including Google Scholar, Baidu Scholar, Web of Science, PubMed, CNKI, and Wan Fang Data. Information from Chinese herbal medicine classics, Yaozhi Data, and the Gaide Chemical Network was also collected. Information related to botany, phytochemistry, pharmacology, toxicity, industrial applications, and processing is summarized in this paper to tap its potentialities and promote its further development and clinical application. More than 320 metabolites have been isolated from PR; saponins, xanthones, and oligosaccharide esters are the main functional metabolites. Pharmacological research shows that its pharmacological action mainly focuses on resisting nervous system diseases, and it also has the functions of anti-oxidation, anti-inflammation, anti-tumor, anti-pathogenic microorganisms and others. The gastrointestinal irritation of its saponins impeded its application, but this irritation can be reduced by controlling the dosage, compatibility with other herbs, or processing. The future progress of PR faces opportunities and challenges. More attention should be paid to the traditional application and processing methods of PR recorded in ancient books. The lack of safety and clinical studies has limited its application and transformation of achievements. Moreover, it is one-sided to take the content of only a few metabolites as the index of processing optimization and quality control, which cannot reflect the full pharmacological and toxicological activities of PR.

KEYWORDS

Polygalae Radix, pharmacological effects, metabolites, applications, toxicity, processing



GRAPHICAL ABSTRACT

Introduction

Polygalae Radix (PR), the dried root of *Polygala tenuifolia* Willd. or *Polygala sibirica* L., is mainly distributed in China, South Korea, Mongolia, and Russia (Jiang et al., 2016). PR is widely used in Japanese Kampo medicine (Tsukada et al., 2021), traditional Korean medicine (Kumar et al., 2013), traditional Chinese medicine (TCM), and Mongolian medicine as a tonic, expectorant, sedative, and antasthmatic. It is known as "Onji" in Japan (Kuroda et al., 2014) and "Wonji" in South Korea, and these terms refer only to the root or root bark of *Polygala tenuifolia* Willd. In China, PR is called "Yuanzhi" and ranks as the third most frequently used single herb in the clinical application of cognitive enhancement prescriptions (Peng et al., 2020a). According to the 2020 Chinese Pharmacopoeia, PR is usually harvested in spring and autumn, the fibrous root and silt are removed, and the product is dried directly or after the wood heart is removed. It possesses bitterness and pungency in flavor and warmth in nature, enters the heart and lung meridian, and holds the effects of calming the nerves and increasing intelligence, coordinating the heart and kidney, eliminating phlegm, and relieving swelling. It is usually used for treating insomnia and dreamful sleep, amnesia, palpitation, and obnubilation caused by heart-kidney imbalance, as well as ungratifying coughing of phlegm, swelling and sores, and breast pain. Other traditional applications of PR are shown in Supplementary Material.

According to Yaozhi Data, more than 800 prescriptions contain PR in diverse dosage forms, especially decoctions, pills, and powders. In 2018, three classic prescriptions containing PR, Kai-Xin-San, Di-Huang-Yin-Zi, and Gu-Yin-Jian, were included in the *Catalogue of Ancient Classic Prescriptions* (the First Batch) by the state administration of TCM. PR is praised as "the key medicine for nourishing life" by Shennong's *Herbal Classic*. As a homologous plant of medicine and food, it shows great health-preserving potential in the fields of healthcare products and medicated diets. Owing to the continuous discovery of pharmacological activities, its application scope in the daily chemical industry tends to deepen and expand, giving it increasing commercial value.

More than 320 metabolites have been isolated from PR, including its main active ingredients—saponins, xanthones, and oligosaccharide esters, as well as other metabolites such as alkaloids, coumarins, lignins, and flavonoids (Wang et al., 2020a). Abundant research on the pharmacological activities of PR mainly focuses on its anti-neurological disease effects (Zhao et al., 2020) such as neurodegenerative diseases (NDD, such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), aging, learning, and memory impairment, spinal cord injury), cardio-cerebrovascular diseases (such as hypoxia-reoxygenation injury, cerebral ischemia-reperfusion, myocardial ischemia, cerebral hemorrhage, arrhythmia) and mental disorders (such as depression, insomnia, anxiety, post-traumatic stress disorder, social disorder). In addition, it has antioxidant, anti-inflammatory, anti-fatigue, anti-tumor, anti-pathogenic microorganisms and other functions (Zheng et al., 2016; Lei et al., 2020; Yu, 2020; Huang and Lin, 2021; Wang et al., 2021a; Zeng et al., 2021a; Gao, 2022; Yang et al., 2022). According to literature reports, it also has certain biological activities in eliminating phlegm, relieving cough, killing sperm, lowering blood pressure, resisting diabetes, contracting the uterus, protecting the stomach, resisting bone loss, obesity, resisting fatty liver, promoting blood coagulation, and resisting radiation.

FIGURE 1
Root **(A)**, aerial part **(B)**, and medicinal pieces **(C)** of *Polygalae radix*.

A comprehensive review is necessary to deepen the understanding of PR, facilitate further study, and guide its rational application. In this study, we reviewed PR from the aspects of botany, phytochemistry, pharmacology, toxicology, industrial applications, and processing (*Graphic Abstract*). In addition, the limitations to current research and the possible directions of future research were also discussed. This review is of great significance for propelling the deep ongoing research and expanding the application scope of PR.

Botany

Morphological feature

PR (Figure 1) is the dried root of two perennials, *Polygala tenuifolia* Willd. and *Polygala sibirica* L. According to the Higher Plants of China (中国高等植物) and Flora of China (中国植物志), their botanical characteristics are shown in Supplementary Table 1. Saponins, PR's main effective metabolite, mostly accumulate in its roots, stems, and leaves, with root bark having dozens of times higher content than xylem and aerial parts (Teng et al., 2009a). The root or root bark of PR is often used medicinally, and aerial parts are used in a few areas and are mainly discarded. The saponin content begins to increase at the flowering stage and peaks at the fruiting stage. The plant should be harvested in April and May, and the total saponin yield of a three-year-old PR is the highest (Teng et al., 2009b).

According to the *Commercial Grades for Traditional Chinese Medicine* distributed by the Chinese Traditional Medicine Association, the medicinal pieces of PR can be generally divided into three specifications: "Yuanzhi tong," "Yuanzhi rou," and "Yuanzhi gun." "Yuanzhi tong" refers to the root bark of the thicker PR with a hollow cylinder shape, which has been loosened by kneading or other means, and its wood heart has been removed. According to the different removal rates of wood core and diameters, the "Yuanzhi tong" is subdivided into five grades, in which the diameter and removal rate of the wood core of the super ones exceed 0.4 cm and 95%, respectively, and the removal rate of the wood core of the lowest grade is ≥ 80%. "Yuanzhirou" is the ruptured root bark of narrower PR that has been smashed with a wood stick and had its wood heart removed. PR, which is too slender to remove the wood heart, is called "Yuanzhigun." Among them, "Yuanzhitong" is considered to be the main type with superior quality; it is more expensive and is the opposite of "Yuanzhigun."

Note that counterfeit products are rampant in the market; they are cheaper and similar in appearance to PR but are very different in flavor and efficacy. These adulterants not only degrade the quality of PR but also compromise the efficacy and safety of related compositions and formulations (Li et al., 2019). To distinguish PR from its adulterated products, we summarized common adulterants and their characteristics in Supplementary Table 2.

Plant resources

PR is mainly distributed in the temperate regions of East Asia and Europe (Figure 2), such as China, South Korea, Mongolia, the Korean Peninsula, and the Russian Federation. In China, Heilongjiang, Jilin, Liaoning, Inner Mongolia, Shanxi, Hebei, Shandong, Henan, Anhui, Jiangsu, Jiangxi, Hubei, Hunan, Sichuan, Qinghai, Gansu, Ningxia,

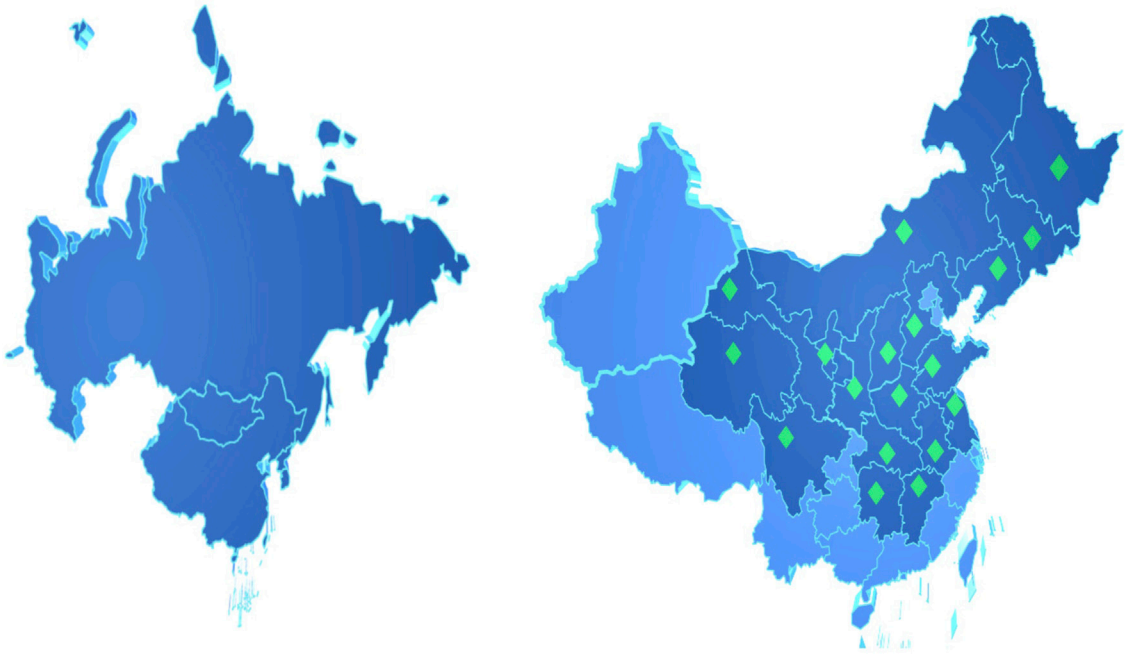


FIGURE 2
Geographical distribution of *Polygalae radix* in the world (left) and in China (right).

and Shaanxi are all its producing areas. Shanxi and Shaanxi have the largest yield (Fan et al., 2020). On 2 April 2022, PR was included in the Catalogue of Genuine Medicinal Materials in Heilongjiang Province. Although it is one of the bulk medicinal materials and 85 traditional export herbs, the slow growth rate and finite production of wild PR cannot meet the huge market demands. As a result of over-exploitation, vegetation destruction, and global warming, wild PR resources are increasingly scarce (Liu et al., 2021). The relevant departments of the Chinese government have brought PR into the national third-class protected wild plants list and issued a policy to prohibit its harvest and trafficking. Apart from setting up nature reserves, improving the *in-situ* protection system, taking *ex situ* protection, and *in vitro* preservation when necessary, it is also a feasible measure to study the suitable growth conditions of PR and replace wild products with cultivated products (Peng et al., 2018; Deng and Jin, 2020). PR is drought-tolerant and likes cool temperatures, which are common on hillsides facing the sun, forest edges, roadsides, and ridges. PR is favorable to be grown where the warmest seasonal and annual precipitation range from 148 mm to 512 mm and 300 mm to 500 mm, respectively, the annual average temperature is between 8.4°C and 15.4°C, the total solar radiation is 502.42~586.15 J/cm, and the altitude is between 100 m and 2,000 m.

Metabolites

As early as 1947, researchers from Shanghai, China, isolated an amorphous acidic saponin from PR, which was hydrolyzed to obtain Tenuigenin A and B (CHOU et al., 1947). In 1964, two kinds of sugar, 5-anhydro-D-sorbitol and N-acetyl-D-glucosamine, were isolated from PR (Takiura and Honda, 1964). In 1977, 3,4,5-trimethoxycinnamic acid (TMCA), 1,2,3,7-tetramethoxyxanthone, 1,2,3,6,7-pentamethoxyxanthone, and 6-hydroxy-1,2,3,7-tetramethoxyxanthone were isolated from PR (Ito et al., 1977). At present, more than 320 metabolites have been isolated and identified from PR, including saponins, xanthones, oligosaccharides, and other metabolites. In this section, we summarize these metabolites and list them in Tables 1–3, and their structures can be seen in Figures 3–7.

Saponins

Saponins are the most important active metabolites of PR and are associated with a wide range of potent pharmacological effects. To date, 70 saponins with oleanane-type pentacyclic triterpene saponins as the parent nucleus, usually linked to glucose, rhamnose, lactose, xylose, galactose, and other sugar types, have been isolated from PR (As shown in Table 1; Figure 3) (Chen and Zhang, 2019). Saponins are unstable and can easily decompose into senegenin, polygalacic acid, tenuifolin, and polygalaxanthone III (PX3) when meeting acid (Gao et al., 2022), possibly explaining the few reports of the monomer. Modern research proves that PR saponins have multiple pharmacological effects, including enhancing immunity, protecting vision, anti-tumor, anti-cardiovascular, and anti-AD (Gao et al., 2022). TLC identification and content determination based on tenuifolin are important methods for the quality control of PR.

Oligosaccharide esters

Oligosaccharide esters in PR are a class of metabolites mainly based on sucrose, glucose, or other trisaccharide small molecules as

TABLE 1 Saponins isolated from PR.

No.	Compound	Parent nucleus	R							Molecular formula	Ref.
			R_1	R_2	R_3	R_4	R_5	R_6	R_7		
1	Onjisaponin A	A	Gal	H	Api	Rha	a	CH_3	CH_2OH	$C_{80}H_{120}O_{39}$	Sakuma and Shoji (1982)
2	Onjisaponin E	A	Gal	H	H	H	b	CH_3	CH_2OH	$C_{71}H_{106}O_{33}$	Sakuma and Shoji (1982)
3	Onjisaponin F	A	H	Ara	Api	H	b	CH_3	CH_2OH	$C_{75}H_{112}O_{36}$	Sakuma and Shoji (1981)
4	Onjisaponin G	A	H	H	Api	H	b	CH_3	CH_2OH	$C_{70}H_{104}O_{32}$	Sakuma and Shoji (1981)
5	Onjisaponin B	A	Gal	H	H	Rha	a	CH_3	CH_2OH	$C_{75}H_{112}O_{35}$	Li (2008), Sakuma and Shoji (1982)
6	Onjisaponin D	A	H	H	Api	H	a	CH_3	CH_2OH	$C_{57}H_{92}O_{27}$	Li (2008)
7	Onjisaponin J	A	H	Ara	e	Rha	a	COOH	CH_3	$C_{85}H_{126}O_{42}$	Li, 2008; Liu (2004)
8	Onjisaponin L	A	Gal	H	e	Rha	a	COOH	CH_3	$C_{86}H_{128}O_{43}$	Li, 2008; Liu (2004)
9	Onjisaponin O	A	Gal	H	H	Rha	b	COOH	CH_3	$C_{77}H_{116}O_{37}$	Li, 2008; Liu (2004)
10	Onjisaponin R	A	Gal	H	Api	H	b	COOH	CH_3	$C_{76}H_{114}O_{37}$	Li, 2008; Liu (2004)
11	Onjisaponin S	A	H	Ara	Api	H	b	COOH	CH_3	$C_{81}H_{122}O_{40}$	Li, 2008; Liu (2004)
12	Onjisaponin T	A	H	Ara	Api	Glc6d	b	COOH	CH_3	$C_{83}H_{124}O_{42}$	Li, 2008; Liu (2004)
13	Onjisaponin V	A	Gal	H	e	b	H	COOH	CH_3	$C_{82}H_{122}O_{41}$	Li, 2008; Li et al. (2008a)
14	Onjisaponin W	A	HM	Ara	e	b	H	COOH	CH_3	$C_{81}H_{120}O_{40}$	Li, 2008; Li et al. (2008b)
15	Onjisaponin X	A	H	Gal	e	Gal	b	COOH	CH_3	$C_{87}H_{130}O_{45}$	Li, 2008; Li et al. (2008a)
16	Onjisaponin Y	A	H	H	H	Rha	a	COOH	CH_3	$C_{69}H_{102}O_{30}$	Li, 2008; Li et al. (2008b)
17	Onjisaponin Z	A	H	H	H	Rha	b	COOH	CH_3	$C_{71}H_{106}O_2$	Li, 2008; Li et al. (2008a)
18	Onjisaponin Vg	A	Gal	H	e	H	b	COOH	CH_3	$C_{82}H_{122}O_{41}$	Li, 2008; Li et al. (2008b)
19	Onjisaponin Wg	A	H	Ara	Api	b	H	COOH	CH_3	$C_{75}H_{122}O_{36}$	Liu et al. (2007)
20	Onjisaponin Pg	A	Gal	H	e	H	H	CH_3	CH_2OH	$C_{76}H_{120}O_{41}$	Liu et al. (2007)
21	Onjisaponin Gg	A	H	H	e	H	b	CH_3	CH_2OH	$C_{76}H_{112}O_{36}$	Liu et al. (2007)
22	Onjisaponin Fg	A	H	Ara	e	H	b	CH_3	CH_2OH	$C_{81}H_{120}O_{40}$	Liu et al. (2007)
23	Onjisaponin Qg	A	Gal	H	e	Rha	H	CH_3	CH_2OH	$C_{70}H_{110}O_{37}$	Liu et al. (2007)
24	Onjisaponin Ng	A	H	H	e	Rha	a	CH_3	CH_2OH	$C_{80}H_{118}O_{38}$	Liu et al. (2007)
25	Onjisaponin Sg	A	H	Ara	e	Rha	b	CH_3	CH_2OH	$C_{87}H_{130}O_{44}$	Liu et al. (2007)
26	Onjisaponin Ug	A	H	Ara	e	Glc	b	CH_3	CH_2OH	$C_{87}H_{130}O_{45}$	Liu et al. (2007)
27	Onjisaponin Tg	A	H	Ara	e	Glc6d	b	CH_3	CH_2OH	$C_{89}H_{132}O_{46}$	Liu et al. (2007)
28	Denegins III	A	Gal	H	H	Rha	a	CH_3	CH_2OH	$C_{75}H_{112}O_{35}$	Li et al., 2022a; Liu (2004)
29	Z-senegin III	A	Gal	H	H	Rha	f	CH_3	CH_2OH	$C_{75}H_{112}O_{35}$	Li et al., 2022b; Liu (2004)
30	Z-senegin IV	A	Gal	H	Api	Rha	f	CH_3	CH_2OH	$C_{80}H_{120}O_{39}$	Li et al., 2022a; Liu (2004)

(Continued on following page)

TABLE 1 (*Continued*) Saponins isolated from PR.

No.	Compound	Parent nucleus	R							Molecular formula	Ref.
			R_1	R_2	R_3	R_4	R_5	R_6	R_7		
31	(Z)-onjisaponin L	A	Gal	H	e	Rha	f	CH_3	CH_2OH	$C_{86}H_{128}O_{43}$	Li et al., 2022b; Liu (2004)
32	(Z)-onjisaponin J	A	H	Ara	e	Rha	f	CH_3	CH_2OH	$C_{85}H_{126}O_{42}$	Li et al., 2022a; Liu (2004)
33	E-onjisaponin H	A	H	H	Api	Rha	a	CH_3	CH_2OH	$C_{74}H_{110}O_{34}$	Li et al. (2006)
34	Z-onjisaponin	A	H	H	Api	Rha	f	CH_3	CH_2OH	$C_{74}H_{110}O_{34}$	Li et al. (2006)
35	Sibiricasaponin E	A	Glc	H	H	Api	$COCH_3$	CH_3	CH_3	$C_{66}H_{104}O_{33}$	Song et al. (2013a)
36	Tenuifolisaponin A	A	Gal6Oy	H	Api	Rha	a	CH_3	CH_2OH	$C_{85}H_{126}O_{42}$	Sun (2005)
37	Tenuifolisaponin B	A	Gal6Oh	H	Api	Rha	a	CH_3	CH_2OH	$C_{86}H_{130}O_{41}$	Sun (2005)
38	Polygalasaponin XXVIII	A	H	H	H	H	H	CH_3	CH_2OH	$C_{53}H_{84}O_{24}$	Sun, 2005; Zhang et al. (1996)
39	Polygalasaponin XXIX	A	Api-Gal	H	H	H	H	CH_3	CH_2OH	$C_{64}H_{102}O_{33}$	Zhang et al. (1996)
40	Polygalasaponin XXX	A	Gal	H	H	Glc	a	CH_3	CH_2OH	$C_{75}H_{112}O_{36}$	Zhang et al. (1996)
41	Polygalasaponin XXXII	A	H	Ara	Api	Rha	a	CH_3	CH_2OH	$C_{79}H_{118}O_{38}$	Liu, 2004; Sun (2005), Zhang et al. (1996)
42	Polygalasaponin XXIV	A	H	H	H	Api	H	CH_3	CH_2OH	$C_{58}H_{92}O_{28}$	Zhang et al. (1996)
43	E-Senegasaponin A	A	Gal	H	Api	H	a	CH_3	CH_2OH	$C_{47}H_{110}O_{35}$	Li et al. (2008a)
44	Arilloside A	A	H	H	H	d	d	CH_3	CH_2OH	$C_{57}H_{72}O_{27}$	Song et al. (2013b)
45	Arilloside D	A	Gal	Ara	H	H	H	CH_3	CH_2OH	$C_{64}H_{102}O_{33}$	Feng et al. (2019a)
46	Polygalasaponin LIII	A	H	Ara	Api	Glc6d	a	CH_3	CH_2OH	$C_{81}H_{120}O_{39}$	Feng et al. (2019b)
47	Myrtifolioside A1	A	H	Gal	Api	Ara	a	CH_3	CH_2OH	$C_{79}H_{118}O_{39}$	Feng et al. (2019a)
48	Compound 73	A	H	xyl	Api	Rha	a	CH_3	CH_2OH	$C_{79}H_{118}O_{38}$	Lv (2007)
49	Compound 74	A	H	H	Api	H	H	CH_3	CH_2OH	$C_{58}H_{92}O_{28}$	Lv (2007)
50	Onjisaponin MF	A	H	H	H	H	a	CH_3	CH_2OH	$C_{63}H_{92}O_{26}$	Ling et al. (2013)
51	Onjisaponin TE	A	H	H	e	Rha	H	CH_3	CH_2OH	$C_{70}H_{110}O_{36}$	Ling et al. (2013)
52	Onjisaponin TF	A	H	H	H	Rha	H	CH_3	CH_2OH	$C_{59}H_{94}O_{18}$	Ling et al. (2013)
53	Onjisaponin TG	A	H	H	e	H	H	CH_3	CH_2OH	$C_{64}H_{100}O_{32}$	Ling et al. (2013)
54	Onjisaponin TH	A	H	H	Api	H	b	CH_3	CH_2OH	$C_{65}H_{96}O_{28}$	Ling et al. (2013)
55	Polygalasaponin XLV	A	Gal	H	H	Glc6d	c	CH_3	CH_2OH	$C_{76}H_{116}O_{38}$	Feng et al. (2019a)
56	Sibiricasaponin A	B	COOH	g	—	—	—	—	—	$C_{36}H_{53}O_{12}$	Song et al. (2013a)
57	Sibiricasaponin B	B	H	m	—	—	—	—	—	$C_{35}H_{55}O_{11}S$	Song et al. (2013b)
58	Sibiricasaponin C	B	H	n	—	—	—	—	—	$C_{35}H_{55}O_{11}S$	Song et al. (2013a)
59	Sibiricasaponin D	B	H	x	—	—	—	—	—	$C_{37}H_{57}O_{12}S$	Song et al. (2013b)
60	Dehydroxypresenegenin	C	CH_3	H	—	—				$C_{30}H_{46}O_6$	Yan (2004)
61	Presenegenin	C	CH_2OH	H	—	—				$C_{30}H_{46}O_7$	Zhou et al. (2014)
62	Tenuifolin	C	CH_2OH	Glc	—					—	$C_{36}H_{56}O_{12}$
63	Polygalacic acid	C	—	H	OH					—	$C_{30}H_{48}O_6$
64	Senegenic acid	D	—	H	—	H	—	—	—	$C_{29}H_{44}O_6$	Zhang et al. (1996)
65	Fallaxsaponin A	D	—	Glc	—	—	—	—	—	$C_{35}H_{54}O_{11}$	Liu et al. (2015)

(Continued on following page)

TABLE 1 (*Continued*) Saponins isolated from PR.

No.	Compound	Parent nucleus	R							Molecular formula	Ref.
			R_1	R_2	R_3	R_4	R_5	R_6	R_7		
66	Hydroxsenegennin	D	CH_2OH	H	—	—	—	—	—	$C_{30}H_{46}O_7$	Yan (2004)
67	Senegenin	D	CH_2Cl	H	H	H	—	—	—	$C_{30}H_{45}ClO_6$	Yan (2004)
68	Platycodin D									$C_{57}H_{92}O_{28}$	Vinh et al. (2020)
69	Micranthoside A					—				$C_{57}H_{92}O_{28}$	Vinh et al. (2020)
70	Zigu-glucoside I					—				$C_{41}H_{66}O_{13}$	Song et al. (2012a)

Note: a = (E)-4-methoxy cinanamoyl; Api = β-D-apiofuranosyl; Ara = β-D-arabopyranosyl; b = (E)-3,4,5-trimethoxy cinnamoyl; c = (E)-3,4-methoxy cinanamoyl; d = acetyl; e = 3-hydroxy-3-mrthylmethyl-5-pentanoic acid ester-5-β-D-apiofur-anosyl; f = (Z)-4-methoxy cinnamoyl; g = D-glucopyranuronic acid; Gal = β-D-galactopyranosyl; Glc = β-D-glucopyranosyl; h = 2-hydroxy-4-methyl-pentanoic acid ester-6-β-D-gal; m = α-D-xyl-4-SO3H; n = β-D-xyl-5- SO3H; Rha = α-L-rhamnopyranosyl; x = α-D-xyl-3-acetoxy-4-SO3H; y = 4-oxide-2-hydroxy-pentanoic acid ester-6-β-D-gal.

the parent nucleus. They form oligosaccharides by linking with rhamnose or glucose through various glycosidic bonds and then further form esters with organic acid constituents such as acetic acid, benzoic acid, and phenylpropenoic acid. They have no more than five sugar molecules in their molecule. Modern research shows that oligosaccharide esters of PR have neuroprotective, antidepressant, anti-dementia, and other central pharmacological effects (Sang et al., 2017). 3′6-Disinapoyl sucrose is another quality control indicator of PR in the *Chinese Pharmacopoeia*. Oligosaccharide esters isolated and identified from PR are listed and shown in Supplementary Table 5; Figure 4.

Xanthones

Xanthone is a general term for secondary metabolites with a tricyclic aromatic hydrocarbon system structure, also known as benzophenanthrone and dibenzo-γ-pyrone. About 70 xanthones have been identified from PR, whose parent nucleus is composed of a benzene ring paralleled with a chromone and has eight substitution sites. Xanthones in PR are mainly simple ketones, with common substituents including hydroxyl, methoxy, and methylenedioxy groups (Wang et al., 2020b). PX3 is one of the index metabolites for content determination of PR in the *Chinese Pharmacopoeia* 2020 edition. The xanthones obtained from PR are shown in Table 2; Figure 5.

Others

Alkaloids, organic acids, volatile oils, coumarins, phenylpropanoids, and steroidals are also present in PR (Zhao et al., 2020), as shown in Table 3; Figures 6, 7. PR also contains trace elements (Li, 2008), such as Zn, Cu, Fe, Mn, K, Ca, and Mg.

Nowadays, studies of metabolite separation from the roots of PR have gradually decreased, and the existing research mostly focuses on saponins and oligosaccharide esters. The attention to xanthones and other metabolites should be improved. This may be related to the relatively low content of these metabolites, and optimizing the technical of extraction, enrichment, and purification may improve the situation. The aerial parts of PR, which have been discarded as waste, are beginning to receive attention and show potential. Tan et al. adopted silica gel, a medium-pressure octadecylsilyl (ODS) chromatographic column, and high-performance liquid chromatography to separate and purify the n-butanol part of the 70% ethanol extract of the aerial part of PR from 13 metabolites of which nine metabolites, including saponin metabolite II, were isolated from plants of *Polygalaceae* for the first time (Tan et al., 2022). Further studies are needed to investigate the differences in chemical composition and content between the aerial parts and roots of PR to further guide their rational development and utilization. Furthermore, the structure-activity relationship of active substances in PR has not been fully revealed; future research is needed to guide the development of lead metabolites, which will help to synthesize or semi-synthesize new derivatives with high efficiency, low toxicity, and safety. In addition, the current extraction and separation technologies mainly use organic reagents such as n-butanol, ethyl acetate, petroleum, and so on, which are irritating to the human body and harmful to the environment. With the advancement of analytical methods and techniques, it is believed that more advanced and environmentally friendly technologies and instruments will be applied.

Pharmacology

Modern pharmacological studies have shown that PR has significant pharmacological activity against nervous system diseases and has anti-oxidation, anti-inflammatory, anti-tumor, and anti-pathogenic microorganism functions, as well as other effects (Zhou et al., 2022), as shown in Supplementary Table 6.

Anti-neurodegenerative effects

Anti-pathologic protein aggregation effects

PR can improve the transport and clearance of Aβ by up-regulating the protein expression of low-density lipoprotein receptor-related protein 1, promoting p21, and down-regulating the receptors for advanced glycation end products and cyclinD1 and E2F1 protein expressions. At the same time, it can inhibit caspase-3, caspase-8, and Bax expression and increase Bcl-2 expression (Yu et al., 2022). The expression of two main enzymes involved in Aβ degradation, insulin-degrading enzyme (IDE) and neprilysin (NEP),

TABLE 2 Xanthones isolated from PR.

No.	Compound	Parent nucleus	R								Formula	Ref.
			R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8		
131	Lancerin	A	H	H	OH	H	H	OH	Glc	H	$C_{19}H_{18}O_{10}$	Liu et al. (2010), Zhou et al. (2014)
132	Onjixanthone I	A	OMe	OMe	OMe	H	H	H	OH	H	$C_{16}H_{14}O_6$	Yukinobu et al. (1991)
133	Onjixanthone II	A	OH	OMe	OH	H	H	OH	OMe	H	$C_{15}H_{12}O_7$	Yukinobu et al. (1991), Zhou et al. (2014)
134	Polygalaxanthone III	A	OH	Glc-6-Api	OH	H	H	OH	OMe	H	$C_{25}H_{28}O_{15}$	Ikeya et al. (1994)
135	Polygalaxanthone IV	A	OH	H	OMe	—	—	Oglc-2-1Rha	OMe	H	$C_{27}H_{32}O_{15}$	Jiang et al. (2011)
136	Polygalaxanthone V	A	OH	H	OH	—	—	Oglc-2-1Rha	OMe	H	$C_{26}H_{30}O_{15}$	Zhou et al. (2014)
137	Polygalaxanthone VI	A	OMe	OMe	OMe	—	—	OGlc	OMe	H	$C_{23}H_{26}O_2$	Zhou et al. (2014)
138	Polygalaxanthone VII	A	OH	OMe	OGlc2-Rha	OH	OMe	H	H	H	$C_{27}H_{32}O_{16}$	Wang et al. (2005)
139	Polygalaxanthone VIII	A	OH	Glc-6-Ara	OH	H	H	OH	OMe	H	$C_{25}H_{28}O_{15}$	Jiang et al. (2005)
140	Polygalaxanthone IX	A	OH	H	OGlc-2-Rha	H	H	H	OH	H	$C_{25}H_{28}O_{14}$	Jiang et al. (2005)
141	Polygalaxanthone X	A	OMe	OMe	OMe	H	H	OGlc-2-Rha	OMe	H	$C_{29}H_{36}O_{16}$	Jiang et al. (2005)
142	Sibiricaxanthone A	A	OH	Glc6-Api	OH	H	H	H	OH	H	$C_{24}H_{26}O_{14}$	Miyase et al. (1999)
143	Sibiricaxanthone B	A	OH	Glc2-1Rha	OH	H	H	H	OH	H	$C_{24}H_{26}O_{14}$	Miyase et al. (1999)
144	7-Phenyl-1-hydroxy-2,3,6-trimethoxyxanthone	A	OH	OCH_3	OCH_3	—	—	OCH_3	—	phenyl	$C_{22}H_{18}O_6$	Jia et al. (2023)
145	1,2,3-Trimethoxy-7-hydroxyxanthone	A	OMe	OMe	OMe	H	H	H	OH	H	$C_{16}H_{14}O_6$	Li et al. (2022b)
146	1,3,6-Trihydroxy-2,7-dimethoxyxanthone	A	OH	OMe	OH	H	H	OH	OMe	H	$C_{15}H_{12}O_7$	Zhang et al. (2016)
147	1,2,7-Trimethoxy-3-hydroxyxanthone	A	OMe	OMe	OH	H	H	H	OMe	H	$C_{16}H_{14}O_6$	Li et al. (2022a)
148	1,2,3,7-Tetramethoxyxanthone	A	OMe	OMe	OMe	H	H	H	OMe	H	$C_{17}H_{16}O_6$	Yukinobu et al. (1991)
149	1,7-Dihydroxy-3-methoxyxanthone	A	OH	H	OMe	H	H	H	OH	H	$C_{14}H_{10}O_5$	Liu et al. (2010)
150	1,7-Dihydroxy-2,3-dimethoxyxanthone	A	OH	OMe	OMe	H	H	H	OH	H	$C_{15}H_{12}O_6$	Wang et al. (2005)

(Continued on following page)

10.3389/fphar.2024.1450733

TABLE 2 (*Continued*) Xanthones isolated from PR.

No.	Compound	Parent nucleus	R								Formula	Ref.
			R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8		
151	1,7-Dihydroxy-2,3-methlendioxyyxanthone	A	OMe	OCH_2O	OCH_2O	H	H	H	H	OMe	$C_{14}H_8O_6$	Jiang and Tu (2004)
152	6-Hydroxy-2,3,6,7-tetramethoxyxanthone	A	H	OMe	OMe	H	H	OMe	OMe	H	$C_{17}H_{16}O_7$	Zhang et al. (2016)
153	6-Hydroxy-1,2,3,7-tetramethoxyxanthone	A	OMe	OMe	OMe	H	H	OGlc	OMe	H	$C_{17}H_{16}O_7$	Yukinobu et al. (1991), Zhou et al. (2014)
154	1,7-Dihydroxyxanthone	A	OH	H	H	H	H	H	OH	H	$C_{13}H_8O_4$	Jiang and Tu (2004)
155	1,7-Dimethoxyxanthone	A	OMe	H	H	H	H	H	OMe	H	$C_{15}H_{12}O_4$	Li (2008)
156	1-Hydroxy-3,7-dimethoxyxanthone	A	OH	H	OMe	H	H	H	OMe	H	$C_{15}H_{12}O_5$	Jiang and Tu (2004)
157	1-Hydroxy-3,6,7-trimethoxyxanthone	A	OH	H	OMe	H	H	OMe	OMe	H	$C_{16}H_{14}O_6$	Jiang and Tu (2004)
158	6,8-Dihydroxy-12,4-trimethoxyxanthone	A	OMe	OMe	H	OH	H	H	H	OH	$C_{16}H_{14}O_7$	Fujita et al. (1992)
159	6,8-Dihydroxy-1,2,3-trimethoxyxanthone	A	OMe	OMe	OMe	H	H	H	H	OH	$C_{16}H_{14}O_7$	Fujita et al. (1992)
160	3-Hydroxy-2,8-dimethoxyxanthone	A	H	OMe	OH	H	H	H	H	OMe	$C_{15}H_{12}O_5$	Yukinobu et al. (1991)
161	1,2,3,6,7-Pentamethoxyxanthone	A	OMe	OMe	OMe	H	H	OMe	OMe	H	$C_{18}H_{18}O_7$	Jiang et al. (2003), Zhou et al. (2014)
162	1,3,7-Trihydroxyxanthone	A	OH	H	OH	H	H	H	OH	H	$C_{13}H_8O_5$	Jiang et al. (2003)
163	1,6,7-Trihydroxy-2,3-dimethoxyxanthone	A	OH	OMe	OMe	H	H	OH	OH	H	$C_{15}H_{12}O_7$	Jiang et al. (2003)
164	3-Hydroxy-1,2,7-trimethoxyxanthone	A	OMe	OMe	OH	H	H	H	OMe	H	$C_{16}H_{14}O_6$	Fujita et al. (1992)
164	7-Hydroxy-1,2,3-trimethoxyxanthone	A	OMe	OMe	OMe	H	H	H	OH	H	$C_{16}H_{14}O_6$	Zhang et al. (2016)
165	2,3,8-Trimethoxyxanthone	A	H	OMe	OMe	H	H	H	H	OMe	$C_{16}H_{14}O_5$	Fujita et al. (1992)
166	1,3,6,7-Tetramethoxyxanthone	A	OMe	H	OMe	H	H	OMe	OMe	H	$C_{17}H_{16}O_6$	Fujita et al. (1992)
167	1,3,7-Trimethoxyxanthone	A	OMe	H	OMe	H	H	H	OMe	H	$C_{16}H_{14}O_5$	Jiang et al. (2003)
168	7-O-Methylmangiferin	A	OH	Glc	OH	H	H	OH	OMe	H	$C_{20}H_{20}O_{11}$	Liu et al. (2010)
169	Euxanthone	A	OH	H	H	H	H	H	OH	H	$C_{13}H_8O_4$	Yang et al. (2000)
170	1,6-Dihydroxy-3,7-dimethoxyxanthone	A	OH	H	OCH_3	H	H	OH	OCH_3	H	$C_{15}H_{12}O_6$	Yukinobu et al. (1991)
171	1,6-Dihydroxy-3,5,7-trimethyoxyxanthone	A	OH	H	OCH_3	H	OCH_3	OH	OCH_3	H	$C_{16}H_{14}O_7$	Miyase and Ueno (1993)
172	Irisxanthone	A	H	H	OH	OCH_3	H	OH	Glc	OH	$C_{20}H_{20}O_{11}$	Chai et al. (2018)
173	Polygalaxanthone XI	A	OH	Glc-2-Api	OH	H	H	OH	OCH_3	H	$C_{25}H_{28}O_{15}$	Li (2008)

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TABLE 2 (*Continued*) Xanthones isolated from PR.

No.	Compound	Parent nucleus	R								Formula	Ref.
			R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8		
174	1,6-Dimethoxy-2,3-methylenedioxyxanthone	A	OCH_3	OCH_2O	OCH_2O	H	H	H	OCH_3	H	$C_{16}H_{12}O_6$	Li (2008)
175	1,3-Dihydroxy-5,6,7-trimethoxyxanthone	A	OH	H	OH	H	OCH_3	OCH_3	OCH_3	H	$C_{16}H_{14}O_7$	Li (2008)
176	7-Hydroxy-1-methoxy-2,3-methylenedioxyxanthone	A	OCH_3	OCH_2O	OCH_2O	H	H	H	OH	H	$C_{15}H_{10}O_6$	Li (2008)
177	1,2,7-Trihydroxy-3,6-dimethoxyxanthone	A	OH	OH	OCH_3	H	H	OCH_3	OH	H	$C_{15}H_{12}O_7$	Li (2008)
178	4-C-β-D-Glucopyrabosyl-1,3,6-trihydroxy-7-methoxyxanthone glycosides	A	OH	H	OH	β-D-Glc	H	OH	OCH_3	H	$C_{20}H_{20}O_{11}$	Wang et al. (2003)
179	4-C-[β-D-apiofuranosyl-(1→6) -β-D-lucopyrabosyl]-1,3,6-trihydroxy-7-methoxyxanthone glycosides	A	OH	H	OH	β-D-Glc-β-D-Api	H	OH	OCH_3	H	$C_{25}H_{28}O_6$	Wang et al. (2003)
180	1-Hydroxy-7-methoxyxanthone	A	OH	H	H	—	—	H	OCH_3	—	$C_{14}H_{10}O_4$	Cho et al. (2012)
181	3,6-Dihydroxy-1,2,7-trimethoxyxanthone	A	OCH_3	OCH_3	OH	OH	—	OH	OCH_3	—	$C_{16}H_{14}O_7$	Cho et al. (2012)
182	1,2,3-Trihydroxy-6,7-dimethoxyxanthone	A	OOH	OH	OH	OH	—	OOH	—	—	$C_{13}H_8O_9$	Yoo et al. (2014)
183	1,2,3,6,7-Pentahydroxyxanthone	A	OH	OCH_3	OCH_3	OOH	—	OH	—	—	$C_{15}H_{12}O_3$	Yoo et al. (2014)
184	4-Hydroxy-1,2,3,7-tetramethoxyxanthone	A	OOH	OCH_3	OCH_3	OOH	—	OOH	—	—	$C_{15}H_{12}O_{10}$	Yoo et al. (2014)
185	1,3,7-Trihydroxy-2,6-dimethanoxyxanthone	A	OH	OMe	OH	—	—	MeO	OH	—	$C_{15}H_{12}O_7$	Xu et al. (2014)
186	7-Hydroxy-1-methoxyxanthone	A	OMe	H	H	H	—	H	H	OH	$C_{14}H_{10}O_4$	Xu et al. (2014)
187	1,7-Dihydroxy-3, 4-dimethoxyxanthone	A	OH	—	OCH_3	OCH_3	—	—	OH	—	$C_{15}H_{12}O_6$	Xu et al. (2014)
188	Sibiriphenone A	B	OH	H	OH	H	H	OH	OMe	H	$C_{20}H_{22}O_{11}$	Zhou et al. (2014)
189	2-Hydroxy-4,6-dimethoxybenzophenone	C	OH	H	OCH_3	H	OCH_3	H	H	H	$C_{15}H_{14}O_4$	Liu et al. (2021)
190	Tenuiphenone A	C	OH	H	OCH_3	H	β-D-Glc-α-L-rha-	H	H	H	$C_{26}H_{32}O_{13}$	Jiang and Tu (2005)
191	Tenuiphenone B	C	OH	β-D-Glc	OH	β-D-Glc	OH	OH	H	H	$C_{25}H_{30}O_{17}$	Jiang and Tu (2005)
192	1,7-Dihydroxy-2,3-methylenedioxyxanthone										$C_{14}H_8O_6$	Shi et al. (2013a)
193	Sibiricaxanthone F										$C_{29}H_{34}O_{16}$	Cho et al. (2012)
194	Tenuiphenone C										$C_{36}H_{62}O_4$	Jiang and Tu (2005)
195	Tenuiphenone D										$C_{38}H_{66}O_4$	Jiang and Tu (2005)
196	Grasshopper ketone										$C_{13}H_{20}O_3$	Tan et al. (2022)

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TABLE 3 Other components isolated from PR.

No.	Compound	Molecular formula	Ref.
197	N-acetyl-D-glucosamine	$C_8H_{15}NO_6$	Wang and Xia (2012)
198	Benzoic acid	$C_7H_6O_2$	Wang et al. (2005)
199	1,5-Anhydro-D-sorbitol	$C_6H_{12}O_5$	Pei et al. (2005)
200	Propyl benzoate	$C_{10}H_{12}O_2$	Li et al. (2005)
201	O-Hydroxybenzoic acid	$C_7H_6O_3$	Jiang et al. (2011)
202	Norharman	$C_{11}H_8N_2$	Jin and Park (1993)
203	N_9-formylharman	$C_{13}H_{12}N_2O$	Jin and Park (1993)
204	1-Carbobutoxy-β-carboline	$C_{13}H_{16}N_2O_2$	Jin and Park (1993)
205	1-Carboethoxy-β-carboline	$C_{14}H_{12}N_2O_2$	Jin and Park (1993)
206	1-Carbomethoxy-β-carboline	$C_{13}H_{10}N_2O_2$	Jin and Park (1993)
207	Sibiricaphenone	$C_{20}H_{28}O_{11}$	Miyase et al. (1999)
208	(28Z)-Tetratriacontenoic acid	$C_{33}H_{66}O_2$	Jiang et al. (2003)
209	3,4,5-Trimethoxybenzeneacrylic acid methyl ester	$C_{13}H_{16}O_5$	Li et al. (2005)
210	Harman	$C_{12}H_{10}N_2$	Jin and Park (1993)
211	Perlolyrine	$C_{16}H_{12}N_2O_2$	Jin and Park (1993)
212	4-Hydroxy-3,5-dimethoxylcinnamate	$C_{12}H_{14}O_5$	Jiang et al. (2011)
213	Tetrahydrocolumbamine	$C_{20}H_{23}NO_4$	Shen et al. (2004)
214	3,4,5-Trimethoxycinnamic acid	$C_{12}H_{14}O_5$	Li et al. (2005), Zhou et al. (2014)
215	Stigmasterol	$C_{29}H_{48}O$	Jiang et al. (2003)
216	A-Sipnasterol	$C_{29}H_{48}O$	Jiang et al. (2003)
217	A-Sipnasterol-3-O-β-D-glucoside	$C_{35}H_{58}O_6$	Jiang et al. (2003)
218	A-Sipnasterol-3-O-β-D-glucoside-6′-O-paltimate	$C_{51}H_{88}O_7$	Jiang et al. (2003)
219	Rhamnetin-3-O-β-D-glucopyranoside	$C_{22}H_{22}O_{12}$	Zhou et al. (2014)
220	B-Daucosterin	$C_{35}H_{60}O_6$	Wang et al. (2003)
221	Vomifoliol	$C_{13}H_{20}O_3$	Tan et al. (2022)
222	3-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)-propan-1-one	$C_{10}H_{12}O_4$	Tan et al. (2022)
223	Robinlin	$C_{33}H_{40}O_{19}$	Tan et al. (2022)
224	3′,5′-Dimethoxy-biphenyl-4-ol	$C_{14}H_{14}O_3$	Tan et al. (2022)
225	Phomolide A	$C_{12}H_{16}O_3$	Tan et al. (2022)
226	Spicellamide A	$C_{32}H_{48}N_4O_8$	Tan et al. (2022)
227	Tenuifolimide A	$C_{37}H_{38}N_2O_{10}$	Chen et al. (2022b)
228	(−)-Medioresinol	$C_{21}H_{24}O_7$	Cheng et al. (2022a)
229	(+)-Pinoresinol	$C_{20}H_{22}O_6$	Cheng et al. (2022a)
230	Syringaresinol	$C_{22}H_{26}O_8$	Cheng et al. (2022a)
231	4-Hydroxy-3-methoxypropiophenone	$C_{10}H_{12}O_3$	Cho et al. (2012)
232	Methyl 4-hydroxy-3-methoxycinnamic acid	$C_{11}H_{12}O_4$	Cho et al. (2012)
233	4-Methoxycinnamic acid	$C_{10}H_{10}O_3$	Cho et al. (2012)
234	Amentoflavon	$C_{30}H_{18}O_{10}$	Cho et al. (2012)

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TABLE 3 (*Continued*) Other components isolated from PR.

No.	Compound	Molecular formula	Ref.
235	Linarin	$C_{28}H_{32}O_{14}$	Cho et al. (2012)
236	2,4,4-Trimethyl-3-formyl-6-hydroxy-2,5-cyclohexadien-1-on	$C_{10}H_{12}O_3$	Cho et al. (2012)
237	Lanierone	$C_9H_{12}O_2$	Cho et al. (2012)
238	Aralia cerebroside	$C_{40}H_{77}NO_{10}$	Cho et al. (2012)
239	1-O-L-arabinopyranosyl-O-(6→1)-β-D-glucopyranosyl-salicylate	$C_{19}H_{27}O_{12}$	Zhou et al. (2008)
240	Canthoside A	$C_{19}H_{26}O_{20}$	Zhou et al. (2008)
241	Methyl 3,4,5-trimethoxycinnamate	$C_{13}H_{16}O_5$	Zhou et al. (2008)
242	1,5-Anhydro-D-glucitol	$C_6H_{12}O_5$	Yu et al. (2014)
243	Methyl pentadecanoate	$C_{16}H_{32}O_2$	Sun et al. (2000)
244	Methyl 9-hexadecenoate	$C_{17}H_{32}O_2$	Sun et al. (2000)
245	Methyl hexadecanoate	$C_{17}H_{34}O_2$	Sun et al. (2000)
246	Methyl isoheptadecanoate	$C_{18}H_{36}O_2$	Sun et al. (2000)
247	Heptadecenoic acid	$C_{18}H_{34}O_2$	Sun et al. (2000)
248	Methyl heptadecanoate	$C_{18}H_{36}O_2$	Sun et al. (2000)
249	Methyl 12,15-octadecadienoate	$C_{19}H_{34}O_2$	Sun et al. (2000)
250	Methyl cis-9-octadecenoate	$C_{19}H_{36}O_2$	Sun et al. (2000)
251	Methyl octadecanoate	$C_{19}H_{38}O_2$	Sun et al. (2000)
252	9,12-Octadecadienoic acid, methyl ester, (E, E)-	$C_{19}H_{34}O_2$	Sun et al. (2000)
253	9,12-Octadecadienoic acid, methyl ester, (Z, Z)-	$C_{19}H_{34}O_2$	Sun et al. (2000)
254	Ethyl oleate	$C_{20}H_{38}O_2$	Sun et al. (2000)
255	(Z)-9-Nonadecenoic acid, methyl ester	$C_{20}H_{38}O_2$	Sun et al. (2000)
256	Methyl nonadecanoate	$C_{20}H_{40}O_2$	Sun et al. (2000)
257	(Z)-Methyl icos-11-enoate	$C_{21}H_{40}O_2$	Sun et al. (2000)
258	Methyl arachidate	$C_{21}H_{42}O_2$	Sun et al. (2000)
259	Methyl behenate	$C_{23}H_{46}O_2$	Sun et al. (2000)
260	Poligapolide	$C_{18}H_{34}O_2$	Yoo et al. (2014)
261	Chondrillasterol	$C_{29}H_{48}O$	Yoo et al. (2014)
262	3α-O-β-Pyranoglucosyl spinasterol	$C_{29}H_{46}O_2$	Yoo et al. (2014)
263	3β-O-β-Pyranoglucosyl Chondrillasterol	$C_{35}H_{57}O_5$	Yoo et al. (2014)
264	3,4,5-Trimethoxy methyl cinamate	$C_{13}H_{16}O_5$	Yoo et al. (2014)
265	3′,4′-Dimethoxy-7-diglucosyl-O-methylenoxy-5-hydroxyl flavol	$C_{30}H_{36}O_{17}$	Yoo et al. (2014)
266	Blumenol C	$C_{13}H_{22}O_2$	Son et al. (2022)
267	9-Epi-blumenol C	$C_{13}H_{22}O_2$	Son et al. (2022)
268	Squalene	$C_{30}H_{50}$	Song et al. (2009)
269	Polygalin D	$C_{28}H_{32}O_{16}$	Song et al. (2013a)
270	Polygalin E	$C_{27}H_{30}O_{16}$	Song et al. (2013b)
271	Polygalin F	$C_{27}H_{30}O_{16}$	Song et al. (2013a)
272	Polygalin G	$C_{33}H_{40}O_{21}$	Song et al. (2013b)

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TABLE 3 (*Continued*) Other components isolated from PR.

No.	Compound	Molecular formula	Ref.
273	Kaempferol	$C_{15}H_{10}O_6$	Song et al. (2013a)
274	Rhamnocitrin	$C_{16}H_{12}O_7$	Song et al. (2013b)
275	Rhamnetin	$C_{16}H_{12}O_7$	Song et al. (2013a)
276	Ermanin	$C_{17}H_{14}O_6$	Song et al. (2013b)
277	Ombuine	$C_{17}H_{14}O_7$	Song et al. (2013a)
278	Ermanin-3-O-β-D-glucopyranoside	$C_{22}H_{24}O_{12}$	Song et al. (2013b)
279	Polygalin A	$C_{23}H_{24}O_{11}$	Song et al. (2013a)
280	Ombuine-3-O-β-D-glucopyranoside	$C_{23}H_{24}O_{12}$	Song et al. (2013b)
281	Ombuine-3-O-β-D-galactopyranoside	$C_{23}H_{24}O_{12}$	Song et al. (2013a)
282	Rhamnocitrin-3-O-β-D-glucopyranoside	$C_{22}H_{22}O_{12}$	Song et al. (2013b)
283	Rhamnocitrin-3-O-β-D-galactopyranoside	$C_{22}H_{22}O_{12}$	Song et al. (2013a)
284	Polygalin C	$C_{28}H_{32}O_{16}$	Song et al. (2013b)
285	Polygalasterol A	$C_{27}H_{47}O_7S$	Song et al. (2012b)
286	Clionosterol	$C_{29}H_{50}O$	Le et al. (2012)
287	Tenuiside A	$C_{19}H_{18}O_{10}$	Shi et al. (2013b)
288	Tenuiside B	$C_{20}H_{22}O_9$	Shi et al. (2013b)
289	Tenuiside C	$C_{20}H_{22}O_9$	Shi et al. (2013b)
290	Tenuiside D	$C_{20}H_{22}O_{11}$	Shi et al. (2013b)
291	Tenuiside E	$C_{26}H_{32}O_{11}$	Shi et al. (2013b)
292	Tenuiside F	$C_{35}H_{52}O_{17}$	Shi et al. (2013b)
293	Mangiferin	$C_{19}H_{18}O_{11}$	Shi et al. (2013b)
294	Polygalapyrone A	$C_{12}H_{10}O_4$	Cheng et al. (2022b)
295	Tenuiside G	$C_{31}H_{44}O_{13}$	Cheng et al. (2022b)
296	Polygalapyrrole A	$C_{12}H_{15}NO_3$	Cheng et al. (2022b)
297	5-Methoxy-6-phenyl-2H-pyran-2-one	$C_{12}H_{10}O_3$	Cheng et al. (2022b)
298	Premnaionoside	$C_{24}H_{40}O_{12}$	Cheng et al. (2022b)
299	Sinapic acid	$C_{11}H_{12}O_5$	Song et al. (2016)
300	Ferulic acid	$C_{10}H_{10}O_4$	Song et al. (2016)
301	P-Hydroxybenzoic acid	$C_7H_6O_3$	Song et al. (2016)
302	P-Coumaric acid	$C_9H_8O_3$	Song et al. (2016)
303	Cinnamic acid	$C_9H_8O_2$	Song et al. (2016)
304	P-Methoxy cinnamic acid	$C_7H_6O_3$	Song et al. (2016)
305	Hexanoic acid	$C_6H_{12}O_2$	Wu (2010)
306	Phenethyl alcohol	$C_8H_{10}O$	Wu (2010)
307	2,5-Dimethylbenzaldehyde	$C_9H_{10}O$	Wu (2010)
308	Stearic acid	$C_{18}H_{36}O_2$	Wu (2010)
309	Oleic acid	$C_{18}H_{34}O_2$	Wu (2010)
310	Palmitic acid	$C_{16}H_{12}O_2$	Wu (2010)

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TABLE 3 (*Continued*) Other components isolated from PR.

No.	Compound	Molecular formula	Ref.
311	Methylsalicylic acid	$C_8H_8O_3$	Wu (2010)
312	Isorhamnetin-3-O-β-D-glucopyranoside	$C_{22}H_{22}O_{12}$	Shi et al. (2013a)
313	Isorhamnetin-3-O-β-D-galactopyranoside	$C_{22}H_{22}O_{12}$	Shi et al. (2013a)
314	Isorhamnetin	$C_{16}H_{12}O_7$	Shi et al. (2013a)
315	Quercetin-3-O-β-D-glucopyranosyl (1→2)-β-D-galactopyranoside	$C_{28}H_{32}O_{17}$	Shi et al. (2013a)
316	Quercetin-3-O-β-D-glucopyranosyl (1→2)-β-D- glucopyranoside	$C_{28}H_{32}O_{17}$	Shi et al. (2013a)
317	5,7-Dihydroxy-8-methoxyflavone-7-O-β-D-glucuronoside	$C_{22}H_{20}O_{14}$	Shi et al. (2013a)
318	Quercetin	$C_{15}H_{10}O_7$	Shi et al. (2013a)
319	Quercetin-3-O-β-D- glucopyranoside	$C_{21}H_{20}O_{12}$	Shi et al. (2013a)
320	Roseoside II	$C_{19}H_{30}O_8$	Tan et al. (2022)
321	Garcimangosone D	$C_{19}H_{20}O_9$	Tan et al. (2022)
322	Ethyl cholestan-22-enol	$C_{29}H_{50}O$	Le et al. (2012)
323	3β-O-β-D-Glucopyranosyl ethyl cholestan-22-enol	$C_{35}H_{60}O_6$	Le et al. (2012)
324	3-O-β-D-Glucopyranosyl clionasterol	$C_{29}H_{50}O$	Le et al. (2012)

can be enhanced by a pectin polysaccharide named RP02-1. RP02-1 can also inhibit Aβ aggregation, and its effect on Aβ aggregation and production made it a candidate leading metabolite for AD (Zeng et al., 2020a). Polygala tenuifolia polysaccharide, another important polysaccharide from PR, reduces Aβ deposition, activates extracellular-signal-regulated kinase (ERK) pathway-related proteins, and enhances synaptic plasticity, which allows it to improve spatial cognitive deficits in AD mice to reduce cellular damage in the hippocampal CA3 region and to maintain the balance of the cholinergic system, thus exerting an anti-AD effect *in vivo* (Li et al., 2024). It is necessary to reveal their structure-activity relationship and study safety for structural modification and optimization as well as for the development of new drugs. 3,6′-Disinapoyl sucrose (DISS) is an oligosaccharide ester of PR that can regulate the mRNA expression of antioxidation and autophagy-related genes to reduce Aβ deposition and neurotoxicity to prolong the lifespan of *Caenorhabditis elegans* (C.elegans) (Tang et al., 2022). However, the lack of positive controls prevents us from determining whether DISS has an advantage over marketed drugs. Interestingly, it has been reported that the extract of PR can prevent axon growth cone collapse and axon atrophy caused by Aβ deposition by inhibiting endocytosis in transgenic mice and cultured neurons, but it has no effect on Aβ deposition. A study shows that significant clinical benefit can be observed in clinical trials only if the Aβ plaque is reduced to a low level (~20 centiloids) (Karran and De Strooper, 2022). This finding reminds suggests making full use of advanced technology and equipment to quantify and monitor the magnitude and velocity of Aβ clearance in future experiments to screen the metabolites that are worthy of further clinical trials, which would help transform scientific research results into clinical application achievements.

It is well known that the presence of alpha-synuclein in the cytoplasm of residual neurons is one of the recognized pathological features of PD, and repeated amplification of more than 35 CAG trinucleotides leads to a long mutant polyQ region in the Huntington protein that causes HD. The therapeutic effect of ambroxol in PD is associated with a reduction in alpha-synuclein. It is more effective in combination with PR and is safe while not affecting its absorption (Yang et al., 2021). This evidence shows that PR and its metabolites can inhibit the secretion and production of Aβ, weaken its toxicity, promote its degradation and clearance, inhibit the hyperphosphorylation of Tau protein, and accelerate the clearance of the Huntington protein and α -synuclein to inhibit the deposition of all these proteins.

Improving synaptic dysfunction effects

The synapse is the hub of neuronal signal transduction, and its function is influenced by neurotransmitters, calcium, presynaptic vesicle dynamics, and postsynaptic signal transduction. Its functional obstruction is one of the culprits of NDD. The therapeutic and relieving effects of PR on symptoms triggered by NDD are closely related to its effects of preventing axonal degeneration and improving synaptic plasticity. M2 microglia can inhibit inflammation and show a neuroprotective effect, and their relative preponderance over M1 can be induced by PR to protect the injured spinal cord from axonal degeneration. Sibiricose A5, DISS, PX3, sibiricose A6, and sibiricaxanthone A were detected in the spinal cord extract, and sibiricose A5 and DISS were found to be the active ingredients (Kuboyama et al., 2021). RP01-1 is a pectin from PR that showed a neurite outgrowth-inducing effect by increasing brain-derived neurotrophic factor (BDNF) expression and promoting AKT, ERK, and cAMP-response element-binding protein (CREB) and phosphorylation (Zeng et al., 2020b). Oligosaccharide esters (OEs) of PR can also promote BDNF, pAkt/Akt, and pCREB/CREB expressions (Niu et al., 2022). The dendritic length and dreblin cluster density were quantified by an *in vitro* high connotation imaging analysis system and immunocytochemical analysis, and the results showed that PR

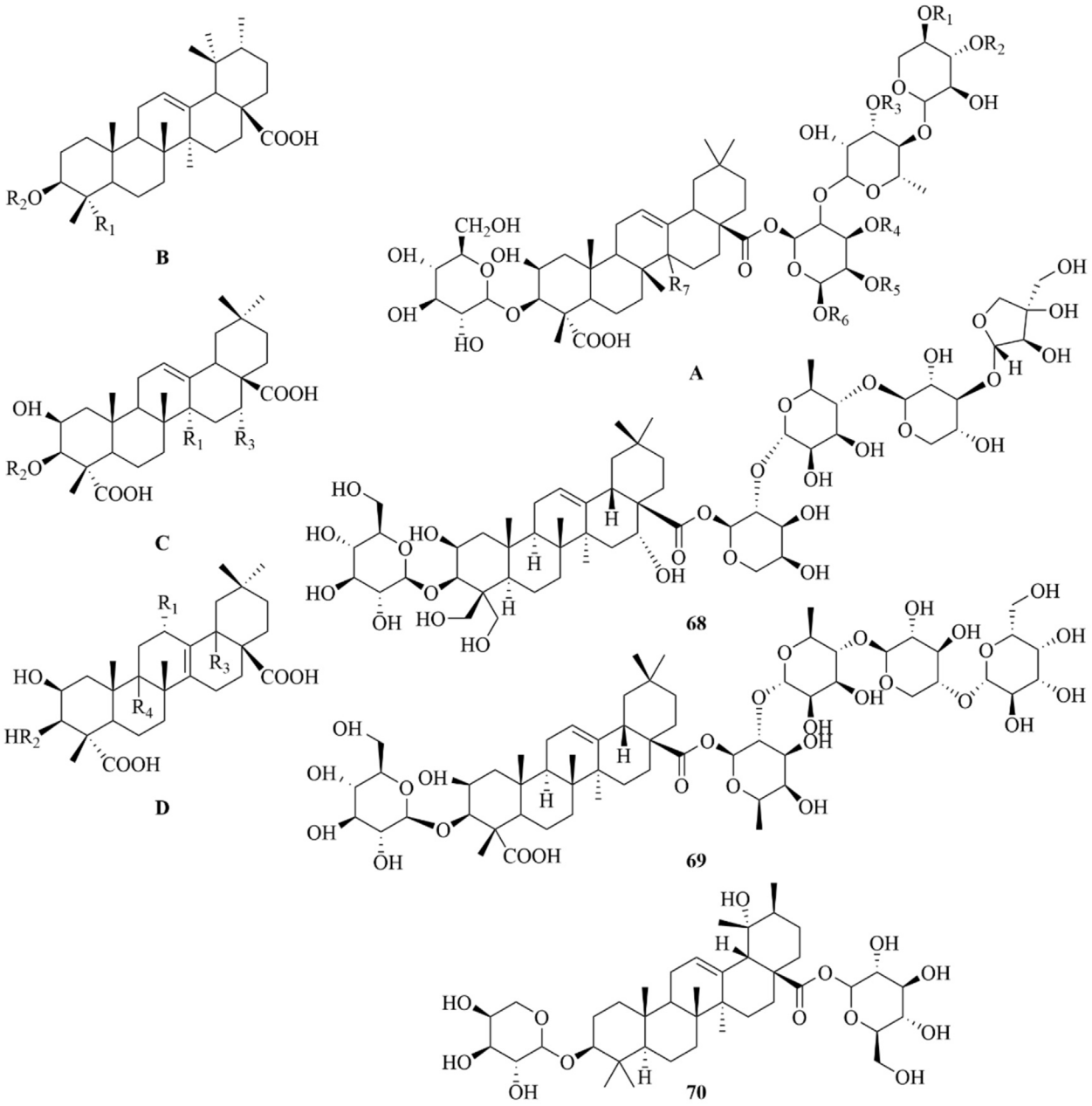

FIGURE 3
Structures of saponins [parent nucleus (A-D). 68–70] in PR.

and tenuifolin could reduce dreblin cluster density by N-methyl-D-aspartic acid-type glutamate receptors, directly improving synaptic plasticity, and PR had a better effect than tenuifolin alone (Koganezawa et al., 2021). The synergistic effects of complex metabolites in PR and other monomers with this effect need further exploration and screening.

Maintaining protein steady-state effects

Due to the close polarity and mutual transformation of saponins, it is difficult to separate and purify the effective metabolites, and fully clarifying the synergistic effect of each metabolite in the extract and the influence of other metabolites on the pharmacokinetics of onjisaponin B. requires further work. Tenuifolin (TEN) can remove damaged mitochondria by up-regulating the mRNA expression of LC3, cathepsin D, Rab7, PTEN-induced putative kinase 1 (PINK1), Parkin, and the protein expression of LC3, PINK1, Parkin, and down-regulating the expression of p62 *in vivo* (Lu et al., 2021). When it was used to treat PC12 cells induced by Aβ25-35, it upregulated p62 expression (Li et al., 2023a). This divergence needs to be further verified and explained. A 2022 study found that TEN could remove damaged mitochondria via activating the mitochondrion phagocytosis mediated by PINK1/Parkin as well. It induced the formation of mitochondrial phagocytes and mitochondrial lysosomes, promoted the transformation from LC3 I to LC3 II, and downregulated p62 expression, and the transfer of Parkin to mitochondria was

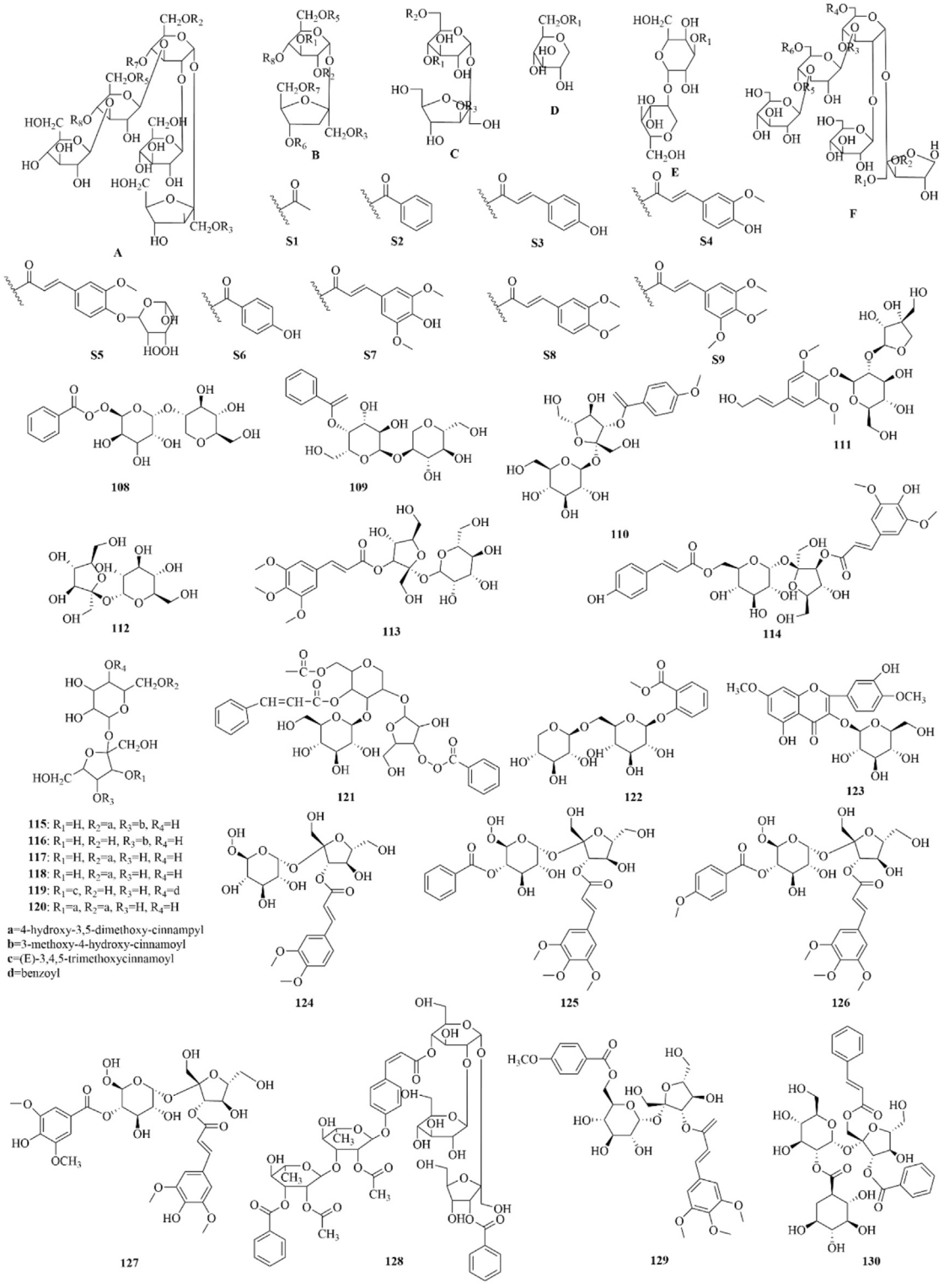

FIGURE 4
Structures of oligosaccharide esters [parent nucleus (A-F), substituent group (S1-S9), 108–130] in PR.

also promoted (Tian et al., 2022). In addition, PR can also inhibit autophagy protection-related proteins to treat spinal cord injury. TEN upregulated growth-associated protein 43 and neurofilament 200, decreased the Beclin-1 and LC3B-II/LC3B-I levels in the spinal cord, and suppressed the level of the PTPN1 protein, which can suppress IRS1 protein to reduce the pAkt and mTOR levels. What is more, the promoting effect of TEN on the functional recovery from spinal cord injury (SCI) rats was blocked by overexpression of

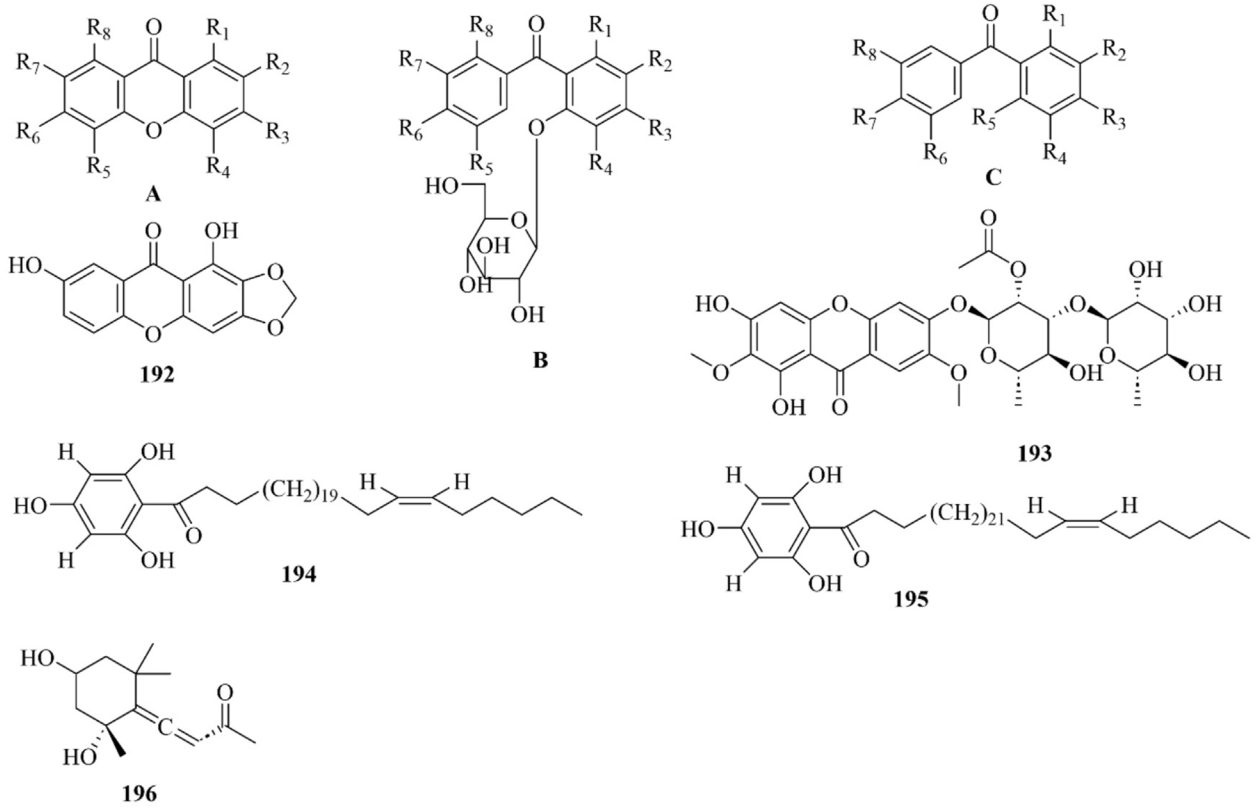

FIGURE 5
Structures of xanthones [parent nucleus (A-C), 192—196] in PR.

PTPN1, an Akt/mTOR antagonist LY294002, and an autophagy inhibitor 3-MA. This indicates that TEN inhibits autophagy by blocking PTPN1 and modulating IRS1/Akt/mTOR signal transduction to promote the functional recovery of SCI rats (Zang et al., 2023).

Regulating energy metabolism effects

Energy metabolism defects are involved in the pathological process of NDD, and maintaining the steady state of energy metabolism is a necessary condition for the normal function of neurons. Mitochondria, the basic organelles of almost all cells in the human body, supply energy, synthesize reactive oxygen species, maintain calcium homeostasis, and affect cell death. In addition to improving glucose utilization, PR can also regulate mitochondrial dysfunction to improve brain energy metabolism and play an important role in treating NDD. It can reverse the phenomena of decreased numbers of mitochondria, structural swelling, vacuole degeneration, distortion or disappearance of the mitochondrial crest, decreased mitochondrial membrane potential (MMP), and enhanced Cyt c expression, apoptosis, and oxidative stress. What is more, GSH, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) expression were increased, caspase-3 expression was downregulated, and tyrosine hydroxylase expression was upregulated. Tenuifolin has a mitochondrial protective function as well, which has been verified in many *in vitro* and *in vivo* studies. It can prevent the loss of MMP, reduce the positive rate of β-galactosidase, increase intracellular ATPase activity, inhibit the increase of inflammatory factors, downregulate the expression of NF-κB and cyclooxygenase-2 (COX-2), upregulate the expression of PPARγ and PGC-1α, and inhibit the activation of caspase-3 and caspase-9 (Wang et al., 2019; Jin et al., 2022; Li et al., 2023b). In recent years, significant changes in lipid metabolism have been observed in the brains of AD models, which may be a new target of NDD (Zhang et al., 2021), but it is not clear whether PR has an effect on this target. In order to further reveal the active mechanism of PR, laser microdissection, in combination with high-performance liquid chromatography-mass spectrometry, can be used to probe the probable lipid changes in the micro area in the brains of AD models before and after treatment.

Antioxidative stress effects

PR has played an anti-AD role in mouse and PC 12 cell models induced by Aβ1-42, which improved the cognitive impairment of mice, increased cell viability, and inhibited apoptosis. A mechanism study showed that PR reduced the contents of reactive oxygen species (ROS) 8-hydroxy-deoxy-guanosine (8-OHdG) and 3-nitrotyrosine (3-NT), activated the PI3K/Akt signaling pathway, and increased the ratios of P-PI3K/PI3K, pAkt/Akt and Bax, enhanced heme oxygenase 1 (HO-1), and promoted nuclear factor E2-related factor 2 (Nrf2) nuclear translocation (Ren et al., 2022). The protective effect of onjisaponin B on a 1-methyl-4-phenyl-1.2.3.6-tetrahydropyridine (MPTP)-induced PD mouse model is achieved through the antioxidant and anti-

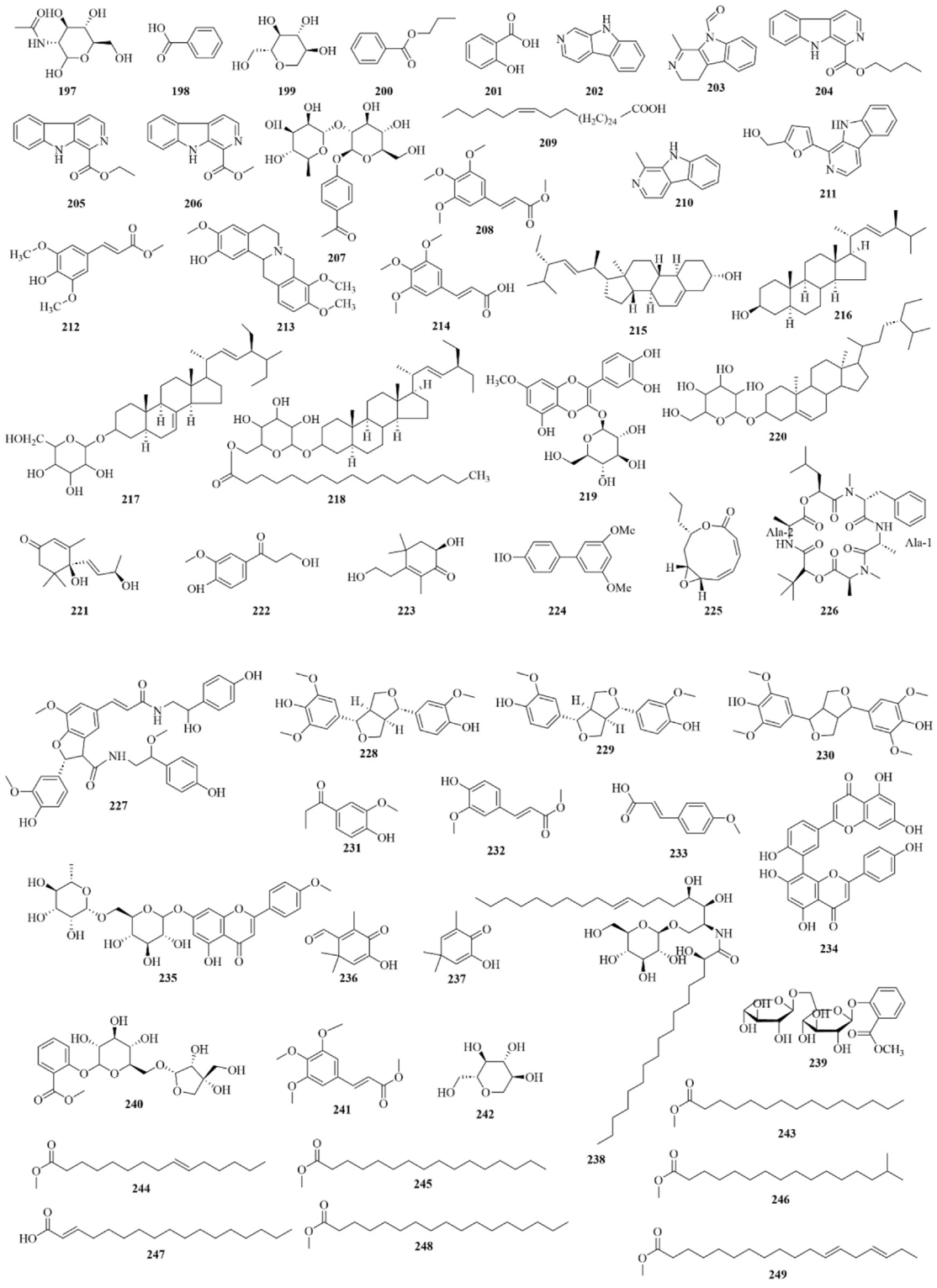

FIGURE 6
Structures of other constituents (197–249) in PR (1).

inflammatory activities mediated by the RhoA/ROCK2 signaling pathway (Peng et al., 2020b). Tenuifolin can prevent the upstream activating factor of NF-κB and the migration of NF-κB to the nucleus and inhibit the release of inflammatory factors and the expression of inducible nitric oxide synthase (iNOS) and COX-2, thus antagonizing nitric oxide (NO)-induced oxidative stress and protecting BV2 and SH-SY5Y cells from Aβ42-induced inflammatory and oxidative stress (Chen and Jia, 2020). It can

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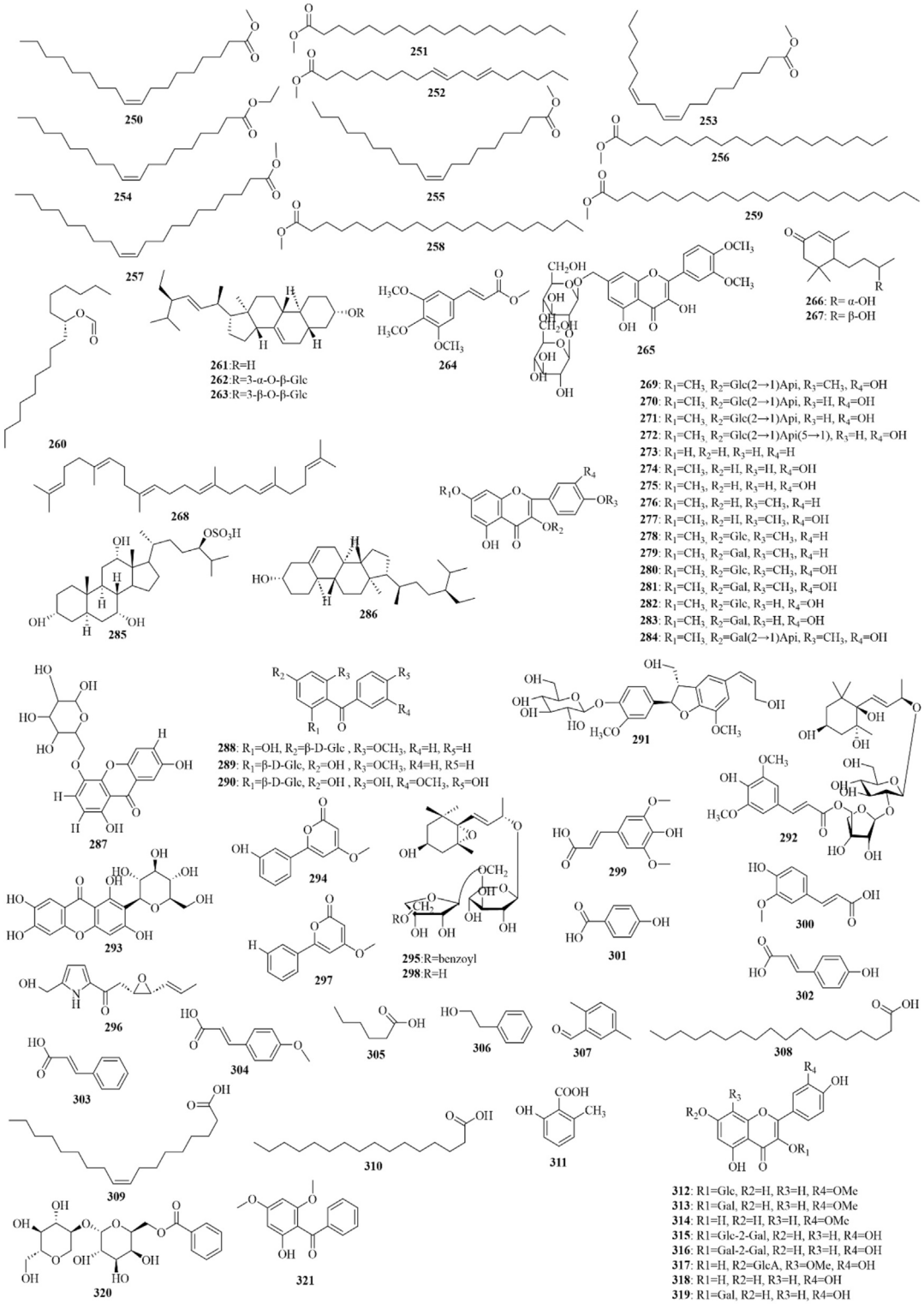

FIGURE 7
Structures of other constituents (250–321) in PR (2).

also improve the behavioral performance of sleep-disordered rats induced by sleep deprivation in a Y maze, object recognition, and an avoidance test, stimulate the production of anti-inflammatory factor IL-10, reduce the production of pro-inflammatory factors IL-1β, IL-6, and IL-18, and activate microglia and increase the expression of 2-related factor 2 and heme oxygenase-1 (HMOX1). At the same time, the expressions of NLRP 3 and caspase-1 p20 were inhibited, the signal cascade of BDNF was

downregulated, and the damage to the hippocampal nerve was alleviated (Jiang et al., 2023).

Anti-central cholinergic system dysfunction effect

The binding of tenuifolin and onjisaponin B with ChEs was studied using various spectral techniques (Gao and Du, 2022). It was found that there was mainly hydrophobic force between tenuifolin and acetylcholinesterase (AChE), which did not affect its structure, quenched its fluorescence dynamically by affecting tryptophan, and had no inhibitory activity on ChEs. The inhibitory effect of tenuifolin on AChE in these two experiments is the opposite, which may be due to the difference between the internal and external environments. More experimental evidence is needed. Onjisaponin B mainly binds ChEs by van der Waals forces and hydrogen bonds and changes their structures, which has cholinesterase (ChE) inhibitory activity by influencing the static quenching of endogenous fluorescence of tyrosine residues. Similarly, due to the difference in the internal and external environments, the enhancement potential of onjisaponin B on the central cholinergic system needs to be verified in more disease models. The aerial part of PR improved learning and memory impairment induced by D-galactose/$NaNO_2$ and scopolamine in mice, increased the levels of ACh, choline acetyltransferase (ChAT), BDNF, IL-10, glutathione, and SOD, and decreased the levels of IL-1β, AChE, and malondialdehyde (MDA) (Wang et al., 2020a). In addition, it also increased the protein and mRNA expressions of BDNF and tropomyosin receptor kinase B in the hippocampus (Zhang et al., 2020). These inspiring results show that scholars should compare the pharmacological effects and mechanisms of the aerial part of PR with its root. Expanding its application scope would be beneficial to reducing waste.

Anti-neuroinflammatory effects

Seventeen saponins, including onjisaponin B, can induce mitosis and inhibit NOD-like receptor thermal protein domain associated protein 3 (NLRP3) inflammatory corpuscles by up-regulating SHP-2 to activate AMPK/mTOR and PINK1/parkin signaling pathways in Aβ1–42-, A53t-α-synuclein-, or Q74-induced BV-2 cells (Qiu et al., 2022). TEN can scavenge free radicals, downregulate NO, ROS, various inflammatory factors [TNF-α, IL-1β, IL-6, COX-2, prostaglandin E2(PGE2)], matrix metalloproteinases (MMP-9 and MMP-2) and other cytotoxic factors, and upregulate transcription factors related to oxidative stress (Nrf2 and HO-1) (Pi et al., 2020). The regulation of inflammatory and oxidative stress-related transcription factors is also one of the mechanisms by which tenuifolin improves the dyskinesia of the demyelinating mouse model induced by cuprizone (CPZ), increasing the myelin content in the corpus callosum, thus alleviating demyelination and treating multiple sclerosis (Li et al., 2023c). Through a combination of network pharmacological prediction and *in vitro* and *in vivo* experiments, it was found that the anti-inflammatory and anti-apoptosis effects of PA may be realized through PPARγ/NF-κB pathway because its effects can be reversed by PPARγ inhibitor GW9662 (Zhao and Jia, 2024). The development of network pharmacology and molecular docking technology makes elucidating the mechanism of action of drugs convenient. With this help, the complex pathways and targets of PR will become clear. Another polysaccharide from PR, PTP70-2, reduces ROS overproduction and MMP dissipation, which may be related to its downregulation of MyD88 and NF-κB signals through the toll-like receptor 4 (TLR4) pathway. Co-incubation with TLR4 inhibitor TAK242 also supports this possibility (Chen et al., 2022a). In addition, the heteropolysaccharide PTBP-1-3 isolated from the crude polysaccharide alkali extract of PR, which can inhibit the activation of microglia/astrocytes induced by lipopolysaccharide (LPS), reduces the production of NO, TNF-α, and IL-1β in a manner similar to minocycline (Zeng et al., 2022). The effective mechanisms of these new polysaccharides need to be further revealed and clarified, laying the foundation for them to be lead metabolites.

Neuron protection and regeneration effects

One study showed the effect of DISS was better than onjisaponin B and tenuifolin (Wang et al., 2021b). Bioinformatics methods and verification and verification based on *in vitro* experiments revealed that DISS can also prevent intracellular calcium overload and abnormal calpain system, increase synaptic protein expression, reduce m-calpain expression, inhibit oxidative stress and iron death, and upregulate BDNF/tropomyosin-related kinase B signal transduction with the help of the and d-galactose combined with Aβ1—42 (GCA) in an induced AD model (Li et al., 2023d).

Other effects

In addition to the mechanisms mentioned above, PR can also prevent and treat NDD by exerting estrogen-like effects that regulate the intestinal microenvironment and the immune system. PR reversed these phenomena in a mouse model of estrogen exhaustion induced by 4-vinylcyclohexene diepoxide and increased the expression of Bcl-2-associated athanogene (BAG1) in the hippocampus without increasing the serum estradiol level (Han et al., 2021). The methanol metabolite of PR containing 17 saponins can reverse the abnormal increase of C3 complement protein, increase beneficial bacteria in the intestine, inhibit harmful bacteria to regulate the intestinal microenvironment, improve spatial memory of mice, and weaken neuroinflammation and aging and improve the behavior of *C. elegans* and prolong its life (Zeng et al., 2021b).

Anti-cardiovascular effects

Modern research shows that TEN can significantly improve neurological function and reduce brain edema, hematoma volume, and hemoglobin level in an intracerebral hemorrhage (ICH) model 72 h after administration with the optimal dosage of 16 mg/kg by regulating 26 differentially expressed proteins mainly related to the complement and coagulation cascade (Wang et al., 2024). Both the root and aerial parts of PR can protect against myocardial injury induced by isoproterenol in rats by inhibiting oxidative stress. There was no significant difference between them, which once again proves that the aerial parts of PR have medicinal value (Fu et al., 2022). TEN has protective effects on brain diseases caused by hypoxia/reoxygenation and ischemia/reperfusion, and its mechanism is related to neuroprotection, inhibition of lipid peroxidation, apoptosis, and iron death. In the H/R model, it can increase the

cell survival rate, enhance the expression of JNK and -c-Jun proteins, inhibit their phosphorylation, upregulate Bcl-2, SOD, GSH, GSH-Px, GPX4, SLC7A11 and MMP, inhibit Bax, caspase-3, NOX, LDH, MDA, ACSL4, and decrease intracellular ROS accumulation and Ca^{2+}, Fe^{2+} levels (Liao et al., 2019; Qiu et al., 2021). Additionally, TEN could cooperate with endothelial progenitor cell transplantation to improve cardiac function in rats with acute myocardial infarction (Jin and Qiao, 2019). Generally speaking, PR has a strong therapeutic effect on cardiovascular and cerebrovascular diseases by regulating calcium channels, inhibiting cell death, regulating mitochondrial function, inhibiting oxidative stress, and inhibiting inflammation. TEN is its main effective metabolite.

Anti-mental disorder effects

Under the influence of COVID-19, in 2020, the number of patients with major depression and anxiety increased by 28% and 26%, respectively (Collaborators, 2021). According to the WHO World Mental Health Report 2022, nearly 1 billion people in the world suffer from mental illness. Statistics show that by December 2023, there were 6.988 million registered patients with severe mental disorders in China. Mental illnesses, such as depression, anxiety disorder, schizophrenia, bipolar disorder, and post-traumatic stress disorder, can not only lead to disability and suicide but can also lead to a significant reduction in life expectancy (Chan et al., 2023). Scholars are looking for and developing drugs to prevent and treat these diseases that bring heavy economic burdens and social problems to individuals, countries, and even the world. PR has attracted much attention because of its anti-mental disorder effect, and the related effects and mechanisms are summarized as follows.

Antidepressant effects

Depression is the most common psychological disorder with high incidence and high mortality, and patients often have significant and persistent depression and pessimism. In addition to the effects of genetics and adverse life circumstances, the pathogenesis of depression involves multiple abnormalities of the monoamine neurotransmitter hypothalamic-pituitary-adrenal (HPA) axis, inflammatory cytokines, neuroplasticity and neurogenesis, and brain structure and function. Depression is triggered when there is a decrease in the level or function of neurotransmitters (e.g., 5-hydroxytryptamine (5-HT), DA, NE) and ACh in the synaptic gaps of the CNS that can affect the body's sense of pleasure, wellbeing, transmission of emotions, and maintenance of learning and memory abilities. PR can inhibit the decreased levels or function of these neurotransmitters, exerting antidepressant effects similar to those of monoamine or triamine reuptake inhibitors. The effects of these two metabolites on animal models are unknown. In addition, polygalatenoside A compounds extracted from other plants have been reported to have antimelanoma potential (Kim et al., 2022). Wang et al. found that the antidepressant mechanism of tenuifolin was related to increasing the expression of 5-HT and decreasing the expression of indoleamine 2; 3-dioxygenase (IDO) in the cerebral cortex and inhibiting the AChE activity and increasing the activity of ChAT in the hippocampus of mice (Wang, 2020). PR can reverse abnormal behavior and enhance expression of LC3-II and beclin1 in chronic restraint stress (CRS) rats in the sucrose preference test, novelty inhibition feeding test, open-air test, and forced-swim test (FST), and it also reduces P62 levels in both in the cortex of mice and prefrontal cortex of rats and reverses microglia activation, astrocyte damage, NLRP3, ASC, caspase-1 protein and pro-inflammatory cytokine expression increases (Zhou et al., 2021).

The antidepressant effect of PR is also related to regulating intestinal flora, neuroprotection, and affecting metabolism *in vivo*. An extract and oligosaccharide ester of PR improved the depressive behavior of rats with chronic unpredictable mild stress (CUMS), increased the levels of NE, 5-HT, dihydroxyphenylacetic acid (DOPAC), and 5-hydroxyindoleacetic acid (5-HIAA) in the hippocampus, and decreased the levels of CRF, adrenocorticotropic hormone, corticosterone, IL-6, and LPS in serum. The results of transmission electron microscope observation show that PR can improve the structure of intestinal flora, restore the function of the intestinal barrier, reduce the release of intestinal endotoxin, and reduce the level of inflammation, thus playing an antidepressant role (Chen et al., 2021; Chen et al., 2023). Another study showed that the extract of PR could inhibit the activation of NF-κB-NLRP3 signal and intestinal inflammation, increase probiotics such as *Lactobacillus* and *Bacteroides*, regulate the content of metabolites and enzyme expression related to the metabolism of tryptophan-kynurenine in the colon, and increase the expression of tight junction protein to balance the gastrointestinal environment (Li et al., 2022a). However, it is necessary to screen the effective monomer metabolites in the extract and further explore whether the metabolites exert synergistic effects by influencing each other's structures or generating co-assemblies. Yunfeng Zhou first discovered the disorder of metabolic pathways such as amino acids, energy, intestinal flora, and purine in the urine and hippocampus of olfactory bulbectomy rats by non-targeted metabonomics. This study confirmed that PR could enhance autophagy, correct the abnormality of the AMPK-mTOR signal pathway, inhibit neuroinflammation, improve neuroplasticity, play an antidepressant role, and improve the depressive behavior in a despair model, chronic unpredictable stress model, and an olfactory bulb resection model (Zhou, 2020). In a word, PR acts on depression through multiple channels and multiple targets, and it has great potential to be developed as an antidepressant.

Sedative, hypnotic, antiepileptic, and anxiolytic effects

Statistics from the WHO show that 27% of people in the world suffer from sleep disorders. Sleep disorder is not only an important pathogenic factor of NDD and mental diseases but also a complication. PR has sedative, hypnotic, antiepileptic, and anxiolytic effects and can be used to improve sleep disorders. PR plays a sedative role in zebrafish by inhibiting 5-HT reuptake, activating GABA, and improving the expression level of GABA transporter 1 (Chen et al., 2020). In the senile insomnia rat model, PR improved the animals' weight, memory, and sleep time, increased the levels of 5-HT water and GABA, decreased Glu level, and downregulated Fuom and Pcp2, which indicated that the effects of *Polygala tenuifolia* on sleep disorders might be related to nerve and metabolic pathways in addition to the GABA signaling

pathway (Ren et al., 2020). PR has been proven to regulate the disorder of circadian rhythm by regulating the CaMKII pathway and treating disorders caused by sleep delay syndrome, extreme nighttime activities, and social jet lag (Haraguchi et al., 2022). Zhang et al. (2023) used the selective antagonist of the GABA benzodiazepine (BZ) site and confirmed that TEN promoted a kind of non-rapid eye movement (NREM) sleep equivalent to physiological NREM sleep through the interaction with GABAA receptor and also confirmed the improvement of sleep disorder of PD mice by TEN. This suggests that animal models with two or more diseases could be used to explore the role of *Polygala tenuifolia*, which is conducive to further understanding its mechanism of treating different diseases with the same treatment. Recently, a study showed that two saponins of PR, YZ-I and YZ-II, had sedative and hypnotic effects, increased the concentration of 5-HT, NE, PGD2, IL-1β, and TNF-α, and inhibited its inflammation to GABAARα2, GABAARα3, GAD65/67, 5-HT1A, and 5-HT2A, as well as DPR, PGD2, iNOS, and TNF-α (Hao et al., 2024). Moreover, the purer YZ-II has a stronger effect on inflammatory factors and inflammatory mediators in a concentration-dependent manner, which suggests that it is necessary to further explore the differences in composition and mechanism of action between the two metabolites and that monomer metabolites also have important research value.

Anti-traumatic stress disorder effects

Note that PR plays a major role in Ren Shen Yang Rong Tang, a decoction used to treat several conditions, for improving the low sociality in NPY-KO zebrafish and increasing its time spent in the fish tank area by inhibiting the activity of HPA-, SAM-, and GABAergic neurons and down-regulating CREB signaling (Kawabe et al., 2022). Systematically screening the metabolites of PR that have therapeutic effects on social disorders, verifying their activities *in vitro* and *in vivo*, and exploring their mechanisms could prove useful in promoting their further development.

Anti-aging and anti-fatigue effects

A study has shown that polysaccharides of PR can prolong the movement time of mice, increase the glycogen storage concentration in the liver and muscle of mice, enhance the activity of lactate dehydrogenase *in vivo*, and inhibit the production of serum urea nitrogen in tired mice, and these effects will be enhanced with the increase in dose (Xie, 2021). However, further studies on the related mechanisms and pathways are needed to reveal the connotation of polysaccharides in PR against exercise fatigue. In the weight-loaded swimming test, hypoxia tolerance under normal pressure, sodium nitrite poisoning survival test, and swimming with load and fatigue rotating rod test, the extracts of PR decreased serum CK, blood urea nitrogen (BUN) and LA, cleared sports metabolites, increased the levels of SOD, GSH, ATP, succinate dehydrogenase (SDH), and malate dehydrogenase (MDH), decreased MDA, and increased the protein expression levels of AMPK and Nrf-2, thus effectively relieving the fatigue and hypoxia of normal mice and hydrocortisone-induced kidney-yang deficiency mice (Wang, 2020c). However, the active monomers contained in PR need to be systematically screened. Overall, PR has the potential to be developed into food additives and health products because of its anti-aging and anti-fatigue effects.

Anti-tumor effects

Research has revealed that PR is composed of Ara, Gal, and Glc (molar ratio: 2.6: 1.8: 1.0) and has α and β configurations. It can induce apoptosis of lung cancer cells, upregulate the cascade reactions of FAS, FAS-L, and FADD to express the caspase family, induce apoptosis, upregulate LC 3B-II, and downregulate P62 to induce autophagy (Yu et al., 2020). In S180 sarcoma cells and S180 tumor-bearing mice, it increased the ratio of Bax and Bcl-2, promoted cytochrome c release and caspase-9/-3 expression, revised the immune organ indexes, the activities of NK cells and lymphocytes, and increased secretion of IL-2, interferon-γ (IFN-γ) and TNF-α (Yu et al., 2021). The RP02-1 also can inhibit the growth of pancreatic cancer cells, regulate Bcl-2 and Bax, and cleave caspase-3 to inhibit the proliferation, migration, and colony formation of cancer cells *in vivo* and *in vitro* with no toxicity (Bian et al., 2020). However, the safety of these polysaccharides needs further evaluation. In addition, targeted metabonomics technology can be used to study the pharmacokinetics of polysaccharides in target organs, revealing their absorption mechanisms and structure-activity relationships, which is helpful in guiding their structural modification.

Song et al. (2019) found the complex euxanthone with Cu (II) has an inhibitory effect on esophagus cancer cells (ECA109), stomach cancer cells (SGC7901), and cervical cancer cells (HeLa) by intercalating and damaging their DNA to inhibit their growth. A study in 2021 used virtual screening, molecular docking, and molecular dynamics simulation to screen and verify that PX3 can inhibit an X-linked inhibitor of apoptosis protein and promote the release of caspases to induce apoptosis, thus treating cancer (Opo et al., 2021). Moreover, 4-O-benzoyl-3′-O-(O-methylsinapoyl)-sucrose from PR can inhibit glucuronidase to relieve side effects such as intestinal bleeding and diarrhea caused by anticancer drugs (Kim et al., 2021). However, these studies have only been verified at the *in vitro* level and lack *in vivo* data.

Anti-pathogenic microorganism effects

PR has an inhibitory effect on severe acute respiratory syndrome coronavirus 2 *in vitro*, with an IC_{50} value of 9.5 g/mL (Ngwe Tun et al., 2022). This shows that it has a potential therapeutic effect on COVID-19. More *in vitro* and *in vivo* experiments are needed to reveal its mechanisms and targets.

Other effects

A chloroform extract of PR could inhibit the main target of diabetes—protein tyrosine phosphatase 1B (PTP1B), and xanthones, sterols, and fatty acids were speculated as the main active substances (Mei et al., 2021). The seed oil of PR can inhibit total cholesterol (TC) and triglyceride levels in plasma and the liver, reduce IL-6 and

TNF-α levels, inhibit the NF-κB signaling pathway, and promote the inactivation of steroid regulatory element-binding protein-1 (SREBP1) and SREBP2 involved in lipogenesis (Xin et al., 2023). Therefore, it can potentially be developed as a dietary supplement for inhibiting metabolic-associated fatty liver disease.

PR can shorten the clotting time of mice, and the activity of ethyl acetate and n-butanol extracts is stronger than that of water extracts. The anticoagulant substances are mainly xanthones and OEs, and oleic acid and linoleic acid play an auxiliary role (Li et al., 2020). However, it is not clear whether PR acts on endogenous, exogenous, or common coagulation pathways, and it is necessary to further study its influence on coagulation factors. Onjisaponin B can target and regulate p65/Cas3 to resist radiation (Wang et al., 2022).

PR also has anti-inflammatory activities *in vitro* and has inhibitory effects on pro-inflammatory cytokines IL-12, p40, IL-6, and TNF-α. What is more, the sugar units at C-3 and/or C-28 of the aglycon in triterpenoid saponins and substitute groups in molecules of phenolic glycosides, especially –OH, –OCH3, and –COOH, were considered to be critical to their anti-inflammatory effect (Vinh et al., 2020). 3-O-(3,4,5-trimethoxy-cinnamoyl), 6′-O-(p-methoxybenzoyl) sucrose ester (TCMB), onjisaponin Fg, and 3,4,5-trimethoxycinnamic acid methyl ester can significantly inhibit NO and PGE2. TCMB, 3,4,5-trimethoxyxanthone, hydrocotoin, and 3,4,5-trimethoxycinnamic acid methyl ester can inhibit PGE2 most significantly. TCMB can decrease the protein levels of iNOS and COX-2 and the mRNA levels of TNF-α, IL-1β, and IL-6. In addition, the results of molecular docking show that it has an affinity for iNOS and COX-2 (Son et al., 2022). Sun et al. (2023) developed an ultra-high performance liquid chromatography-photo-diode array-electrospray ionization-quadrupole-time-of-flight-mass spectrometry-lipoxygenase-fluorescence detector (UPLC-PDA-ESI-Q-TOF-MS-LOX-FLD) method that can rapidly screen small molecular inhibitors of LOX and other enzymes and determined for the first time that tenuifoliside A, sibiricaxanthone B, 7-O-methylmangiferin, polygalaxanthone Ⅲ, onjisaponin B, onjisaponin F, and onjisaponin Z are the active anti-inflammatory metabolites. These experiments brought new anti-inflammatory metabolites of PR into our view, although they have only been verified *in vitro*. More animal experiments and clinical trials are needed to further validate these anti-inflammatory effects and provide a scientific basis for its selection as a natural anti-inflammatory metabolite for clinical treatment. Its structure can be optimized to enhance its targeting and stability, making it better applied to the development of anti-inflammatory drugs.

The extract of PR (1.2 mg/mL), consisting of eight OEs, two xanthones, and two saponins, had good 2,2-diphenyl-1-picrylhydrazyl (DPPH)·and 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonate (ABTS+)·scavenging capacity with a scavenging rate over 80%, and the synergistic effect of oligosaccharide esters and xanthones was presumed to be the material basis (Guo et al., 2019). Future scholars will be able to study the effects of different extraction methods and processes on the antioxidant activities of these polysaccharides. The aerial part of PR can improve the learning and memory ability of model mice, reduce the MDA content in serum, heart, brain, liver, and kidney tissues, and increase SOD, CAT, GSH-Px, and total antioxidant capacity in serum and tissues (Zhang et al., 2019).

In summary, PR has broad-spectrum pharmacological activity, and its related pharmacological research has achieved fruitful results, which provides a sufficient scientific basis for its application. Its further development offers many opportunities and challenges. First, the pathogenesis of various diseases, especially nervous system diseases and cancers, is extremely complicated, and the molecular mechanisms and material basis of PR are still not clear. Advanced technologies, such as spatial chemoproteomics and the combination of deep learning and ultra-high resolution confocal laser scanning microscopy, can be used to observe the changes in cell morphology and function of PR, which is helpful to further understand its pharmacological process and find its targets. In addition, Qualcomm quantitative metabolomics can be combined with LC-MS to screen and identify effective monomer metabolites and small molecular metabolites in the extracts and effective parts of PR. Second, in recent years, with the iteration of science and technology and the development of analytical methods, an increasing number of active metabolites have been separated from PR, especially those with anti-inflammatory activity, further revealing the material basis of pharmacological activities of PR. However, the effects of some metabolites are only reflected *in vitro*; in view of the different metabolic environments *in vivo* and *in vitro*, their activities need to be verified *in vivo* in animal models. Third, disease models based on nematodes, cells, fruit flies, rats, and mice are used to study pharmacological activities at present. In the future, rhesus monkeys, domestic dogs, and other animal disease models with genetic relationships and metabolic pathways that are more similar to humans can be used for the pharmacological study of PR, which will be conducive to improving the success rate of clinical transformation. Fourth, more toxicological and clinical experiments should be carried out on some metabolites that have shown good pharmacological activities *in vitro* and *in vivo* to provide a scientific basis for ensuring their safety and effectiveness and promoting their further development and rational application. Finally, the aerial parts of PR, which are always discarded, also have rich metabolites and similar pharmacological effects. Future research can systematically compare the differences in activity and metabolites between these parts and the roots of PR and provide scientific support for their medicinal value.

Toxicity

Although PR is rich in active ingredients and has systemic pharmacological effects, its toxic and side effects have been reported in ancient and modern literature, which limits its application to some extent. Modern pharmacological studies have found that the toxicity of PR is mainly aimed at the gastrointestinal tract. PR shows irritation to the gastrointestinal tract in rat and mouse models, which can reduce their activities and food intake, affect their hair, inhibit small intestine movement and gastrointestinal emptying, cause gastrointestinal flatulence, stomach volume expansion, intestinal wall thinning, necrosis, mucosal defects, inflammatory cell infiltration, reverse peristalsis, direction, blood vessel congestion, endothelial cell swelling, and other pathological tissue changes and deaths. The results of biochemical indexes showed that PR could inhibit the levels of gastrin (GAS), motilin, substance P (SP), PGE2, Ca2+-ATPase,

pepsin activity, and intermittent cells of Cajal (ICC), cause slow waves in gastric electrogram amplitude, and increase the levels of vasoactive intestinal peptide (VIP), somatostatin (SS) and NO (Cui et al., 2021). Fortunately, the toxicity of PR can be reduced or eliminated by compatibility with other herbs or processing to promote the transformation of toxic substances or introduce new substances to counter its irritation to the gastrointestinal tract. For example, the content of magnolol in gastrointestinal fluid increases after the compatibility of PR and *Magnolia officinalis* cortex, which can inhibit the increase of NO content and VIP expression, regulate the metabolism of energy, amino acids, and fat, and reduce its gastrointestinal toxicity (Ma et al., 2019; Ma et al., 2021). Licorice-simmered PR can improve its irritation to zebrafish and rabbits by reducing DISS and PA, inhibiting the increase of inflammatory factors, and relieving the congestion and swelling of rabbit eyes as well as the damage of iris, cornea, and conjunctiva caused by raw PR (Chen et al., 2024). The aerial parts of PR have no acute toxicity (Sun et al., 2020). The existing toxicological research on PR and its active metabolites is not enough to completely prove its safety. It is necessary to carry out a series of systematic toxicological experiments, including but not limited to skin irritation experiments, mutagenic experiments, and teratogenic experiments.

Industrial applications

PR is one of the medicinal and edible species approved by the Chinese government for use as a raw material in health foods. More than 70 health products contain PR or its extracts in the Yaozhi Data, most claiming to improve sleep quality. Other products purport to increase bone density, enhance immunity, delay aging, combat fatigue, clear throat, and improve memory. Yue. et al. developed a metabolite beverage with flavedo and polysaccharides of PR that has a good effect on exercise-induced fatigue (Yue et al., 2020). PR is also utilized for medicated diets with high nutritional value, such as Yuanzhi Lianfen Zhou, which is made of PR, polished japonica rice, and lotus seed. It has efficacy in nourishing the heart, improving intelligence, hearing, and eyesight, and relieving amnesia and insomnia.

According to Gaide Chemical Network statistics, *Polygala tenuifolia* extract and *Polygala tenuifolia* root extract are used as skin conditioners in about 207 and 394 cosmetics, respectively, with top safety and no acne risk. These products are various, including liquid foundation, toning lotion, cream, essence, hair tonic, cleansers, shampoo, and foot bath powder. For example, *Polygala tenuifolia* root extract acts as a skin conditioning agent in "The History of Whoo Cheongidan Hwa Hyun Lotion (后天气丹花献滋养乳)."

In addition, PR was made into an antibacterial sleep-aiding bamboo cellulose fiber for home textiles with the effect of helping sleep [CN202211232475.4] (Hu et al., 2023), an atomized liquid with sedative and soothing effects [CN202211087989.5] (Zhu and Liu, 2022), a composition for promoting skin regeneration and improving skin wound surface [CN202180020964.0] (Pu, 2022), a sleep-assisting tea capable of improving sleep [CN 202180020964.0] (Zhao, 2022), pills for improving intelligence and height [CN202210494926.5] (Zhou, 2022), a mask capable of whitening and removing freckles [CN202210505643.6] (Wang, 2022), a composition capable of curing the effects of drink [CN202211063577.8] (Zhang et al., 2022), and other products. Interestingly, PR can also be used as an additive in pig feed to increase the food intake and body weight of growing-finishing pigs [CN202210343455.8] (Zhan et al., 2022).

To sum up, PR has important practical and economic value and can be used to produce many products. We offer the following suggestions to maximize PR applications: First, with the awakening of public awareness of healthcare, a medicated diet shows great potential. Future research could mine medicinal diet data containing Yuanzhi from ancient and modern literature, explore effects and elucidate mechanisms via *in vivo*, *in vitro*, and clinical experiments, summarize differentiated effects across populations, and establish a unified quality control system. These efforts would solidify the foundations for industrialized mass production and market launch. In the second place, the application of PR in cosmetics is still in a marginalized position at present, and most products only utilize the cleaning effect of saponins; future development could address its antioxidant, anti-aging, and anti-inflammation effects and increase its use in products such as maintaining skin barrier stability and improving complexion. The nude mouse model can be used to investigate the effects of these metabolites on skin conditions. Next, effective metabolites and plant additives can be extracted from the aerial and other nonmedicinal parts of PR, reducing waste. Last but not least, the safety of the active ingredients and effective parts of PR should be investigated through the evaluation of the tolerance, toxicological safety, metabolism, and residue of the target animals, which can promote its application in the form of additives in health food, cosmetics, and food.

Processing research

After processing, the chemical composition and structure and the properties of TCM have changed, and the toxicity is suppressed, which is more in line with the needs of TCM to adapt to the time, local conditions, and people. As a traditional Chinese medicine with superior efficacy and obvious toxic side effects, PR should be processed before clinical application. The ancient processing methods of PR include purification processing methods of removing the wood heart, pounding or grinding into powder, plain stir-frying methods of stir-frying slightly, stir-frying to yellow, baking, and stir-frying to scorch, as well as stir-frying with many different adjuvants (Gao et al., 2020). According to many ancient books, licorice, ginger juice, wine, wheat, black bean juice, bile, rice-washed water, honey, and other auxiliary materials are often used in PR processing. Ancient processing methods of PR are listed in Supplementary Table 3. In addition, there are records of processing PR with two or three of the above adjuvants; the method of decocting PR with black beans and licorice together was recorded in "Jing Yue's Complete Work" (景岳全书). The common processed products of PR in modern times mainly include stir-fried PR, deep-fried PR, stewed PR, PR stir-fried with licorice juice, *Magnolia officinalis* cortex juice or honey stir-fried PR and, cinnabar mixed PR (Wu et al., 2024). Among them, PR stir-fried with

Magnolia officinalis cortex juice and stewed PR are not included in the processing standards of Chinese herbal pieces, and few studies have examined PR mixed with cinnabar. The modern PR processing methods are shown in Supplementary Table 4.

Removing the wood heart

Modern scholars differ on whether to remove the xylem of PR. On the one hand, they think that although the content of effective metabolites is low in xylem, it also has medicinal value (Liu et al., 2012) and should not be removed. On the other hand, they think that the xylem should be removed because the concentrations of its effective metabolites are several times lower than the phloem (Yang et al., 2018). The amino acids and small molecular organic acids contained in the xylem act on NMDA receptors by acting on 91 potential targets such as SLC6A1, GRM5, GRIA1, and SLC1A1, which makes people feel depressed or anxious (Lou, 2023). At present, the various Chinese herbal medicine quality standards have no compulsory requirement to remove the xylem, and more experimental data are required to reach a consensus on this dispute.

Effect of processing on chemical constituents of PR

The increase of extractive substances after PR processing may be due to the introduction of liquiritin, glycyrrhizic acid, glucose, and other metabolites that increase with the addition of adjuvants, which can be used as a quality control index (Zhu et al., 2019; Cui et al., 2020). Saponins and oligosaccharide esters in PR are unstable and can be hydrolyzed during the processing. One is deglycosylated, and its glycosidic bonds in C3 or C28 were broken, and the other has its ester bonds broken, resulting in the generation of the second-level glycosides and (or) glycosides with less toxicity (Gao et al., 2021). In addition, licorice can promote the hydrolysis of some saponins in PR into onjisaponin Z and tenuifolin, and stewing PR with licorice can promote the absorption and distribution of oligosaccharides and increase their bioavailability (He et al., 2023; Xiong et al., 2023). The changes in the material basis after processing have important implications for reducing toxicity and increasing the efficiency of PR (Zhao et al., 2021). However, the potential co-assembly phenomena, regularity, and supramolecular mechanisms between various excipients and chemical metabolites of PR need further study.

Processing technology research of PR

The *Chinese Pharmacopoeia* and local processing standards do not specify processing parameters of different PR processed products, and adjuvant dosages and terminal points are not uniform. Currently, the terminal points are judged subjectively by color or whether products are sticky to hands. With the development of analytical technology and understanding of effective substances of PR, its technological optimization indexes have evolved from single extraction content to comprehensive indexes. For example, Yuan et al. (2021) optimized the technological parameters of PR stir-frying with licorice or honey based on seven metabolites. Song et al. (2023) optimized the water addition, licorice dosage, and drying process parameters based on the comprehensive score of five metabolites and established the relationship between the color and metabolites of stewed PR, which provided an important reference for determining the terminal point. However, it is necessary to carry out pre-production tests and scale-up verifications on the optimized processing technology to adapt to modern production and form a standardized operation flow for each processing technology of PR.

Conclusion and perspectives

PR is a natural product with a long medicinal history, rich chemical metabolites, wide pharmacological effects, and high medicinal value. Research has achieved fruitful results and provides a scientific basis for its wide application in medicine, healthcare products, and cosmetics. However, some shortcomings need to be solved in follow-up studies.

As a popular best-selling herb at home and abroad, the scarcity of wild resources and the long artificial planting cycle put PR in short supply. The aerial parts and wood heart of PR, which were often discarded in the past, have been shown to have medicinal potential, which suggests that their chemical constituents and pharmacological effects should be studied and compared with the root bark of *Polygala tenuifolia* to expand the source of PR and rationally utilize resources.

PR is widely used in medicine pairs, composition, compound prescriptions, healthcare products, and cosmetics. However, the compatibility mechanisms of related drug pairs, compositions, and compound prescriptions have not been fully revealed, and the interaction between the chemical metabolites of the prescriptions is still unclear and needs further study. In addition, its active ingredients need to be evaluated for safety in order to expand its applications in cosmetics and health products.

With the awakening of modern health awareness, the related medicated diets recorded in ancient books and documents show great potential. Future research can study the compatibility principle, proportion, and applicable populations and promote the emergence of innovative products.

PR contains more than 320 metabolites, of which saponins, oligosaccharide esters, and xanthones are the main metabolites. The low content of xanthones may be the reason for less research into these compounds, so more advanced technologies could be used to extract, enrich, separate, and purify them, and more attention should be paid to this kind of metabolite. Few studies report on the structure-function relationships of the active metabolites in PR, limiting the understanding of the potential modifications and optimizations of the structure of its lead metabolites and hindering the artificial synthesis of its active metabolites and the development of related drugs and health products.

The pharmacological effects of PR, especially its effects against nervous system diseases, have been widely studied, but the transformation from scientific research results to clinical results is rarely seen. Future research could reveal the monomer composition of its active sites, explore its synergistic effects, and conduct safety inspections and clinical research on its active metabolites.

Limited toxicological studies show that PR has no toxicity, but it causes gastrointestinal irritation, probably due to its saponins.

Studying the metabolism of saponins in blood, urine, feces, bile, cerebrospinal fluid, and target organs is helpful in clarifying its toxicological mechanism. Furthermore, strengthening the research and safety analysis of the safe range of PR and its active ingredients is conducive to its development and application in drugs, health products, and cosmetics.

Only a few of the many PR processing methods reported in ancient books have been used and supported by modern research. Comparing the chemical composition and pharmacological effects of different processed products of PR recorded in ancient books is beneficial to the inheritance and innovation and provides a reference for its application. In addition, optimizing the processing technology of PR by selecting pharmacological activity indexes *in vivo* based on different medicinal purposes helps obtain targeted processed products.

To sum up, this study reviews and analyzes the botany, phytochemistry, pharmacological action, toxicity, industrial applications, and processing research of PR and looks forward to its future development, aiming at providing a reference for its sustainable development and rational application.

Author contributions

HK: writing–original draft. LK: investigation, writing–review and editing. AH: investigation, validation, and writing–review and editing. AY: investigation and writing–review and editing. HJ: investigation, validation, and writing–review and editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

The project was funded by Heilongjiang Touyan Innovation Team Program, the National Famous Old Traditional Chinese Medicine Experts Inheritance Studio Construction Program of the National Administration of TCM [No. (2022) No. 75], The Seventh Batch of National Old Traditional Chinese Medicine Experts Academic Experience Inheritance Program [No. (2022) No. 76], the National Training Program for Traditional Chinese Medicine Characteristic Technology Inheritance Talents [No. (2023)], Construction of key disciplines of Chinese medicine processing [No. (2023) No. 14061230012], the Traditional Chinese medicine Processing technology inheritance base project. The stomach and intestine icons utilized in the GRAPHICAL ABSTRACT were generated using Servier Medical Art, provided by Servier (https://smart.servier.com/), and were licensed under https://creativecommons.org/licenses/by/4.0/. Some small icons were added on them to convey information. In addition, the texts in this paper were polished and significantly improved by Stork's Writing Assistant (https://www.storkapp.me/writeassistant/).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2024.1450733/full#supplementary-material

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frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 03 October 2024
DOI 10.3389/fphar.2024.1397872

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OPEN ACCESS

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RECEIVED 08 March 2024
ACCEPTED 24 June 2024
PUBLISHED 03 October 2024

CITATION
Rahman U, Younas Z, Ahmad I, Yousaf T, Latif R, Rubab U, Hassan H, Shafi U and Mashwani Z-u-R (2024) Enhancing health and therapeutic potential: innovations in the medicinal and pharmaceutical properties of soy bioactive compounds.
Front. Pharmacol. 15:1397872.
doi: 10.3389/fphar.2024.1397872

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Enhancing health and therapeutic potential: innovations in the medicinal and pharmaceutical properties of soy bioactive compounds

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An extensive examination of the medical uses of soybean bioactive components is provided by this thorough review. It explores the possible health advantages of isoflavones with phytoestrogenic qualities, like genistein, which may lower the risk of cancer. The review highlights the different roles and possible anticancer activities of phenolic compounds, phytic acid, protease inhibitors, lignans, and saponins, among other bioactive components. It also addresses the benefits of dietary fiber and oligosaccharides derived from soybeans for intestinal health, as well as the impact of soy protein on diabetes, obesity, cancer, and cardiovascular health. Conjugated linoleic acid (CLA) has anticancer and cholesterol-lowering properties; its involvement in promoting metabolic processes is also examined. Pinitol is highlighted in the study as a blood sugar regulator with promise for controlling insulin signaling. In this review, we aim to affirm soybeans' potential as a high-functional, well-being food by examining their recently discovered therapeutic and pharmacological capabilities, rather than to improve upon the previous studies on the reported nutritional advantages of soybeans.

KEYWORDS

soybean, pinitol, isoflavones, therapeutic, cancer, diabetes, peptides

1 Introduction

Glycine max or soybean, is a noteworthy subtropical food crop that is prized for its high-quality oil and protein-rich composition, which contribute to its economic significance. Soybeans are widely available and used over the world as a grain and as the main source of raw materials for the production of soymilk and tofu (soybean curds). Soybeans are a Chinese crop that have been cultivated for millennia. They are ranked among the top five main plant foods in ancient Chinese history, along with barley, millet, wheat, and rice (Lee et al., 2020; Swallah et al., 2023). Soybean processing produces a number of useful products, including as oil, bran, flour, soluble extract, and textured protein. In spite of these potential benefits, soybeans have not received enough attention in industrial products and human diets (Swallah et al., 2023). Nonetheless, as more people become aware of soy and its benefits for their health, soy products are becoming more widely available in the functional food market (Feng et al., 2021). This increase in demand is in line with the growing understanding that plants are abundant in phytochemicals, bioactive, non-nutritive, and

TABLE 1 Literature review on therapeutic potential of some major isoflavone and other phytochemicals extracted from soybean.

Bioactive	Dose	Model	Major effects	Results	References
Gaidzein	Genisten at 100 μm	*In vivo* female ICR model	Improved DSS, Expression of IL-1β, IL-6, IL-7, and TNF-α	Isoflavone mediated DSS induced inflammation, linked to improved antioxidant functioning along with TLR4/MyD88 inhibition	Noda et al. (2012)
Genistein	50–100 μm	*In vitro* model	Elviation of SOD, CAT and GSH lvels along with reduced LPS induced NO production	Modulation of inflammatory response via inhibition of PGE2 and NO along with modulation of vital genes	Blay et al. (2010)
Genistein	100 mg/kg of body weight	*In vivo* wistar rat model	Reduction in colonic COX-1 expression and MPO activity	Oral administration of genistein showed anti-inflammatory response	Seibel et al. (2009)
Genistein	200 μm	*In vitro*	TLR mediated IL-6 prudction DNA binding and upregulation of p53 protein	Reduced IL-6 mRNA production along with inhibtry effects for p53	Dijsselbloem et al. (2007)
Genistein mixed with diet	100 mg/kg	*In vivo*	Lowered colonic weight, rectal bleeding and diarrhoea ratio	Alleviation of DSS- caused injury, inflammation and gut dysfunction	Zhang et al. (2017)
Morphine-5	10 mg/kg/day	*In vivo*	Activation of adiponectin and stimulation of peroxisomes proliferator	Reduction of blood glucose levels	Yamada et al. (2012)
SFPs	—	*In vivo*	Inhibition of dipeptidyl peptidase, salivary alpha-amylase and intestinal alpha-glucosidase	Regulation of postprandial glucose response	González-Montoya et al. (2018)

perhaps beneficial compounds (Swallah et al., 2021). This paradigm shift has led to the emergence of the concept of "functional foods."

In these goods, substances originating from plants are added to various forms in an effort to provide advantageous qualities and functions to the body. Soybean represents the confluence of nutritional value and health-promoting characteristics, having been one of the first foods generally recognized for such advantages (Swallah et al., 2023).

Recent research has generally ignored other features of soybeans, such as their nutritional value, in favour of examining the potential medicinal and therapeutic uses of soy bioactive compounds. From a nutritional perspective, foods made from soy have been demonstrated to be beneficial in treating a variety of lifestyle disorders (Wu et al., 2017). In addition to improving bone health, these nutrients can reduce the risk of prostate, colorectal, and breast cancers, among other cancers. Additionally, eating soy has been associated with a lower risk of cardiovascular diseases, type II diabetes, obesity, cognitive decline, renal dysfunction, menopausal symptoms, atherosclerosis, and coronary heart disease, partly because it lowers low-density lipoprotein levels (Xiao, 2011). Recent research suggests that soy consumption may inhibit the activity of delta-6 desaturase (D6D), an essential enzyme involved in the endogenous synthesis of long-chain polyunsaturated fatty acids (LC-PUFA), despite the well-documented effects of increased soy consumption on triglycerides and cholesterol (Gonzalez-Soto et al., 2021). This nuanced perspective highlights the diverse nutritional possibilities of soy-based foods and how they can contribute to overall health promotion in addition to their well-known medicinal and therapeutic advantages.

The primary objective of this review is to systematically compile evidence-based data regarding the significance of bioactive compounds present in soybeans for human health. This compilation aims to explore the pharmacological and therapeutic potential of these materials, perhaps leading to future applications in both industrial and clinical settings.

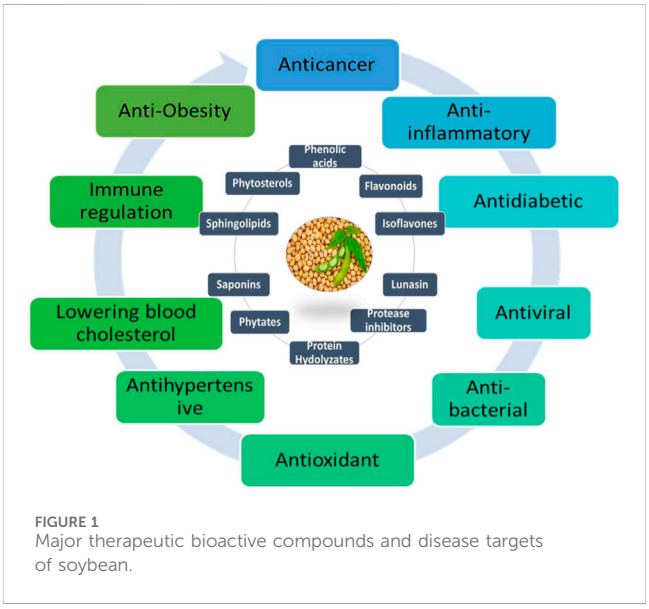

FIGURE 1
Major therapeutic bioactive compounds and disease targets of soybean.

2 Bioactive profiles of soybean

Foods typically have trace levels of bioactive compounds, which are dietary components that may be used to treat oxidative stress, metabolic disorders, and reduced pro-inflammatory states (Swallah et al., 2020). Researchers are constantly monitoring these compounds to assess potential health impacts as shown in Table 1 (Ng et al., 2013). Soybeans have a wide spectrum of

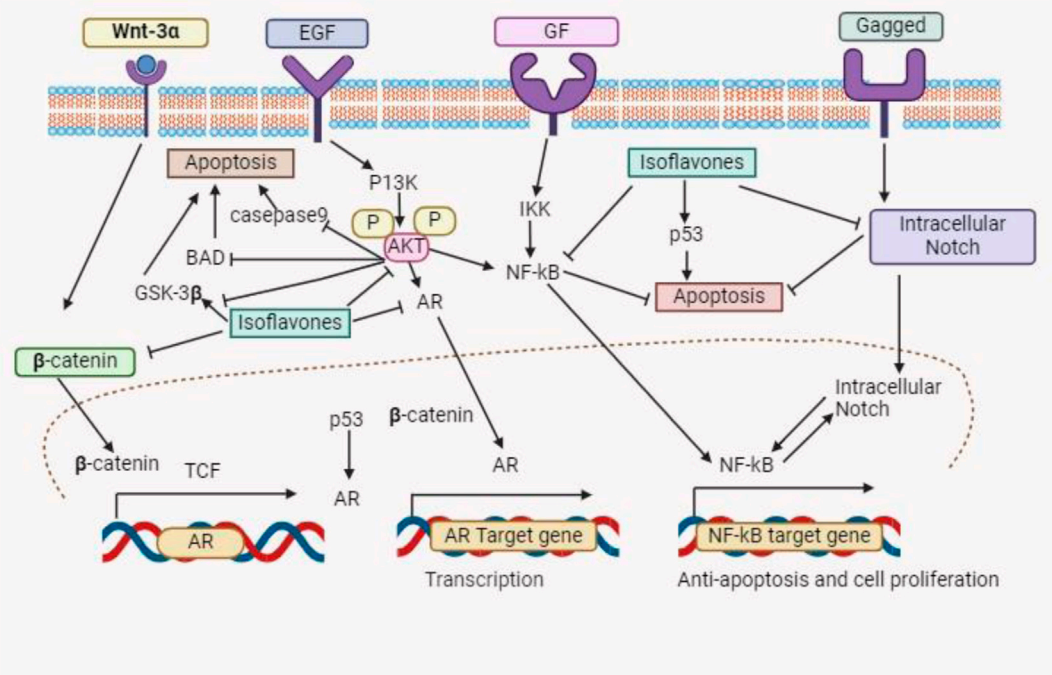

FIGURE 2
This schematic diagram shows different pathways which indicate that the isoflavones have the therapeutic potential to induced cancer cell death.

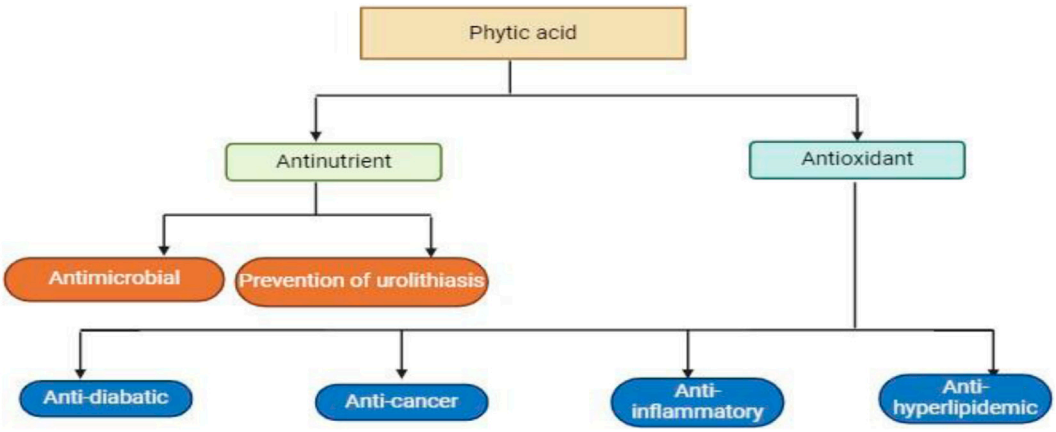

FIGURE 3
This diagram show that Phytic acid have the therapeutic potential to decreased and control the following mentioned diseases in the figure.

bioactive macromolecules, which have been connected to several health advantages. Frequent consumption has been linked to a decreased risk of several illnesses, such as osteoporosis, cardiovascular disease, cancer, and cognitive impairment. It has also been linked to menopausal symptoms.

The primary bioactive components of soybeans are phytosterols, isoflavones, proteins or peptides, saponins, carotenoids, tocopherols, and protease inhibitors, according to Chatterjee et al. (2018); Wu et al. (2017). Additionally, soybeans contain other constituents, including but not limited to glycinin, Kunitz trypsin inhibitor, Bowman-Birk inhibitor, peroxisomal proteins, hemagglutinin, SbPRP protein, neutral PR-5 protein, ferritin, isoflavone-deprived soy peptide, defense proteins (such as β-glucan-binding protein, calmodulin, glysojanin, lunasin, and disease resistance protein), enzymes (tyrosine ammonia-lyase and phenylalanine ammonia-lyase), defensive enzymes (cysteine proteinase, isocitrate lyase, isoflavone synthase, chalcone reductase, and vestitone reductase), UDP-glucose (including betaglucosidase, flavonoid 3-O-glucosyltransferase, 5′-adenylylsulfate reductase, isoflavone conjugate-hydrolyzing β-glucosidase, ATP sulfurylase, and 2-oxoglutarate-dependent dioxygenases genes), polysaccharides, glyceollins, anti-carcinogenic daidzein-rich fraction, and

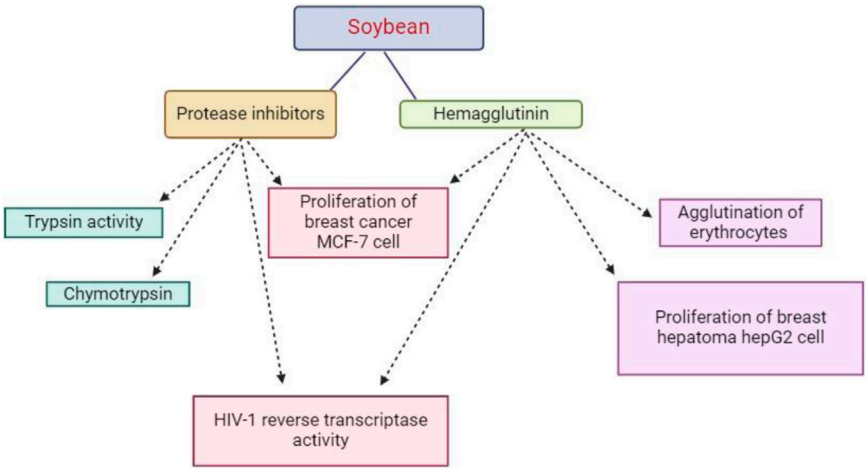

FIGURE 4
This diagram indicate that protease inhibitors have some pharmacological and therapeutic activities against some diseases.

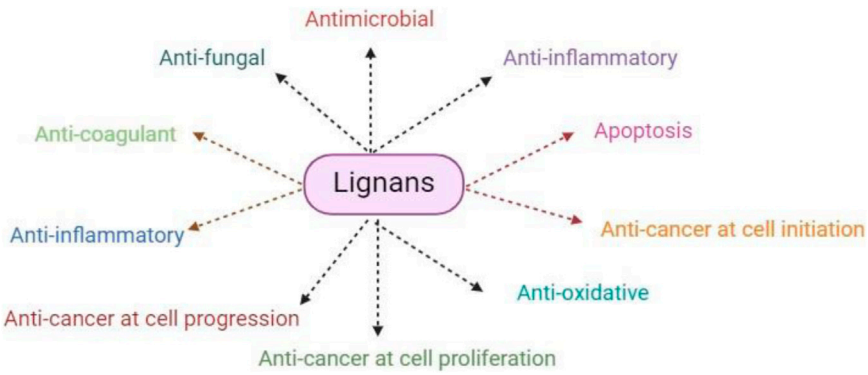

FIGURE 5
This figure explained the overall pharmaceutical and therapeutic potential of lignans against different diseases and pathogens.

isoflavones (Ng et al., 2013). Soybeans' wide variety of bioactive compounds emphasizes both its intricate nutritional composition and potential health advantages as shown in Figure 1.

3 Phenolic compounds

Tannic acid, flavonoids, phenolic acids, and hydroxycinnamic acid derivatives are examples of phenolic chemicals, often known as phenolics, which are antioxidants present in the majority of plants (Król-Grzymała and Amarowicz, 2020). Tannic acid, which is naturally occurring as gallic acid, is a polyhydroxyl phenol ester (Easwar Rao and Viswanatha Chaitanya, 2020). Moreover, soybeans contain isoflavone, a byproduct of flavonoids and phenolic acids (Li et al., 2011).

3.1 Isoflavones

In addition to lignans, isoflavones are phytochemicals generated from plants that are activated by the gut flora and have physiological activity comparable to that of estrogen (Hu et al., 2020). Classify them as phytoestrogens (Hu et al., 2020). Classified the various isoflavones present in soybean hypocotyls into four classes according to their chemical structures: i) aglycons (daidzein, genistein, and glycitein); ii) glycosides (daidzin, genistin, and glycitin); iii) acetyl glycosides; and iv) manonyl glycosides. Isoflavones have been shown in numerous studies to activate estrogen receptors in the vagina, oocytes, and mammary glands; in addition, depending on their physiological environment or chemical makeup, they may have estrogenic or antiestrogenic actions (Nakai et al., 2020). As an antiestrogen, isoflavone, for example, has been associated with a lower incidence of prostate and breast cancers and, both *in vivo* and *in vitro*, has antioxidant properties similar to those of vitamin C and E (de Jesus et al., 2018; Sivoňová et al., 2019). Moreover, tyrosine protein kinase is inhibited by the isoflavone that an oncogene produces (Aichinger et al., 2016). According to research by Benkerroum (2020), genistein stands out among soybean isoflavones in that it has the ability to successfully inhibit the growth of cells associated with cancers of the breast, colon, lung, prostate, and skin *in vitro*. Furthermore, by blocking

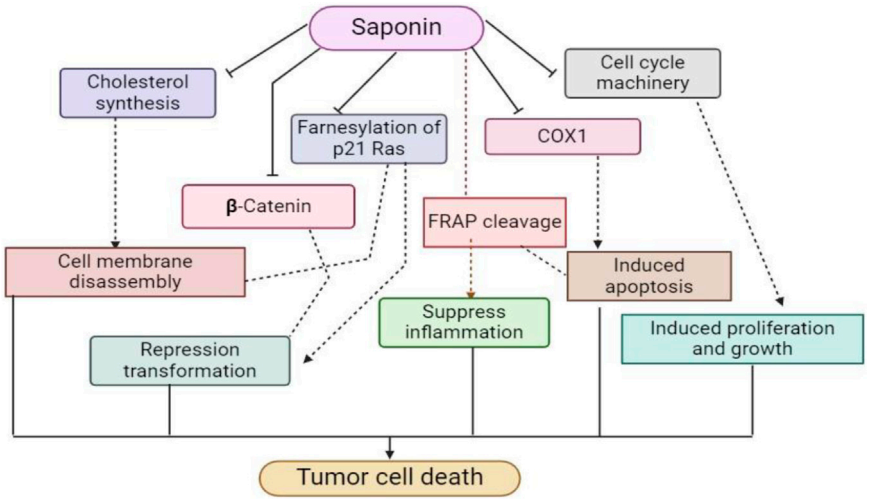

FIGURE 6
This schematic diagram shows the anti-cholesterol lowering mechanism and anti-cancer mechanism of saponin derivatives at cellular levels.

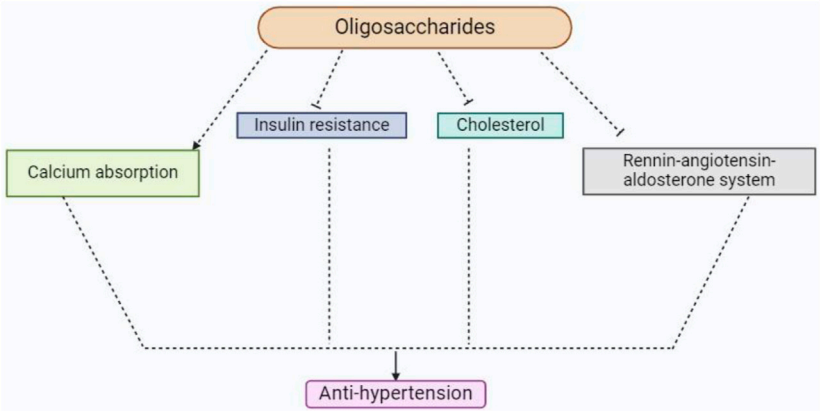

FIGURE 7
This diagram indicates the anti-hypertension activity of soybean oligosaccharides and their derivatives.

vasculogenesis, which halts the passage of oxygen or nutrients, genistein inhibits the formation of boils (Martínez-Poveda et al., 2019).

Ziaei and Halaby (2017) found that studies on the impact of isoflavone consumption on the menstrual cycle in Western women reduce the incidence of breast cancer. Furthermore, isoflavones have a slight estrogenic effect that reduces menopausal symptoms without having a negative impact on health (Ahsan and Mallick, 2017). Isoflavones show promise as cholesterol-lowering medicines because they have the ability to cut blood cholesterol by up to 35% as shown in Table 1 (Taku et al., 2007). On the other hand, animal protein called casein has been linked to elevated blood cholesterol levels (Koury et al., 2014). When people consume insufficient amounts of protein, their stored fat is converted to protein, which raises blood fat levels and blood cholesterol (Ramdath et al., 2017).

About 15% of menopausal women in the US undergo estrogen therapy, a practice linked to a higher risk of malignancies of the reproductive organs (Palacios et al., 2019). Due to its ability to replace estrogen in this population, soybeans a natural dietary source are becoming more and more popular as an alternative (Messina, 2016). By boosting vitamin D activity, decreasing calcium loss from bones, and improving calcium absorption, estrogen plays a critical role in lowering the incidence of osteoporosis (Dall and Britt, 2017). Phytoestrogens are the isoflavones found in soybeans that are structurally and functionally similar to estrogen (Li et al., 2011). According to Tuli et al. (2019), isoflavones, in particular genistein, have significant anticancer properties. They promote normal cell division while suppressing the division of cancer cells by weakly attaching to estrogen receptors. In contrast to the frequent adverse effects of estrogen therapy, isoflavones reduce menopausal hot flashes without causing hyperlipidemia or altering the muscle layers in the breast and uterus (Ahsan and Mallick, 2017). In addition, isoflavones counteract osteoporosis, a common issue

TABLE 2 This table show different bioactive peptides of soy proteins and their therapeutical and pharmaceutical potential by using different types of models (Chatterjee et al., 2018).

Soy protein source	Bioactive peptides	Properties	Tested models
βCG	YVVNPDNDEN YVVNPDNNDEN LAIPVNKP LPHF	Hypocholesterolemic ACE inhibition	HepG2 human liver cell Assay for ACE inhibitory activity *in vitro*
βCG (α′-subunit)	Sulfur-13: MITLAIPVNKPGR Methylparaben: MITLAIPVN MITL is soymetide-4 KNPQLR; EITPEKNPQLR; RKQEEDEDEEQQRE	Immunostimulating FAS inhibitor	Phagocytosis assay FAS inhibition research; mouse adipocyte 3T3-L1
βCG (β-subunit)	YPFVV is soymorphin-5 YPFVVN is soymorphin-6	Anti-diabetic Immunostimulant	Diabetic KKAy mice High-level plus-maze examination in male ddY mice
Glycinin	IAVPGEVA IAVPTGVA LPYP SPYP HCQRPR	ACE inhibition Phagocytosis	ACE inhibitory assay Human polymorphonuclear leukocytes
Glycinin (A4 and A5)	LPYPR	Hypocholesterolemic	Mice given a 50 mg/kg dosage for 2 days
Lunasin	SKWQHQQDSCRKQKQ GVNLTPCEKHIMEKIQ GRGDDDDDDDDD	Anti-cancer Anti-oxidative Anti-inflammatory	MCF-7 breast cancer cells undergo TEN-mediated apoptosis; antioxidants in Caco-2 cells
Birk-bowman inhibitor		Chemoprevention by anti-cancer proteinase inhibition	Stop the production of ROS in PC-3, LNCaP, BRT-55T, and 267B1/Ki-ras prostate cancer cells
Defatted soy protein	X-MLPSYSPY	Anti-cancer	Stop P388D1 murine macrophages at the G2/M stage
Soy protein	YVVFK; IPPGVPYWT; PNNKPFQ; NWGPLV	Hypotensive	Spontaneously hypertensive rats
Genetically modified soybean protein	LLPHH; RPLKPW	Antioxidative; Antihypertensive	Hypertensive rats
Black soybean protein	IQN	Inhibition of adipogenesis	Mouse adipocytes 3T3-L1
Soymilk	RQRK; VIK	Anti-inflammatory	RAW 264.7 macrophages from mice
Fermented soybean	VAHINVGK	Inhibition of ACE	Assay for ACE inhibitory activity
Chymotrypsin Korean fermented soybean paste	HHL	Hypotensive	Spontaneously hypotensive rats
Soy sauce with fermentation	SY GY	Hypertensive	Rats with spontaneous hypertension

among elderly women, by increasing bone density and preventing bone reabsorption (Akhlaghi et al., 2020). Figure 2 summarizes the several physiological roles that isoflavones play.

3.2 Phenolic acids

Eight phenolic acids, including p-hydroxy benzoic acid, vanillic acid, gentisic acid, salicylic acid, chlorogenic acid, and cinnamic acid (Ferreira et al., 2019), are abundant in soybeans. Notably, browning—an unwanted dietary impact that depletes nutrients and modifies color and flavor—can arise from the hydrolysis of chlorogenic acid to caffeic acid (Liu et al., 2019). However, according to Gao et al. (2017), both caffeic acid and chlorogenic acid have the ability to prevent the development of nitrosamines both *in vitro* and *in vivo*. Moreover, these phenolic acids show that they can stop the rat liver's production of aflatoxin B1 (Benkerroum, 2020). Phenolic acids, which function as antioxidants, can also prevent reactive oxygen species from damaging DNA (Kiokias et al., 2020). These phenolic acids have a dual character that draws attention to both their possible detrimental effects on food quality and their beneficial effects on increasing antioxidant activity and thwarting damaging processes.

4 Phytic acid

Phytic acid is composed of six phosphate groups that are symmetrically connected to a myo-inositol ring. It is sometimes referred to as myo-inositol hexaphosphate (IP6) (Silva and Bracarense, 2016). Although found (Gao et al., 2017) in many plants, phytic acid is particularly prevalent in grains and legumes; 2.58% of soybean seeds have phytic acid in them (Hummel et al., 2020). Phytic acid hydrolyzes during food processing to produce myo-inositols with fewer phosphate groups, IP1, IP2, and IP3 (which contain one, two, and three phosphate groups, respectively) (Gupta et al., 2015).

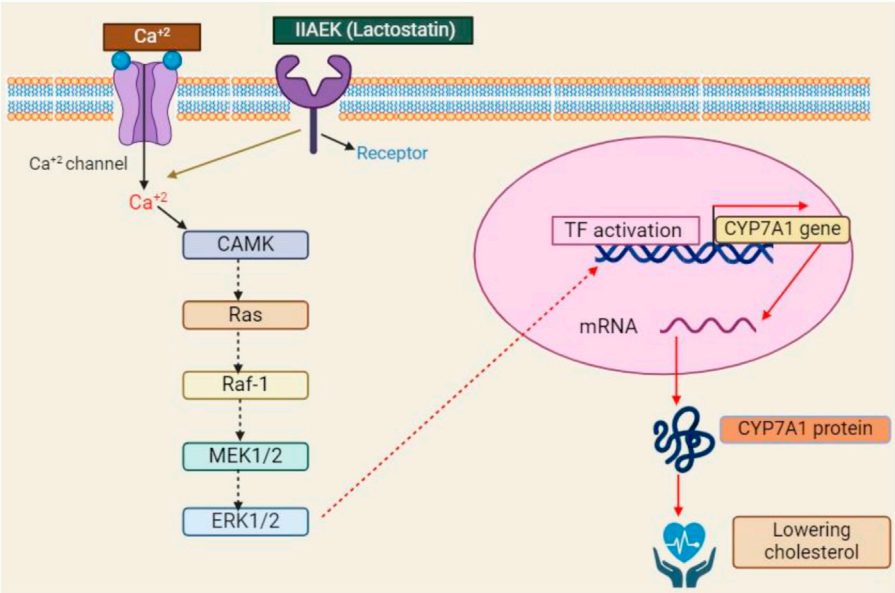

FIGURE 8
Cholesterol is the main cause of many diseases such as blood pressure and cardiovascular diseases, so this schematic diagram show that soybean proteins and peptides have the potential to lower the cholesterol level in the body.

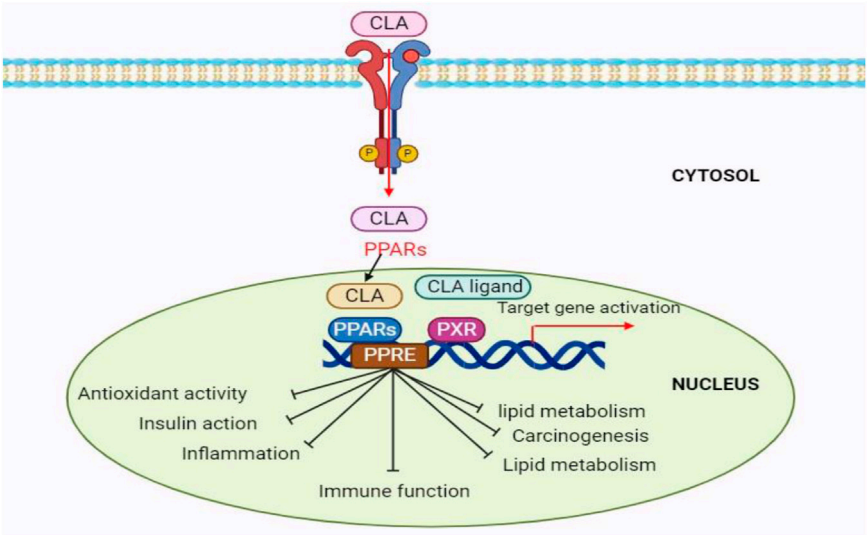

FIGURE 9
The schematic illustration of the CLA-regulated biological pathway during carcinogenic, adipose, diabatic, antioxidant, anti-inflammatory and cardiovascular diseases.

Humans excrete 0.5–0.6 mg/L of phytic acid in their urine, which represents 1–3 percent of the total amount consumed (Marolt and Kolar, 2020). Phytic acid, which is widely distributed in the outer shell of grains and beans, chelates with divalent ions such as Fe2+, Zn2+, Mg2+, and Ca2+ to prevent absorption in the small intestine (Gupta et al., 2015). Phytic acid also functions as a non-nutritional ingredient, preventing the body from using minerals and blocking vital digestion enzymes like α-amylase, trypsin, and pepsin by adhering firmly to the protein base (Popova and Mihaylova, 2019). Phytic acid was once thought to be non-nutritional because of its influence on mineral absorption, but more recently, its antioxidant, anticancer, and lipid-lowering properties have made it popular (Abdulwaliyu et al., 2019) Figure 3.

The storage of cations and phosphorus is one of the biologically active functions of phytotic acid (Kumar et al., 2021; Zhao, 2019) report evidence of iron-induced oxidative damage occurring *in vivo*,

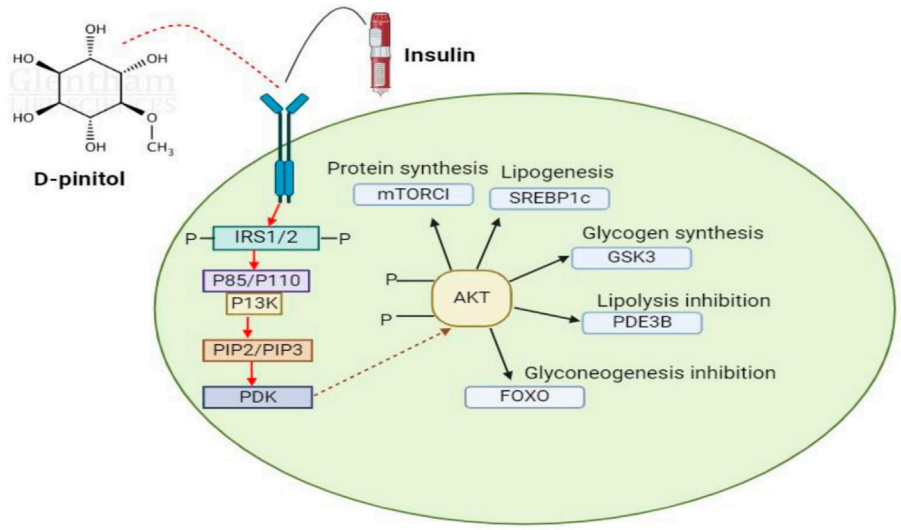

FIGURE 10
This schematic diagram show that D-pinitol have some pharmaceutical and therapeutic potential against cancer, diabetes, anti-cholesteric effects and anti-inflammatory properties.

wherein iron can cause lipid or cell oxidation through the formation of hydroxyl radicals. Conversely, phytic acid can bind to iron, which prevents hydroxyl radical production and halts cellular oxidation (Abdulwaliyu et al., 2019). One way that this antioxidant effect is being studied in relation to food processing is through studies on the delivery of phytic acid to minimize oxidation during food processing.

Furthermore, because phytic acid activates the expression of tumor-suppressor genes including p53 and WAF-1/p21, those who consume more grains and vegetables high in phytic acid had a decreased risk of colorectal cancer. By inhibiting the growth of cancer cells and encouraging cell differentiation, phytonic acid also has anticancer action (Vucenik et al., 2020). Furthermore, lower inositol phosphates, such as IP3 and IP4 (which have three and four phosphorus groups, respectively), influence the body's communication systems and have a major biological role in controlling cell-to-cell responses (Mukherjee et al., 2020).

5 Protease inhibitors

Soybeans and other plant systems such as grains, grass, potatoes, fruits, vegetables, peanuts, and corn contain protease inhibitors (PIs) (Srikanth and Chen, 2016). According to Hellinger and Gruber (2019), soybeans include Kunitz and Bowman-Birk types of PIs that block the actions of chymotrypsin, elastase, and serine proteases. Soybean PIs, which were once thought to be antinutritional inhibitory factors, have drawn interest lately due to their possible anticancer effects (Gitlin-Domagalska et al., 2020).

Trypsin inhibitors have been demonstrated to suppress the production of free radicals, preventing cells from suffering oxidative damage, which is the primary mechanism by which PIs contribute to health benefits (Srikanth and Chen, 2016). In particular, the Bowman-Birk type PI, which is well-known for its chymotrypsin inhibitory effect, inhibits the function of the tumor promoter 12-o-tetradecanoylphorbol-13-acetate, which in turn inhibits the expression of the oncogene MYC, decreases hydrogen peroxide production, and protects DNA's helical structure while preventing DNA oxidation (Gitlin-Domagalska et al., 2020).

Notwithstanding structural variations, recent studies have demonstrated the anti-carcinogenic properties of compounds present in other plants, including soybean PIs, retinoids, garlic acid, nicotinic acid, tamoxifen, and epigallocatechin gallate. These compounds work by inhibiting the generation of superoxide radicals or H2O2 by tumor promoter factors, as shown in Figure 4. Moreover, trypsin inhibitors found in soybeans facilitate the release of insulin, which controls blood sugar levels (Cristina Oliveira de Lima et al., 2019).

6 Lignans

Lignans, which are found in plants in small amounts, help to build the framework of the cell wall when they are bound. After consumption, intestinal bacteria transform them into enterolactone or enterodiol, which are then eliminated in the urine as conjugates of glucuronides (Frezza et al., 2020). Flax seeds and soybeans are good sources of lignans or lignan precursors. According to Rodríguez-García et al. (2019), lignans are categorized as phytoestrogens because they have chemical structures similar to those of estrogen and can control estrogen levels. According to research, a significant lignan consumption may reduce the body's free estrogen content, which may reduce the risk of breast cancer caused by estrogen (Peterson et al., 2010).

Regarding (Vinardell and Mitjans, 2017), there is evidence that suggests lignins can stop breast cancer cells from proliferating in tissue culture systems. To be more precise, lignans block the action of 7-α-hydroxylase, which helps to produce bile acid from cholesterol, or they block the activity of 5α-reductase and 17β-

hydroxysteroid dehydrogenase, which are involved in estrogen biosynthesis and metabolism. This combined effect may reduce the incidence of colon cancer and cancer linked to sex hormones, respectively (Chang et al., 2019). Furthermore, by working in concert with flavonoids and other phytochemicals, the ingestion of foods high in lignan can strengthen their anticancer qualities which are given in Figure 5 (Rodríguez-García et al., 2019).

7 Saponin

According to Shi et al. (2004), out of all the edible legumes, soybeans have the greatest saponin content. According to Moses et al. (2014), saponins that include a covalently linked non-saccharide can be classified as either triterpene or steroid saponins. Based on the skeleton of the non-saccharide section, triterpene saponins are further classified as oleanane, ursane, dammarane, and cycloartane (Vincken et al., 2007; Kamo et al., 2014) Eleven distinct saponins, six from group A and five from group B, are extracted from the soybean hypocotyl (Neacsu et al., 2020).

L-arabinose, L-rhamnose, D-xylose, D-glucuronic acid, D-galactose, and D-glucose are among the monosaccharides of soyasaponins. The hypocotyl and germ layer contain high concentrations of saponins, whereas the outer skin contains none at all (Lim, 2012; Kamo et al., 2014) states that the type and amount of soyaponins vary among species, with group B saponins ranging from 0.26% to 2.75% and group A saponins between 0.36% and 0.41%. Furthermore, during germination, the concentration of group B saponin increases (Guajardo-Flores et al., 2012). The amount of saponin is decreased when microorganism enzymes ferment, yet there is little data on how heating or processing affects saponin content (Tangyu et al., 2019).

Recent studies have reevaluated saponin, which was previously thought to be a bitter, non-nutritional chemical. These studies have shown saponin's physiologically active roles. This puts it in the forefront as a functional nutrient and includes immune system stimulation, cholesterol lowering, and anticancer benefits (Zaynab et al., 2021). Particularly, soybean saponin shortens the duration of exposure to the mesentery, which speeds up the absorption of hazardous chemicals and lessens their toxicity (Tian et al., 2018). Because soybean saponin and cholesterol have similar molecular structures, they prevent cholesterol from being absorbed and promote its release (Ramdath et al., 2017).

Furthermore, saponin and vitamin E (tocopherol) work together to improve blood circulation and prevent skin imperfections (Choudhry et al., 2016). In addition to lowering blood levels of low-density lipoprotein (LDL, or "bad cholesterol") and facilitating smoother blood flow, vitamin E also keeps brown spots, usually referred to as age spots, from developing on the faces of middle-aged and older people (Galmés et al., 2018).

Moreover, saponins function as antioxidants similarly to phytic acids, preventing cell damage caused by free radicals (Ganesan and Xu, 2017). Notable is their ability to lower the rates of DNA mutations, especially in avoiding colon cancer (Tin et al., 2007). With a molecular composition similar to licorice saponins, soybean saponins may have anticancer properties that are being studied (Wang et al., 2019). Interestingly, saponins decrease DNA synthesis in tumor cells, increase killer cell activity, (Figure 6) function as a cell poison specific to sarcoma, and slow the growth of cervical and epidermal cancer cells (Fuchs et al., 2017). According to recent research, group B saponins from soybeans have an inhibitory effect on HIV infection (Li et al., 2018).

8 Dietary fiber and soy oligosaccharides

Dietary fiber, which is difficult for the body's enzymes to digest, is found in large amounts in soybeans. Dietary fibers are classified as either water-insoluble (cellulose and lignin) or water-soluble (pectin and gum) (Dhingra et al., 2012). Colonic microbes degrade water-soluble dietary fiber to produce vital short-chain fatty acids such as propionic, butyric, and acetic acids. These fats help colonic cells absorb cholesterol and are essential nutrients for them (Markowiak-Kopeć and Śliżewska, 2020). However, by encouraging bowel movements and improving intestinal function, water-insoluble dietary fiber can avoid constipation (Bae, 2014).

According to Hussain et al. (2020), the cell wall of soybean shells is made up of pectin and insoluble fibers, with a high concentration of water-soluble fibers. Many physiological processes are made possible by the properties of dietary fiber, including as swelling, water retention, absorption of organic molecules, ion absorption and exchange, and breakdown by intestinal microbes (Rowland et al., 2018). One of the most significant benefits of soybean dietary fiber is its capacity to lower cholesterol (Ramdath et al., 2017). Additionally, as per (Axelrod and Saps, 2018), soybean fiber plays a crucial role in controlling constipation, promoting consistent bowel movements, and reducing the transit time of food through the intestines.

Approximately 4% stachyose and 1% raffinose make up the soluble oligosaccharides known as soybean oligosaccharides, which are found in soybeans. Although they are few in young plants, their concentrations greatly increase as the plants age. Humans cannot digest these oligosaccharides, which cause flatulence and encourage the large intestine to produce gas (Salarbashi et al., 2019). In spite of this, they have drawn notice for encouraging the development of advantageous bacteria in the intestines (Pan et al., 2018).

In addition to supporting vitamin synthesis in the gut, soybean oligosaccharides and dietary fiber also prevent the growth of pathogenic bacteria and the creation of amines and ammonia (Rowland et al., 2018). They function as growth promoters for Bifidobacterium, a beneficial bacterium that improves immunity, stimulates peristaltic movement in the gut, facilitates absorption and digestion, and reduces inflammation. In order to maintain intestinal pH, inhibit the growth of dangerous bacteria, enhance bowel movements, avoid constipation, and preserve intestinal function, Bifidobacterium creates lactic acid (Piqué et al., 2019). Furthermore, according to Rowland et al. (2018), Bifidobacterium lessens the generation of carcinogens like phenol, skatole, and indole, inhibits the absorption of hazardous substances like ammonia and H2S, and lessens the effects of insulin resistance and high cholesterol on hypertension (Figure 7).

9 Soy protein and peptides

As the main source of the protein that makes up a large amount of human diet, soybeans are a rich supply of vital and high-quality

amino acids that come from plants (Singer et al., 2019). Consumption of soy protein has not kept pace with production, even after decades of research on its technological uses dating back to the 1950s (Kearney, 2010). According to Rizzo and Baroni (2018), soy protein is used in a variety of physiologically functional foods, including dairy products, meat alternatives, infant formulas, sports drinks, and fortified grain products. Soy protein has gained interest recently as a phytochemical because of its potential to cure diabetes, cancer, obesity, and cardiovascular disease (Forni et al., 2019).

Serum cholesterol levels, which are impacted by dietary lipids and protein, have a significant correlation with the development of arteriosclerosis (Liu et al., 2019). When it comes to lowering serum cholesterol levels, plant-derived protein works far better than its counterpart generated from animals. Particularly soy protein has been shown to have a cholesterol-lowering effect, thus it's best to consume both forms of protein in moderation (George et al., 2020).

Proteins are the source of bioactive peptides, which are essential for maintaining health because they are physiologically active and serve as a source of nutrition. Many soybean peptides have been discovered in the past 10 years, as Table 2 illustrates. According to Chatterjee et al. (2018), these peptides have a variety of characteristics, such as being hypocholesterolemic, anti-diabetic, hypotensive, capable of phagocytosing and boosting the immune system, anti-inflammatory, chemopreventive, and antioxidant. Consumer preference for items originating from natural sources is expanding, in part because bioactive peptides show fewer negative effects in humans when compared to medications and synthetic food additives (Sanjukta and Rai, 2016). Figure 8 show how soy protein reduced the cholesterol level.

10 Lecithin

Complex lipids called lecithins are widely distributed in foods and tissues such as liver, brain, egg yolk, and soybean oil. They are essential to many metabolic functions. They help in the removal of waste products, aid in the absorption of fat-soluble vitamins and nutrients, and solubilize cholesterol to lower blood cholesterol levels (Li et al., 2017). Furthermore, studies on lecithin have demonstrated its efficacy in avoiding diabetes, preserving renal function, restoring liver function, and enhancing digestibility (Lecomte et al., 2017). Research has shown that adding lecithin to a low-fat, low-cholesterol diet will considerably raise HDL cholesterol levels while reducing LDL cholesterol by 15% when compared to a low-fat diet alone. Beyond its function in the body to dissolve, cleanse, and transport lipids, lecithin helps maintain skin health by clearing waste, triglycerides, and fatty materials from blood vessels (Van Hoogevest and Wendel, 2014). Moreover, lecithin functions as an antioxidant to reduce oxidative damage to vitamin A (Félix et al., 2020). According to Moré et al. (2014), it also has the beneficial impact of avoiding senile dementia and enhancing brain function.

Moreover, soybean lecithin is essential for stopping the brain's acetylcholine from being reduced. Administration of lecithin has been shown to raise acetylcholine levels in several studies (including one with rats). Phosphatidylcholine has a beneficial effect on the cerebrum's increased activity, which causes an increase in the consumption of acetylcholine. In addition to influencing lipid metabolism and fat absorption, phosphatidylcholine is involved in nerve activity. Phosphatidylinositol, on the other hand, is involved in liver metabolism, cell division, proliferation, and hormone expression. The primary building block of phosphatidylcholine, choline functions as a precursor to acetylcholine, which helps to avoid forgetfulness (Kim et al., 2021).

11 Conjugated linoleic acid (CLA)

CLA is a class of derivatives of unsaturated fatty acids, with isomers called 9-cis and 12-cis octadecadienoic acid, respectively, according to their geometric configurations and positions (Kim et al., 2016). Naturally occurring linoleic acid contains two double bonds, which results in the production of eight isomers, most of which are trans fatty acids. More than 98% of all CLA isomers are composed of these isomers (Hwang et al., 2021). Due to its potential as an anticancer agent, particularly in halting the development of skin cancer in mice, CLA was originally identified in fried ground beef (Kim et al., 2016). Later, studies on animals shown its efficacy against a range of cancers, including breast and colon cancers caused by different carcinogens (den Hartigh, 2019). The 9-cis, 11-trans octadecadienoic acid isomer of CLA possesses potent anticancer properties among the eight isomers (den Hartigh, 2019).

Moreover, CLA has antioxidant qualities that are comparable to butylated hydroxytoluene and better than α-tocopherol, which may protect cell membranes from damage by free radicals and have anticancer effects (Hwang et al., 2021). Furthermore, CLA inhibits atherosclerosis by causing significant decreases in triglycerides, LDL cholesterol, and total cholesterol. This effectively reduces the development of atherosclerotic plaques (Bialek et al., 2017). According to Martín-González et al. (2020), adding CLA to livestock feed promotes growth, increases lean meat content, decreases body fat, and improves feed efficiency. Commensal microorganisms, especially gut bacteria, help convert linoleic acid into CLA in animal meat or milk (Di Rienzi et al., 2018). The control of adipocyte differentiation, insulin resistance, lipid metabolism, carcinogenesis, inflammation, and immunological functions is linked to the activation of CLA-mediated CYP7A (Figure 9).

12 Pinitol

Pinitol, also known as D-Pinitol or 3-O-methyl-D-chiro-inositol, is a naturally occurring blood sugar regulator that may be found in pine needles and legumes. It is commonly utilized in several cultures' traditional diabetes treatments. A methyl group is attached to the third carbon of chiro-inositol, a structural isomer of myo-inositol, via an ether bond (Li et al., 2021). By removing the methyl group from the third carbon, gastric acid causes pinitol to change into chiro-inositol (Dumschott et al., 2019). After entering the bloodstream, chiro-inositol participates in insulin and galactosamine signaling, which supports healthy energy metabolism (Bevilacqua and Bizzarri, 2018). Low chiro-inositol concentrations are seen in diabetics who have poor glucose tolerance or insulin resistance; conversely, artificial chiro-inositol treatment lowers insulin resistance, indicating improved sugar metabolism and blood sugar management (Morley et al., 2017).

As a result, swallowed pinitol might function similarly to restore normalcy in the metabolism of sugar (Sánchez-Hidalgo et al., 2021). Notably, patients with type 2 diabetes have used pinitol as an oral hypoglycemic medication to control their blood sugar levels.

Because of its antioxidative action on nitric oxide-mediated signaling, D-pinitol (DP) has been shown to be able to prevent diabetes-induced endothelial rupture in cardiovascular artery arteries. Uncertainty surrounds the mechanism underlying DP's antihyperglycemic effects. The estimated antihyperglycemic action of DP is depicted in Figure 10. Plant treatments are becoming more and more popular in the field of DP-associated pharmacology due to their pharmacobiological properties, especially their anti-inflammatory and antioxidative qualities. The various *in vivo* and *in vitro* actions of pinitol help to avoid and lessen oxidative and inflammatory diseases (Antonowski et al., 2019).

Translocation of GLUT4 from endoplasmic reticulum to plasma membrane of mainly skeletol muscle is considerd the major target for insulin to maintain glucose levels. Both *in vitro* and *in vivo* experiments proved that pinitol increases the glucose uptake along wth GLUT4 translocation to the membrane. GLUT 4 due to its sensitivity to insulin plays important role in the glucose homeostasis by transporting to skeletal muscles (Ishiki and Klip, 2005). It is reported that p13K/Akt signaling pathway is mainly involved in this transportation and diabetes management by phosphatase signaling cascade (Dang et al., 2010). Therefore, D-pinitol is proved to have reduced the plasma glucose levels. It is also imperative to note that this P13K/K signaling pathway is also implicated in variety of other disease pathways like cancer, cardiovascular disorders and other neurological diseases, which are also regulated by D-pinitol by effectively reducing the concentration of blood glucose through synthesis of glycogen (Dang et al., 2010).

13 Therapeutic potential of soybean

This diverse array of bioactive compounds in soybean make it a good candidate to be used for various diseases. Following are some major diseases for which soybean bioactive have been used major diseases.

13.1 Cancer

Cancer is an abnormal cell growth that can either stay in one place inside the body or spread to other parts of it (Sanjukta and Rai, 2016). According to a study by Garg et al. (2016). From the functional viewpoint and bioactive components of soybean, dietary factors—such as food types, diversity, portion sizes, preparation techniques, and overall caloric balance—are responsible for one-third of all cancer cases. It is therefore still essential to find the bioactive substances that can stop or slow the growth of cancer cells to promote a healthy and high-quality life (Bhandari, 2014). The idea that high soy intake is linked to significantly lower incidence of cardiovascular disease and cancer is supported by a substantial body of research (Nagata et al., 2017). Still, because of their purported anticancer properties, the majority of the data points to soy isoflavones, with genistein being the key chemical of interest (Kim et al., 2014). As a well-known tyrosine kinase inhibitor (Singh et al., 2019), genistein has been shown to activate transcription factors, such as estrogen receptors, and to help induce the expression of genes in breast cancer cells (Banerjee et al., 2008). One of the suggested mechanisms involves the inhibition of topoisomerase and angiogenesis through the suppression of cell division and the disruption of the extracellular matrix degradation that envelops the formation of arteries and tumors (Singh et al., 2019; Varinska et al., 2015). Isoflavones will therefore scavenge against oxidants implicated in carcinogenesis in their role as an anticancer agent. Because genistein binds and adheres to estrogen receptor (ER) isoforms, activates peroxisome proliferator-activated receptors (PPARs), has epigenetic and genome-wide effects, and induces apoptosis, it is known to exhibit mild estrogenic action, which may inhibit the growth of cancer cells (Chen and Chien, 2014; Fürst et al., 2012). A case-control research has shown that a high soy intake during youth is associated with a lower risk of breast cancer in adulthood (Shu et al., 2001). Miso (fermented soybean paste) was fed to F344/DuCrj rats, and despite miso having 2.2% sodium chloride (NaCl) by weight, there were less precancerous colonic crypts (Masaoka et al., 1998). Rats were given a diet containing 2.2% or 4.4% wt/wt of NaCl but no miso showed no difference in the number of aberrant crypt foci as compared to the untreated controls, who were fed neither miso nor NaCl (Masaoka et al., 2000). However, a later study found no evidence that eating soy foods such as boiled beans, miso soup, and tofu protected against cancer (specifically, breast cancer) (Nishio et al., 2007). A study on soy consumption and Chinese women stands in stark contrast to this. Consuming soy has been shown in the study to have anti-inflammatory properties (Wu et al., 2012; Yan et al., 2010) a further meta-analysis also revealed a lower incidence of colon cancer (Ryan-Borchers et al., 2006). Study examined how soy isoflavones affected postmenopausal women's immune systems. According to the authors, the administration of isoflavone resulted in a decrease in the plasma concentration ($P < 0.5$) and an increase in the B cell population ($P < 0.05$) of 8-hydroxy-2-deoxy-guanosine, an oxidative marker of DNA damage. The scientists came to the conclusion that giving postmenopausal women two soy capsules, each containing 235 mg of soy extract and 17.5 mg of isoflavones, over a period of 12 weeks (total dose of isoflavones: 70 mg/day) increased the number of B cells and showed promise in preventing DNA damage. However, after using soy supplements to alleviate menopausal symptoms, patients with early-stage breast cancer showed no discernible improvement in menopausal symptom scores (MacGregor et al., 2005). But soy peptides have also been supported in a variety of experimental models for their potential to aid in the treatment of a wide range of cancer types, including breast and prostate cancers, which are particularly interesting due to their sensitivity to sex steroid hormones (Lule et al., 2015; Pabona et al., 2013; Singh et al., 2014). Subsequent research has shown that lunasin and Bowman-Birk inhibitor (BBI), two minor 2S fraction of soy proteins, are linked to the majority of anticancer soy peptides (Lule et al., 2015; Pabona et al., 2013; Singh et al., 2014).

One well-known type of serine protease inhibitor found in large quantities in legumes, particularly soybeans, is called Bowman-Birk inhibitor. They have long been thought of as antinutrients that interfere with the process of normally breaking down protein into amino acids because they can block the activities of chymotrypsin and trypsin. As a result, they are invariably rendered inactive during

the manufacturing of soy products, including soy milk and animal feed. Numerous species, including humans, have demonstrated its anticarcinogenic properties, as have tissue types like the liver, colon, breast, prostate, oesophagus, and so on (Chen et al., 2014). On HT-29 colon cancer lines, Bowman-Birk inhibitors had antiproliferative properties (Clemente et al., 2012; Fereidunian et al., 2014) as well as in animal models of carcinogenesis, by means of their effects on endogenous proteases and by the development of endoplasmic reticulum stress-mediated apoptosis, or cell death, in response to endoplasmic reticulum stress (Clemente and del Carmen Arques, 2014). Therefore, following both proteasomal inhibition and antiangiogenesis, ROS-induced mitochondrial damage triggers apoptosis, which is the mechanism by which Bowman-Birk inhibitors achieve their anticancer action (Cruz-Huerta et al., 2015; Fereidunian et al., 2014; Souza et al., 2014). The Tagalog word "Lunas," which means "for a cure," is where the term "lunasin" originated. The Gm2S-1 gene encodes a distinct 43-amino acid peptide sequence that is generated from soy and is bioactive.

It has been demonstrated that (SKWQHQQDSCRKQKQG VNLTPCEKHIMEKIQGRGDDDDDDDDD) functions as a chemopreventive peptide in both *in vitro* and *in vivo* settings (Chatterjee et al., 2018; Lule et al., 2015) by interacting with non-acetylated H3 and H4 histones, which in turn prevents acetylation and so expresses its anticarcinogenic action (Singh et al., 2014; Wang and De Mejia, 2005). According to reports, lausenin can be found in soy products such as tempeh, su-jae, tofu, soymilk, and soy newborn formula. It has also been proposed that lausenin can prevent skin, colon, breast, prostate, and liver cancers in animal models and cell cultures (Sanjukta and Rai, 2016). According to a study using a cell culture model, varying gene expression in response to lunasin administration with and without lipopolysaccharide stimulation influences diverse biological activities and its signaling pathways, which are not dependent on histone acetylation (Dia and De Mejia, 2011). Moreover, lunasin enhanced the tumoricidal activity of natural killer cells in both *in vitro* and *in vivo* cancer models by collaborating with immunostimulatory cytokines in immunotherapy for lymphoma (Chang et al., 2014). A review was conducted on lunasin's ability to protect human HepG2 cells against oxidative damage. The author noted that when carcinogens are not present, the cellular morphology does not change. Therefore, adding lunasin did not directly affect the cellular morphology, but it did hinder the transformation of the cells when carcinogens are present. Nevertheless, in human HepG2 cells exposed to oxidative stress generated by tert-butyl hydroperoxide, Lunasin showed chemoprotective activity once more (Fernández-Tomé et al., 2014).

13.2 Antidiabetic and antiobesity effects

Type I diabetes, or insulin-dependent diabetes, occurs when the pancreas is unable to secrete insulin, and type II diabetes, or non-insulin-dependent diabetes, occurs when there is an imbalance between the absorption of blood sugar and insulin secretion. Diabetes is a well-known metabolic disease that is characterised by an increased level of blood sugar (Hamid et al., 2015). Type II diabetes in particular is a global epidemic that is affecting nearly every aspect of our society. By lowering blood glucose, soy protein has demonstrated a crucial role in diabetes. Bioactive chemicals derived from soy are thought to regulate the metabolism of fat and glucose by interacting with oestrogen receptors and appearing to have an antidiabetic impact (Garg et al., 2016; Mohamed, 2014). Obesity is the medical term for the state in which extra body fat has accumulated to the point where it could be harmful to health. Insulin resistance and type II diabetes are frequently associated with obesity and hyperlipidemia. Therefore, all soy bioactive components with hypolipidemic properties can also exhibit antidiabetic and antiobesity properties, and their efficacy has been shown in many animal models (Chatterjee et al., 2018). Triglycerides may be less likely to accumulate in the liver as a result of soy protein (Hunter and Hegele, 2017). Leu-Pro-Gly-Pro and Pro-Tyr-Pro-Arg, two peptides found in soybean glycinin protein, have shown a significant anti-obesity potential (Singh et al., 2014). By activating PPAR, a crucial transcription factor in controlling the expression of genes during glucose homeostasis, fatty acid oxidation, and lipid metabolism, soy protein can promote insulin resistance and lipid levels. Using an *in vitro* model, (Yang et al., 2011) investigated the antiobesity impact of black soy peptides. In comparison to mice fed a high-fat diet without black soybean (22.6 g), the authors observed that rats fed with both a high-fat diet and black soybean peptides ((2, 5, or 10%) for 13 weeks) gained less body weight. Although their exact mode of action is unknown, soy-derived isoflavones have been demonstrated to be useful in treating type II diabetes mellitus by reducing blood glucose levels (hyperglycemia) (Niamnuy et al., 2011). They also have antioxidant and α-glucosidase inhibitory properties. It has been demonstrated that the hypocholesterolaemic soy-derived peptides (IAVPGEVA, LPYP, and IAVPTGVA) enhance glucose metabolism by promoting glucose uptake via glucose transporters type 1 and type 4, or GLUT-1 and GLUT-4, in a hepatic cell (Lammi et al., 2015; Tsou et al., 2012). Furthermore, studies conducted *in vitro* and *in silico* have demonstrated the effectiveness of soy-derived peptides (IAVPTGVA) as a serine exopeptidase inhibitor, dipeptidyl peptidase-4 (DPP-4). In order to maintain glucose homeostasis, DPP-4 stimulates the hydrolysis of two essential polypeptides: an insulinotropic polypeptide that is glucose-dependent and glucagon-like (Lammi et al., 2016). A meta-analysis investigation on the impact of soy isoflavone supplementation on non-Asian postmenopausal women was carried out by Yang et al. (2011). According to the studies, supplementing with soy isoflavones may help lower body weight, moderate blood sugar levels, and regulate insulin levels in plasma. Similar to this, a study on type II diabetic women that fed bread (120 g) fortified with soybean flour—that is, replacing 30% of the wheat flour with soybean flour—for 6 weeks found no discernible impact on the profile (Moghaddam et al., 2014).

13.3 Hypocholesterolaemic and antiatherosclerosis effects

The condition known as hypercholesterolaemia, or high cholesterol, refers to elevated blood cholesterol levels. Consequently, there are higher than normal blood lipid and bad cholesterol (low-density lipoproteins, or LDL) levels. This is a well-known risk factor for coronary heart disease (CHD), which is one of the main causes of death in the west and globally (Messina, 2016;

Singh et al., 2014). It has been proposed that cholesterol functions as a molecular pattern linked to harm when it activates the NLRP3 inflammasome, (Duewell et al., 2010; Progatzky et al., 2014), which promotes colitis and colorectal cancer (Du et al., 2016). Even while dietary proteins, such as those found in soy foods, are thought to help stimulate the effect of blood cholesterol concentrations, there is still debate regarding the cholesterol-lowering (or hypocholesterolemic) effects of soy protein (Messina, 2016; Singh et al., 2014). Nonetheless, a multitude of studies support soy protein as the primary nutrient accountable and refute soy isoflavones (Messina, 2016). Studies on animals and cell cultures revealed that isoflavones had a beneficial effect on reducing atherosclerosis and lowering cholesterol levels in (LDL) low-density lipoprotein by preventing oxidation and increasing (HDL) high-density lipoprotein in the body. In (Millar et al., 2017) Numerous findings have confirmed the hypocholesterolaemic activity of dietary proteins and their bioactive peptides, including modifications to bile acid secretion, alterations in liver cholesterol metabolism, hormonal effects, and control of cholesterol receptors (Singh et al., 2014). It has been demonstrated that cholesterol metabolism is essential for increasing T-cells' adaptive immunological response (Tall and Yvan-Charvet, 2015). Particularly, it has been demonstrated that oxysterols, the byproduct of cholesterol metabolism, block the synthesis of cholesterol by binding to the liver's X receptor β (LXRβ). Oxysterols become sulphated upon T-cell activation, which causes them to no longer cling to LXRβ. Therefore, to boost proliferation in the acquired immune response, T-cell cholesterol metabolism promotes cholesterol production (Bensinger et al., 2008; Kloudova et al., 2017). Due to their potential as a substitute for traditional hormone replacement treatment, their capacity to lower cholesterol, and their antiatherogenic health effects, soy protein, and isoflavones garnered international attention and were extensively discussed in 1995 (Messina, 2010). Subsequent research revealed that consuming 25% soy protein on a daily basis could help lower blood levels of triglycerides, low-density lipoprotein (LDL), and serum cholesterol by 12.9%, 10.5%, and 9.2%, respectively (Bhoite et al., 2021). Another meta-analysis showed that while isoflavone phytoestrogens had little effect on patients with normal serum cholesterol concentrations, they did lower the plasma cholesterol concentrations of those with increased levels (Wang et al., 2013). The effects of soy meals on blood cholesterol levels after oral administration to both people and animals were taken into consideration while evaluating their benefits on cardiovascular illnesses (Bhoite et al., 2021). As a result, these dietary proteins undergo protease breakdown in the stomach, releasing bioactive peptides that may lower cholesterol levels. Similarly, the American Diabetes Association (ADA) suggests that consuming 26–50 g of soy protein daily (instead of animal protein) for 5 weeks would be sufficient to prevent and treat cardiovascular disease (CVD) (Bhoite et al., 2021; Koutelidakis and Dimou, 2016). According to a study by Torre-Villalvazo et al. (2009), dietary soy protein (i.e., with an adjusted protein concentration purity of 90.6% casein, 86% soy protein) can reduce cardiac triglyceride and cholesterol concentrations as well as suppress cardiac ceramide concentrations by lowering lipid accumulation and inhibiting the expression of serine palmitoyltransferase, the key enzyme in sphingolipid biosynthesis, in the hearts of obese mice. Therefore, consuming soy protein could be a helpful dietary treatment approach to stop lipotoxic cardiomyopathy. A major storage protein found in soybeans, 7S globulin, was shown in an *in vivo* investigation involving rats to drastically reduce plasma cholesterol content by 35% (Lule et al., 2015). Contradictory findings revealed that nonprotein fraction, isoflavones, and saponins were unable to demonstrate any beneficial effects in lowering cholesterol (Adams et al., 2004). This was clarified when rats were given LPYPR derived from soybean glycinin subunit at a dose of 50 mg/kg without isoflavones for 2 days, and the rats' serum total and LDL cholesterol levels decreased by 25% (Chatterjee et al., 2018). A tetrapeptide (LPYP) from the soy protein glycinin was used in a later investigation to demonstrate a hypercholesterolaemic effect (Kwon et al., 2002). Additionally, the Mangano et al. (2013) study clarified that while the administration of soy protein (18 g/d) and isoflavone tablets (105 mg/d isoflavone aglycone equivalents) to 131 healthy, ambulatory older women was effective in reducing inflammatory markers (IL-6), it did not improve serum lipid levels, which are thought to be a critical risk factor in coronary heart disease (CHD). In a similar vein, (Wang et al., 2013) proposed a theory that soy isoflavone, specifically genistein, is implicated in the pathophysiology of Kawasaki disease, which is associated with children aged 5 and under and causes inflammation of the blood arteries throughout the body, especially in the heart. This peptide functioned as a competitive indicator of 3-hydroxy-3-methylglutaryl CoA reductase, or HMG-CoA reductase, which is the primary enzyme that limits the rate of cholesterol biosynthesis (Chatterjee et al., 2018; Pak, Koo et al., 2012). A further animal model study found that feeding pigs a lunasin-enriched soy extract in addition to casein reduced their levels of low-density lipoprotein (LDL) cholesterol when compared to the group that only received a casein diet, which indicated a faulty LDL receptor gene (Galvez, 2012). In the last few years, peptide extract, protein complexes, and dietary supplements have been added to lunasin-enriched soy extract to create a variety of functional foods. Dietary supplements made from soybeans, such as Lunasin XPR® (peptide extract), LunaSoyTM (protein complex), and LunaRich®, are utilized commercially as potential ingredients in functional foods that lower cholesterol and as dietary supplements for heart disease and general cellular health (Lule et al., 2015; Udenigwe and Aluko, 2011). It is important to remember, nevertheless, that studies on soy protein's ability to lower cholesterol have been conducted using *in vivo* models and cell lines. Additionally, clinical trials must be conducted in order to determine the protein's safety and effectiveness in therapeutic applications (Lule et al., 2015).

13.4 Antihypertension effects

The third most significant risk factor for serious health issues, including early mortality, globally is hypertension, or high blood pressure, which is a result of the angiotensin-converting enzyme (ACE), a crucial component in blood pressure management (Zhong and Schleifenbaum, 2019). Angiotensin-I converting enzyme (ACE), the first step in the renin-angiotensin system, is inhibited by antihypertensive or ACE inhibitory peptides. This affects the negative feedback effects of angiotensin II and controls blood pressure and fluid balance in the body. By cleaving the dipeptide

at the C-terminus, the dipeptidyl carboxypeptidase activities of ACE transform the dormant decapeptide angiotensin I into the active vasoconstricting octapeptide angiotensin II, which raises blood pressure (De Mejia and Ben, 2006; Natesh et al., 2003). It is proposed that one of the most important treatment strategies for managing hypertension is ACE inhibition. In addition, a number of risk factors, including heart failure, myocardial infarction, coronary heart disease, and stroke, are thought to be the fundamental cause of hypertension (Singh et al., 2019). Notably, a study on the ACE-Inhibitory activity of soy protein isolates in the prevention of hypertension in an *in vivo* model showed that, when 25 g of non-soy protein were substituted with 25 g of soy nuts (i.e., soy nuts containing 25 g of soy protein and 101 mg of aglycone isoflavones), hypertensive women and normotensive postmenopausal women's blood pressure increased, all of which were attributed to the cardioprotective effect (Margatan et al., 2013; Welty et al., 2007). Using *in vitro* and *in silico* models, (Zhao et al., 2019) investigated new ACE inhibitors generated from soybean proteins. Despite being a strong ACE inhibitory peptide, dimethylglycine (DMG) had no deleterious effect on HEK-293 cells. The outcomes of the molecular docking analysis showed that DMG and ACE's activities (Ala354, Glu384, Gln281, His353, His513, Lys511, and Tyr520) interacted well. According to the authors, DMG exhibited strong action compared to ACE, with an IC50 value of 3.95 ± 0.11 mM. Using human platelets that had been separated, black soybean extract also demonstrated a potent inhibitory effect on platelet aggregation brought on by collagen in an *in vitro* model (Kim et al., 2011). The review also covered the investigation on the functional characteristics and antihypertensive-inhibitory activity of produced soy whey protein and fractions. Using ultrafiltration, the whey proteins were divided into several fractions; the unfractionated whey protein had the strongest inhibitory activity of the angiotensin-I converting enzyme. Once more, the whey protein fraction (>50 kDa) showed superior solubility, stability, and emulsion activity; in contrast, the unfractionated sample showed the highest percentage of nineteen (19%) of angiotensin-I converting enzyme inhibition (Lassissi et al., 2014). About 33 peptides with angiotensin-I converting enzyme (ACE) inhibitory action were found in *Lactobacillus* plantarum strain C2 fermented soymilk, according to a study by Singh and Vij (2017); Acharjee et al. (2015) studied confirmed that postmenopausal women who were categorized by metabolic syndrome status experienced a greater decrease in blood pressure, inflammatory markers, and molecule adhesion after receiving soy protein (25 g) and isoflavones (101 mg) supplements. Additionally, a study found that consuming foods high in soy fibre, such as biscuits supplemented with soy fibre (about 100 g/day for 12 weeks), resulted in a significant reduction in body weight and lipid content in an adult who was overweight or obese. These conditions are known to be symptoms of hypertension, obesity, and hyperlipidemia, among other conditions (Hu et al., 2013). Additional research on animals revealed that soybeans may have a preventive role against cardiovascular disease when the foetus is exposed to isoflavones through the mother's diet (Bonacasa et al., 2011). In contrast to chemosynthetic pharmaceuticals, ACE-inhibitory peptides derived from plants and animals may prove to be a viable substitute due to their safety, growing demand, and cost-effectiveness (Singh et al., 2014).

14 Conclusion

Soybeans have considerable health and therapeutic potential due to their varied range of bioactive chemicals. A wide variety of bioactive substances, including as proteins, saponins, carotenoids, phytosterols, isoflavones, and tocopherols, are found in soybeans and have been shown to have major health advantages. As phytoestrogens, isoflavones have antioxidant properties and lower the risk of cancer. Phenolic substances are involved in the regulation of estrogen and antioxidant activity. Saponins have anticancer properties and help lower cholesterol. Once thought to be antinutritional, protease inhibitors now exhibit anticancer and antioxidant qualities. Phytic acid lowers cholesterol and possesses antioxidant properties, albeit impeding the absorption of minerals. Fiber and soy oligosaccharides support intestinal health. Lecithin promotes brain health, lowers cholesterol, and facilitates the absorption of nutrients. CLA lowers cholesterol and has anticancer properties; pinitol controls blood sugar. The novel medical and pharmaceutical uses of soy bioactive components have great promise for improving human health and well-being as long as research is conducted. Including soy in diet may be a natural, all-encompassing way to improve general health and ward against a number of chronic illnesses.

15 Future perspective

The use of soy in therapeutic applications and health enhancement shows significant promise for the future. Researchers are discovering special ingredients in soy called bioactive compounds that have amazing health benefits. These substances may be used to treat and prevent a number of illnesses, including diabetes, heart problems, and some forms of cancer. Researchers anticipate using these bioactive molecules for other purposes in the future, recommending different courses of action for people depending on their particular needs. Furthermore, it's possible that these soy chemicals will be included into better and new medications, administered using cutting-edge technology to provide better outcomes with fewer adverse effects. As we move forward, there are a lot of opportunities for developing better and more effective solutions for general well-being thanks to the potential of soy bioactive components.

Several studies have shown how technological developments have led to the global spread of genetically modified (GM) crops. Genetically engineered soybeans that are widely planted either appear on our plates in a variety of culinary forms or are blended with other components to make processed foods. Regarding the impact of these genetically engineered crops and commodities on health, opinions vary. Concerns about the environment and public health have led to calls for stringent laws that are meant to lessen risks. Notably, cutting edge genetic engineering methods like genome editing have lately been used to develop novel ways to gene correction that do not rely on conventional genetic modification techniques. This new generation of genetically modified crops is being brought about by these technologies in conjunction with sophisticated biotechnological instruments. It is expected that traditional crop breeding will eventually converge with biotechnology,

genetic engineering, and molecular breeding technologies due to the world's growing population and issues like food scarcity, climate change, and pollution. With the use of soy bioactive components in cutting-edge drug delivery systems, nanotechnology is set to play a critical role in maximizing pharmaceutical efficacy and reducing side effects. Furthermore, nanotechnology can be used to solve issues with the development of highly functional health foods.

Author contributions

UbR: Writing–original draft. ZY: Visualization, Writing–review and editing. IA: Conceptualization, Software, Writing–review and editing. TY: Investigation, Writing–review and editing, Validation. RL: Visualization, Writing–review and editing, Validation. UmR: Visualization, Software, Writing–review and editing. HH: Visualization, Data curation, Formal Analysis, Investigation, Writing–review and editing. US: Visualization, Data curation, Validation, Writing–review and editing. Z-u-RM: Supervision, Validation, Writing–original draft, Writing–review and editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 24 October 2024
DOI 10.3389/fphar.2024.1421136

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OPEN ACCESS

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RECEIVED 21 April 2024
ACCEPTED 20 September 2024
PUBLISHED 24 October 2024

CITATION
Orozco-Barocio A, Sánchez-Sánchez MA, Rojas-Mayorquín AE, Godínez-Rubí M, Reyes-Mata MP and Ortuño-Sahagún D (2024) Phytochemicals from Cactaceae family for cancer prevention and therapy. *Front. Pharmacol.* 15:1421136. doi: 10.3389/fphar.2024.1421136

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Phytochemicals from Cactaceae family for cancer prevention and therapy

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Cancer is a global health issue, increasingly prevalent and a leading cause of mortality. Despite extensive research, conventional treatments remain aggressive, often damaging healthy cells, and exhibit limited efficacy. Addressing drug resistance and enhancing treatment effectiveness are critical challenges in advancing cancer therapy. This review examines the potential of natural plant compounds, particularly phytochemicals and their derivatives, in developing novel anticancer agents. These metabolites have a long history in traditional medicine, with 42% of molecules approved for cancer treatment between 1981 and 2019 being either natural products or derivatives. The Cactaceae family, which comprises more than 1,500 species, represents a largely untapped source of potentially useful chemopreventive and anticancer agents. Although more than 3,000 plants and their derivatives have contributed to chemotherapeutic development, cactus species have received limited attention until recently. Emerging evidence highlights the anticancer potential of fruits, stems, and cladodes from various cactus species. This review provides a comprehensive and current overview of experimental studies on Cactaceae in cancer research, aiming to pave the way for the development of innovative, natural cancer therapeutics and contribute to the ongoing battle against this formidable disease.

KEYWORDS

anticancerigens, tradicional medicine, Cactacea, phytochemical, antitumor

1 Introduction

Non-communicable diseases presently constitute the predominant cause of global mortality, with cancer anticipated to emerge as the primary cause of death and a significant impediment to increased life expectancy across all nations in the 21st century (Bray et al., 2024). The World Health Organization (WHO) projects cancer as either the first or second leading cause of premature mortality, under the age of 70, in

177 out of 183 countries, and the third or fourth leading cause of death in a further 23 countries (World Health Organization, 2024). Consequently, cancer is escalating worldwide, and is one of the main causes of mortality, with an estimated 35 million new cases forecasted for 2050, signifying a 77% surge from 2022 levels (Bray et al., 2024).

Chemotherapy remains a cornerstone of cancer treatment and requires the use of different chemotherapeutic agents targeting different cancer properties and pathways (Iqbal et al., 2017). Nonetheless, the emergence of multidrug resistance represents a significant obstacle to successful cancer therapy and the containment of metastasis (Costea et al., 2020). Mechanisms of multidrug resistance include increased drug efflux, drug inactivation, detoxification pathways, alteration of target structures, inhibition of apoptosis, involvement of cancer stem cells, miRNA dysregulation, epigenetic modifications, DNA repair defects, tumor heterogeneity, microenvironmental influences, epithelial-mesenchymal transition and modulation of reactive oxygen species (Bukowski et al., 2020). To improve the efficacy of cancer treatment, agents must be developed that are able to circumvent the mechanisms of drug resistance. To this end, researchers are investigating the potential of natural products in combating various facets of drug resistance (Keyvani-Ghamsari et al., 2020).

Phytochemicals and their derivatives, obtained from plant sources, have long been employed in traditional medicine practices (Rizeq et al., 2020). These compounds, rich in beneficial nutrients and biologically active metabolites, have garnered significant interest for their potential roles in preventing and treating a myriad of ailments, including hypercholesterolemia, diabetes, cardiovascular disease, hypoglycemia, hypolipidemia, edema, joint pain, weight control, visual disorders, neuroprotective effects, and asthma (Shoukat et al., 2023). With an increasing focus on alternative botanical medicines, there is growing recognition of their potential in developing anticancer therapies with reduced side effects (Dey et al., 2019; Majolo et al., 2019; Choudhari et al., 2020; Khan et al., 2019). Notably, a substantial proportion of cancer treatments (around 42%) approved between 1981 and 2019 were either natural products or their derivatives, highlighting their significance in drug development. Significantly, 114 of the 247 anticancer molecules released (46%) during the same period were derived from natural products and their derivatives (Newman and Cragg, 2020). Despite the extensive diversity within the cactus family, comprising over 1,500 species, only a limited number have been investigated for their chemopreventive and anticancer properties, presenting an untapped avenue for further exploration (Harlev et al., 2013; Allegra et al., 2019).

The term “cactus” covers a diverse collection of succulent species, comprising about 130 genera and 1,500 species within the family Cactaceae (order *Caryophyllales*). As the second largest family in the Neotropics, the Cactaceae exhibit a broad spectrum of morphologies and sizes, characterized by rapid growth, adaptation to nutrient-poor soils and low water requirements (da Silva-Santos, et al., 2021; Santos Díaz et al., 2017; Bravo-Hollis and Sánchez-Mejorada, 1978). The extensive diversity of the Cactaceae is notably documented in regions such as Mexico, which harbors 586 species, as well as in the southwestern United States of America, the central Andes (including Peru, Bolivia, southern Ecuador, northeastern Chile, and northwestern Argentina), Brazil, Paraguay, Uruguay, and Argentina (Das et al., 2021; Santos Díaz et al., 2017; Bravo-Hollis and Sánchez-Mejorada, 1978).

Cactaceae, a remarkable plant family has evolved fascinating adaptations to thrive in arid and semi-arid regions across the world. The Cactaceae family includes two main subfamilies: *Cactoideae and Opuntioideae*. *Cactoideae* represent the largest and most diverse subfamily containing specialized succulents and includes several genera such as *Hylocereus*, *Cereus*, *Pilosocereus*, *Melocactus*, *Stenocereus, Cephalocereus*, *Myrtillocactus, Lophophora*, and *Lophocereus.* The subfamily Opuntioideae, which comprise leafless, stem-succulent plants with numerous spines, includes two small genera, *Pereskia* (Pereskioideae) and *Maihuenia* (Maihuenioideae) (da Silveira Agostini-Costa, 2020; Santos Díaz et al., 2017; Bravo Hollis and Sánchez-Mejorada, 1991). Cacti are widely used and exploited in different cultures around the world: as food (bread and cakes, juice, ice cream, yogurt, gummy candies, refreshing and alcoholic beverages), cosmetics, even as ornamental plants, as fodder for ruminants, hedges, living fences, firewood, as construction material, and in folk medicine (Monteiro, et al., 2023; Shoukat, et al., 2023; Martins, et al., 2023; García-Cruz, et al., 2022; De Araujo, et al., 2021; da Silva Santos, et al., 2021; Das et al., 2021). Traditionally, people find utility in various cacti parts. Primarily, the cladodes, stem, fruits, and roots are used. Subsequently, the flowers, mucilages, and seeds also serve for practical purposes. (da Silveira Agostini-Costa, 2020; Das et al., 2021; Bravo Hollis and Sánchez-Mejorada, 1991).

Diverse studies have confirmed the traditional use of cactus species against several disorders, such as: metabolic disorders, as a blood sugar lowering, diabetes mellitus, obesity, hypertension, antiatherogenic, antihyperlipidemic, antihypercholesterolemic, antioxidant and diuretic, cardioprotective, hepatoprotective, wound disinfectant, dysentery treatment, adjuvant in venomous snake bites, elimination of parasites, antimicrobial, anti-inflammatory, analgesic, wound healing, anti-ulcer, anti-proliferative properties, and anti-cancer (Shoukat, et al., 2023; Martins, et al., 2023; Madrigal-Santillán et al., 2022; García-Cruz, et al., 2022; De Araujo, et al., 2021; da Silveira Agostini-Costa, 2020; Ramírez-Rodríguez, et al., 2020; Harlev, et al., 2013). In a recent review, Das et al. show a list of 42 cactus species ethnobotanically used as botanical medicines and the parts of the cactus used (Das et al., 2021).

Cactus plants synthesize diverse chemical metabolites in response to various environmental stimuli, encompassing polyphenols, alkaloids, betalains, terpenes, and fatty acids, with nutritional, pharmacological, and food-related applications (Das et al., 2021; Harlev et al., 2013; Gandía-Herrero et al., 2016; Santos Díaz et al., 2017; da Silveira Agostini-Costa, 2020; Madrigal-Santillán et al., 2022). Scientific literature has elucidated the therapeutic potential of cactus species in combating degenerative diseases, notably cancer, supported by extensive research efforts (Harlev et al., 2013; Gandía-Herrero et al., 2016; Santos Díaz et al., 2017; Iqbal et al., 2017; da Silveira Agostini-Costa, 2020; Madrigal-Santillán et al., 2022; Shoukat et al., 2023; Martins et al., 2023). Moreover, numerous chemotherapeutic agents utilized in cancer treatment derive from plant sources, with carotenoids, terpenoids, and alkaloids among the primary metabolites (Shoukat et al., 2023).

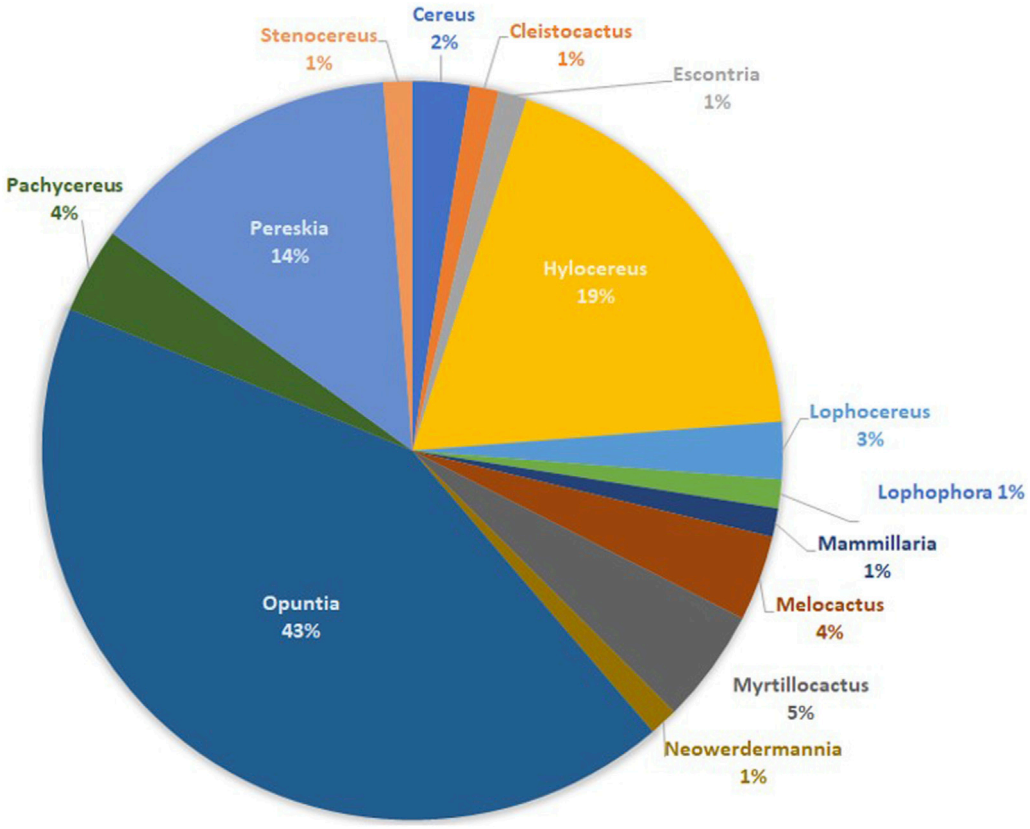

FIGURE 1
The most important Cactaceae genera, analyzed according to the percentage of original references (N = 80).

Actually, cactus fruits, stems, and cladodes have garnered significant attention for their anticancer properties, supported by international reports and emerging scientific evidence (Das et al., 2021; Daniloski et al., 2022; Madrigal-Santillán et al., 2022; De Araujo et al., 2021; da Silveira Agostini-Costa, 2020).

This work aimed to provide an up-to-date and comprehensive review of the experimental work carried out to date with plants of the Cactaceae family in cancer research. A literature search was performed in the PubMed, Web of Science, Scopus, and Science Direct databases. The keywords and phrases used in the search were "medicinal plants AND cancer", "plant extract AND anti-cancer", "Cactaceae AND cancer" and "cactus AND cancer". The species that were included after analyzing the above keywords were those that reported the traditional use of the plant or its parts against cancer and those that reported anticancer effects of the plant extracts or metabolites in both *in vivo* and *in vitro* models. Care was taken to ensure that only cactus species whose taxonomic identification data were registered in an academic institution with an identification document were included. Care was also taken to ensure that all research articles were published in peer-reviewed journals. Likewise, the intention of this review is to compile the information known so far about cacti in cancer treatment, to present the scientific methods used and the phytochemicals identified in order to stimulate further research on the possible mechanisms of action of these plants in the biology of cancer and the influence on immunological mechanisms.

2 Cactaceae genus with antitumor effects of extracts and metabolites derived from

After an extensive search and selection of references reporting anti-cancer effects of extracts from various Cactaceae plants, we compile in this review the information from eighty original references from 14 genera of the Cactaceae family (Figure 1). Three genera have been studied the most so far: *Opuntia*, *Hylocereus* and *Pereskia*, accounting for about 76% of the studies. However, if we analyze the different species included in the studies for each genus (Figure 2), we find that two of the three genera with the most studied species are again *Opuntia* (with eight studied species) and *Pereskia* (with five studied species) and also including *Mammillaria* (with six studied species).

2.1 Genus *Cereus*

Cereus species are characterized as large, tree-like, columnar cacti exhibiting four to ten pronounced ribs, thick stems, large white flowers and delicious pink or red fruits that emerge from the edges of the ribs. These plants show remarkable resilience to temperatures from −5°C to 45°C and exhibit tolerance to various soil compositions. Their natural habitat spans the southwestern United States of America, Mexico, Central America, and Brazil,

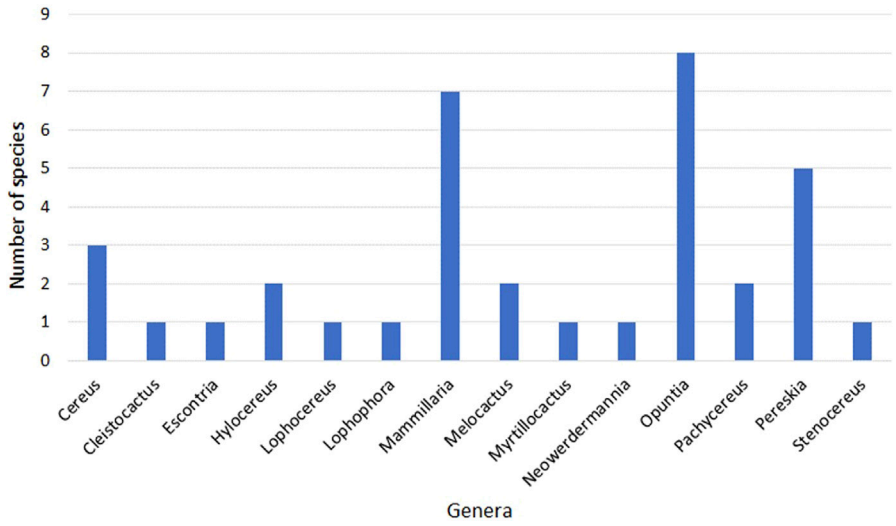

FIGURE 2
Number of different species by genus investigated in anticancer studies.

comprising numerous species within the *Cereus* family (De Araujo, et al., 2021; Harlev, et al., 2013; da Silva-Santos, et al., 2021) (Figure 3B). Notably, *Cereus* cacti have garnered attention as potential crop plants and are also recognized for their medicinal properties, particularly in the treatment of breast cancer, with emphasis on *Cereus quadrangularis* Haw. taking center stage (Harlev, et al., 2013). In addition, extracts from *Cereus hildmannianus* cladodes are used in folk medicine for diverse purposes such as weight loss, cholesterol reduction, diuretic effect, treatment of pulmonary disorders, rheumatism, wound healing, and lithiasis management (da Silva-Santos, et al., 2021). *Cereus jamacaru* DC, commonly known as mandacaru, has been investigated for its anti-cancer and anti-tumor potential. It has been shown to reduce the viability of sarcomas, inducing a tumor reduction of 86.07%, and mitigating the cytotoxic effects of cisplatin without inducing mutagenic or cytotoxic damage in murine blood cells and human lymphocytes (De Araujo, et al., 2021; Vencioneck Dutra, et al., 2018) (Supplementary Table S1). Additionally, studies by Jacomini et al. (2015) have shown the importance of *Cereus peruvianus* Mill. (Figure 3A). callus cultures as a source of polyunsaturated fatty acids (PUFAs) and their remarkable antiproliferative activity. The raw extract inhibited the growth of Caco-2 cells (colon adenocarcinoma cells) and showed an antiproliferative effect with a CC50 value of 250 μg/mL (Jacomini, et al., 2015) (Supplementary Table S1).

2.2 Genus *Cleistocactus*

The genus *Cleistocactus*, which belongs to the family Cactaceae, comprises flowering plants that are indigenous to the mountainous regions of South America, particularly Peru, Uruguay, Bolivia and Argentina, at altitudes of up to 3,000 m above sea level. The etymology of the genus name comes from the Greek term “kleistos”, “ which means “closed” and is attributed to the characteristic of its flowers scarcely opening. These cacti typically have tall, slender stems, which often branching extensively with numerous ribs, closely spaced areoles, and spines. The flowers are tubular, with tips that seldomly open, typically revealing only the protrusion of the style and stamens (Lowry, 2016) (Figure 3D). Notably, prior to Mina et al.’s study in 2020, there was scant information regarding the biological activity or phytochemical composition of the genus *Cleistocactus*. Mina and colleagues assessed the cytotoxic potential of the crude alkaloid fraction extracted from *Cleistocactus winteri*, commonly known as the golden rat tail, against three human cancer cell lines: hepatocarcinoma (HepG2), breast adenocarcinoma (MCF-7), and colon adenocarcinoma (Caco-2). Their investigation revealed significant cytotoxic effects, with IC50 values of 26.53 μg/mL, 23.8 μg/mL, and 13.07 μg/mL, respectively. Notably, these values demonstrate nearly equivalent cytotoxic activity to that of Doxorubicin, employed as a positive control in the study (Mina et al., 2020) (Figure 3C) (Supplementary Table S1). These results could stimulate further research on the cytotoxic properties of the extracts from this Cactaceae family.

2.3 Genus *Escontrias*

Escontria chiotilla, colloquially known as jiotilla, is an arboreal cactus species indigenous to southern Mexico specifically in the dryland of the states of Michoacán, Guerrero, Puebla, and Oaxaca (Bravo-Hollis and Sánchez-Mejorada, 1978) and characterized by numerous branching stems. Notably, its small flowers possess a unique “gold leaf” appearance with campanulate morphology and receptacle tubes, showcasing a metallic sheen attributed to translucent, membrane-like bracts. These cup-shaped, yellow flowers bloom twice annually, from February to May and from July to September. The fruits, resembling large grapes, exhibit purple-brown or purple-red hues in both peel and pulp, featuring a bittersweet flavor. Papery bracts encase the fruit, which harbors ovoid-shaped, black seeds measuring 1–1.5 mm in length and

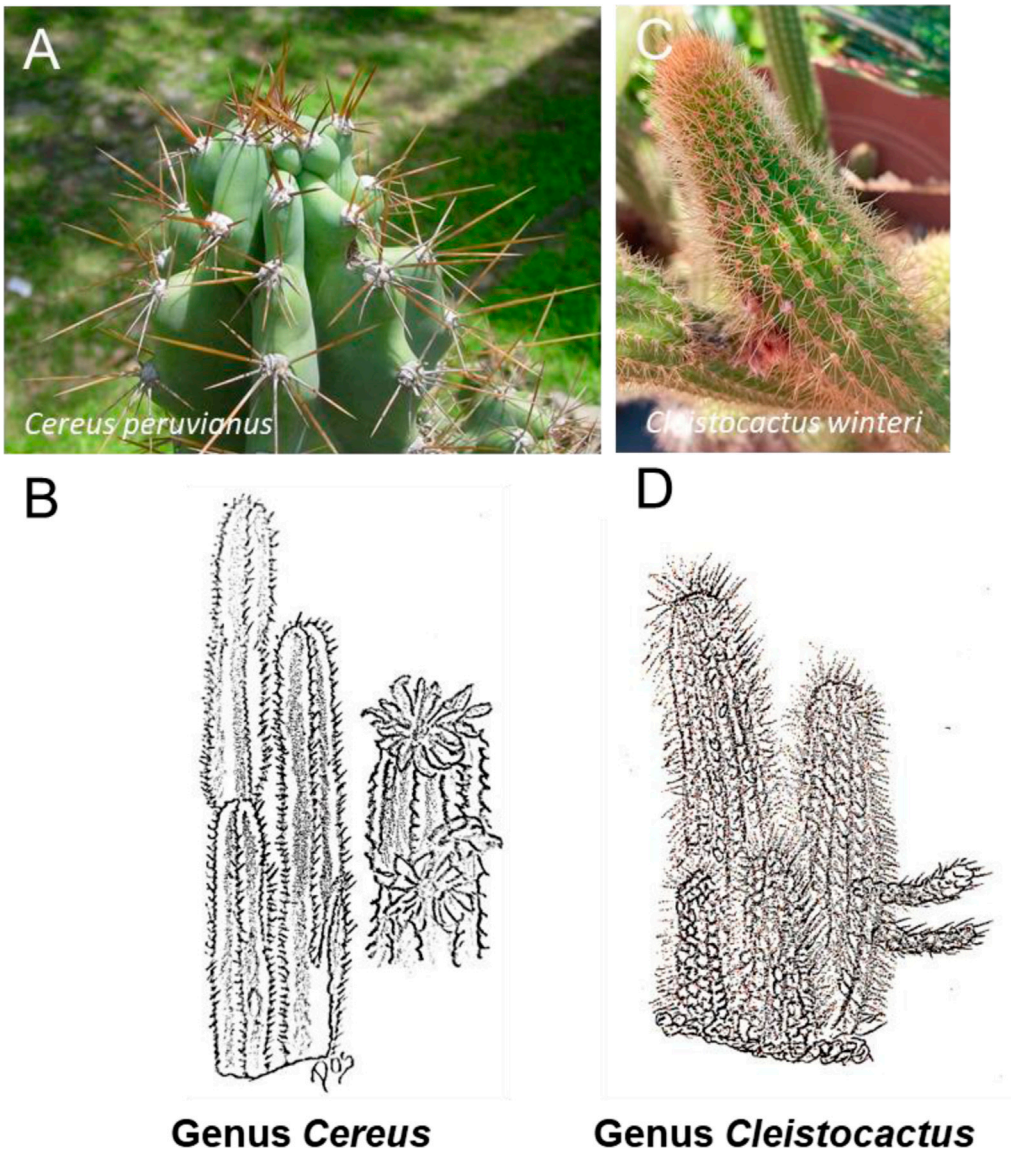

FIGURE 3
Schematic representative draw **(B, D)** and detailed picture **(A, C)** for the genus *Cereus* **(A, B)** and *Cleistocactus* **(C, D)** of cactaceae family. The most common representative of the Cactaceae from the genus *Cereus* is the *Cereus peruvianus*, also known as the Peruvian apple cactus or the Queen of the Night. From the genus *Cleistocactus* one of the most common representatives is the *Cleistocactus strausii*, also known as the "Silver Torch" or "Wooly Torch."

0.8–1.0 mm in diameter. Harvested primarily from April to May and from September to November, these fruits, marketed under different names such as "jiotilla", "chiotilla" or "quiotilla" hold significant cultural and economic value in the region (Bravo-Hollis and Sánchez-Mejorada, 1978; Cruz-Zamora, et al., 2017) (Figures 4A–C). The fruits of *Escontria chiotilla* are consumed both fresh and in processed forms such as jam, ice cream and alcoholic beverages, distinguishing their flavor from that of other cactus species (Ramírez-Rodríguez, et al., 2020).

To date, only one study has investigated the cytotoxic activity of the clarified fruit juice of *E. chiotilla* in four different human adenocarcinoma cell lines (Sandate-Flores, et al., 2020) (Supplementary Table S1). No cytotoxicity was observed in the normal cell control (NIH/3T3 cell line), and the MCF-7 breast cancer cell lines and the Caco-2 colon cell lines also showed no cytotoxicity. The fruit juice of *E. chiotilla* showed a higher level of cytotoxicity only in the liver cancer cell line HepG2 and decreased cell viability in the prostate cancer cell line PC-3. Therefore, further research is needed to investigate this selective activity.

2.4 Genus *Hylocereus*

Hylocereus, commonly referred to as white-fleshed pitahaya or dragon fruit, belongs to the epiphytic cactus species in the Cactaceae family and is the most widely cultivated within its genus. The plant

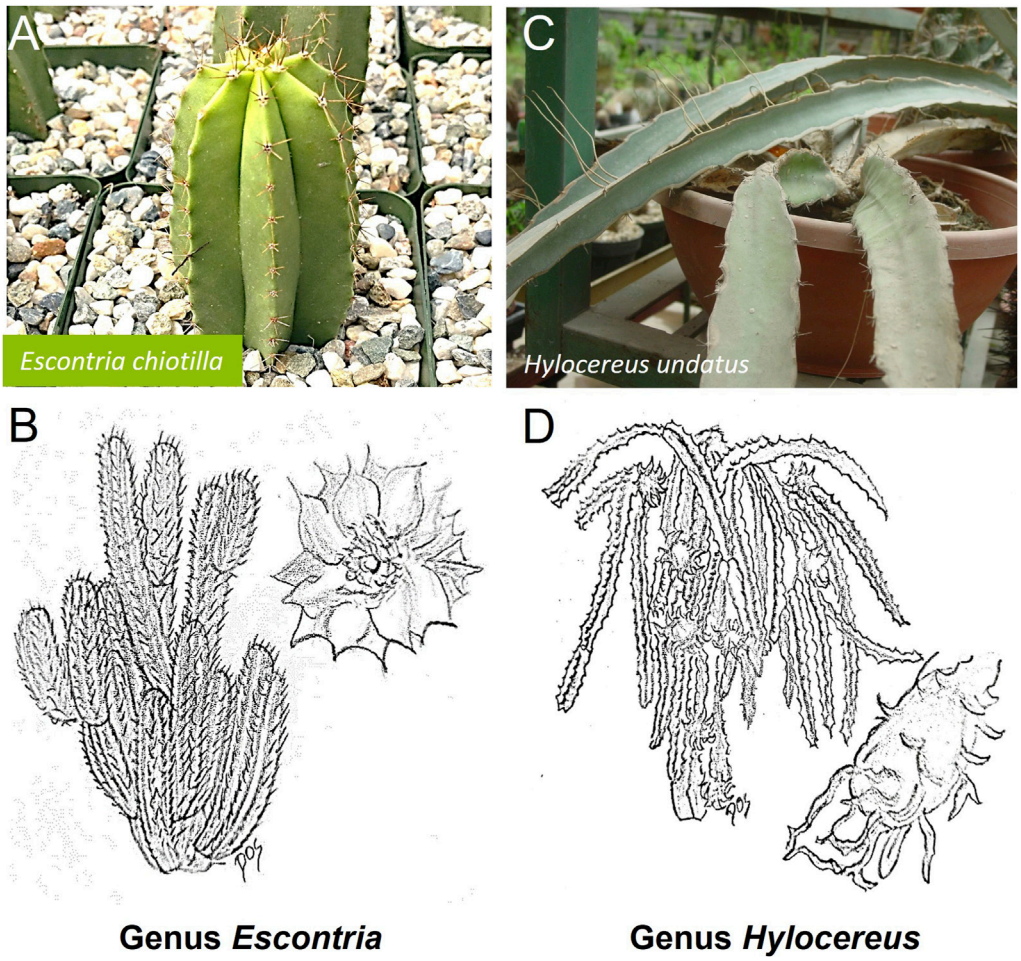

FIGURE 4
Schematic representative draw **(B, D)** and detailed picture **(A, C)** for the genus *Escontria* **(A, B)** and *Hylocereus* **(C, D)** of cactaceae family. The most common representative of the Cactaceae from the genus *Escontria* is *Escontria chiotilla*, commonly referred to as "Chiotilla" or "Chichituna.". From the genus *Hylocereus* is *Hylocereus undatus*, known as the "Dragon Fruit" or "Pitaya."

features climbing, jointed, and fleshy stems with triangular shapes consisting of three undulating wings with horny scalloped margins, reaching lengths of up to 6 m. Its nocturnal flowering produces large, fragrant bell-shaped flowers pollinated by bats and moths, measuring 30 cm long and 23 cm wide, adorned with a green, lobed stigma. The ellipsoidal pitahaya fruit, 10–12 cm in diameter and 11–30 cm in length, showcases a bittersweet flavor and vibrant colors, with bracts covering the outer skin and juicy, fleshy pulp containing evenly distributed small, shiny seeds (Verona-Ruiz, et al., 2020; Bravo Hollis and Sánchez-Mejorada, 1991). Taxonomically, the *Hylocereus* genus comprising about 17 species, with nine registered in Mexico, and is native to tropical regions of Central and South America, thriving in diverse climates and soils worldwide (Ramirez-Rodríguez, et al., 2020; De Araujo, et al., 2021) (Figure 4B). Cultivated varieties, distinguished by skin and flesh color, include *H. undatus* with red skin and white flesh (Figure 4C), *Hylocereus polyrhizus* with red skin and red flesh, and *Hylocereus megalanthus* with yellow skin and white flesh. Both fruits and flowers contain pharmacologically active compounds, making *Hylocereus undatus* a captivating cactus species prized for its ornamental appeal and culinary delights (Joshi and Prabhakar, 2020) Figure 4D.

In 2006 Wu et al., compared the antiproliferative activity of dragon fruit (pitahaya) peel and flesh on B16F10 melanoma cells, being better the effect of the fruit peel. The inhibition of the proliferation was dose-dependent in relation to a higher content of flavonoids and betalains (Wu, et al., 2006) (Supplementary Table S1). Later on, several others have reproduced the results in other cancer cell lines, by using extracts from different *Hylocereus* species, and going deep into the identification of the active metabolites and the mechanisms of the effects. Yellow piraya extract (*H. undatus*) has showed a high inhibitory effect on breast cancer MCF-7 cell proliferation (Jayakumar and Kanthimathi, 2011) (Supplementary Table S1). Also, extracts of peels of dragon fruit from *H. undatus* and *H. polyrhizus*, which contained 3 to 5 times of higher flavonoid and polyphenol content compared to fruit flesh, showed good antiproliferative activity against cancer cells of human gastric adenocarcinoma cell line (AGS) and human breast adenocarcinoma cell line (MCF-7) (Kim, et al., 2011) (Supplementary Table S1). In addition, dragon fruit peel extract presents a cytotoxic effect against PC3 (human prostate cancer cell line), Bcap-37 (human breast cancer cell line) and MGC-803 (human gastric cancer cell line) in a dose-dependent manner.

The IC50 value ranged from 0.61 to 0.73 mg/ml. *Hylocereus polyrhizus* showed a better cytotoxic effect than *H. undatus* on MGC-803 cells (Luo, et al., 2014) (Supplementary Table S1).

In addition to its antiproliferative effects, different extracts from the pulp and the peel of the dragon fruit exhibits proapoptotic effects. An hydroalcoholic extract (50:50 ethanol-water) from the pulp of fruit pitahaya from *H. polyrhizus* induces a decrease in cell proliferation in MCF-7 (ER+) cell line, in which a cell cycle analysis revealed an increase in G0/G1 phase followed by a decrease in G2/M phase, but also an induction of apoptosis, by suppressing BRCA1, BRCA2, PRAB, and Erα gene expression. Interestingly, MDA-MB-435 cells (ER−) were not affected by the extract (Guimarães, et al., 2017). In another study using *H. undatus* pulp and peel extracts and determining by using Liquid Chromatography Mass Spectrometry (LC-MS) and Gas Chromatography–Mass Spectrometry (GC-MS) the main metabolites of the extracts, the total phenolic and flavonoid contents in the peel extract were significantly higher than those in the pulp extract. Although, both extracts displayed cytotoxic activity against MCF-7 and Caco-2 cancer cells after 48 h of treatment at IC50 values ranging from 14 to 53 μg/mL with high selective indices against normal WI-38 and MCF-10A cell lines. In addition, the apoptosis potential of the anticancer effects was also evaluated. In this case, the increase in apoptosis was revealed by the overexpression of p53, BAX, and caspase-9 and the downregulation of antiapoptotic Bcl-2 mRNA and protein expressions (Salam, et al., 2022) (Supplementary Table S1).

When aqueous and methanolic extracts from the pulp of pitahaya (*Hylocereus costaricensis, H. undatus, H. megalanthus*) were compared with different human cancer cell lines, moderate cytotoxic activity against cancer cells was observed, with no detectable effects on normal cells. Methanol extracts predominated, except for Caco-2 cells, where the aqueous extract at 500 μg/mL showed the highest cytotoxic activity. Methanol extracts at the same concentration showed optimal results against HT29 colon, HepG2 liver and DU145 prostate cancer cells, while the effects on normal prostate epithelial cells remained negligible (Pasko, et al., 2021a) (Supplementary Table S1). Subsequent studies extended these findings and investigated the cytotoxic activity of dragon fruit extracts on additional human cancer cell lines derived from different tissues and exhibiting different metastatic properties (including skin, prostate and gastrointestinal cancer cell lines). In addition, the antioxidant and anti-inflammatory activities of the extracts were investigated. The extracts showed significant effects on the viability of cancer cells, especially in colon and prostate cancer cells. In particular, the cytotoxic activity against colon cancer cells Caco-2 and HT-29 was significantly higher for the water extracts than for the methanol extracts, both for DI and DT. Conversely, prostate cancer cells DU145 showed higher activity with the methanol extracts than with the water extracts, with DI exerting a stronger effect on cell viability than DT. In addition, the cytotoxic effect of water and methanol extracts from DT fruit on high-grade metastatic prostate cancer cells (PC3) was significantly stronger compared to DI (Pasko, et al., 2021b) (Supplementary Table S1).

More recently, Shyamalagowri et al. (2022) prepared silver nanoparticles (AgNPs) using the aqueous extract of dragon fruit (H. undatus) peel and investigated their cytotoxic activity *in vitro*. They observed a significant cytotoxic effect of DFPAE-AgNPs against HepG2 cell lines with extensive cell death after 24 h at different concentrations (10–50 μg mL) (Shyamalagowri, et al., 2022) (Supplementary Table S1). However, when the medicinal value of natural dyes extracted from two species (*H. polyrhizus* and *H. undatus*) of dragon fruit was investigated on Ehrlich ascites carcinoma (EAC) cell line, neither species showed any remarkable cytotoxic effect on EAC cell viability at different concentrations (25, 50, 100, 200 and 400 μg/mL) (Ashaduzzaman Nur, et al., 2023) (Supplementary Table S1). El-Nashar et al. (2024) also identified flavonoids, phenolic acids, anthocyanin glycosides, lignans, stilbenes and coumarins in peel, pulp and seed extracts of white piraya (*H. undatus*) by HPLC-ESI/MS-MS. In addition, the synergistic cytotoxic potential with cisplatin against cervical cancer cells was investigated to increase efficacy and optimize treatment outcomes. In addition, the cytotoxic effect was investigated by MTT assay against prostate (PC3), breast (MDA-MB-231) and cervical cancer (HeLa) as well as against normal Vero cell lines. The seed and peel extracts showed a remarkable cytotoxic effect against all tested cell lines. They also showed downregulation of Bcl-2 and overexpression of P53 and BAX in HeLa cells treated with Pitaya extracts, which eventually activated the apoptosis process (El-Nashar, et al., 2024). Consequently, much more research is needed to clearly determine the nature of the extracts and the phytochemical metabolites that induce the cytotoxic and proapoptotic properties.

2.5 Genus *Lophocereus*

Lophocereus schottii (Engelm) Britton & Rose, is a species of cactus endemic to Mexico. It thrives in warm climates and is typically associated with coastal dunes and xerophyte shrublands. This cactus species is characterized by 5–10 ribs and reaches a height of 2–4 m. Colloquial names include cardona, muso, sina, sinita, cactus star, mochi and viejo (meaning “old man”) (Figure 5B). Traditional medicinal practices include the use of decoctions made from the stems of *L. schottii.* (Figure 5A). These preparations are employed for treating various diseases, including cancer, diabetes, ulcers, sores, stomach disorders and tuberculosis. Notably the stems possessing five ribs are preferred for these therapeutic purposes. Chemical investigations have revealed the presence of alkaloids such as lophocerine and pilocerine in this species (Bravo-Hollis and Sánchez-Mejorada, 1978; Argueta, et al., 1994; Djerassi, et al., 1958; Shetty, et al., 2012; da Silveira Agostini-Costa, 2020). These metabolites contribute to its pharmacological properties. In laboratory studies, we explored the effects of an ethanolic extract derived from the stems of *L. schottii* on murine L5178Y lymphoma. Following oral, intratumoral and intramuscular administration to inoculated mice, this extract resulted in prolonged survival and a reduction in tumor mass (Orozco-Barocio et al., 2013) (Table 1). Recently, our studies have highlighted lophocerine as a promising anticarcinogenic phytochemical. *In vitro* experiments demonstrated that both the ethanolic extract and its polar fraction (PFLs) from *L. schottii* exhibited remarkable antiproliferative activity. In particular, the polar fraction showed higher efficacy. It inhibited cell viability in stimulated splenocytes and L5178Y cells significantly more than in non-stimulated cases. UPLC-MS analyzes revealed that the polar fraction, which was enriched with 86% lophocerine, contained the

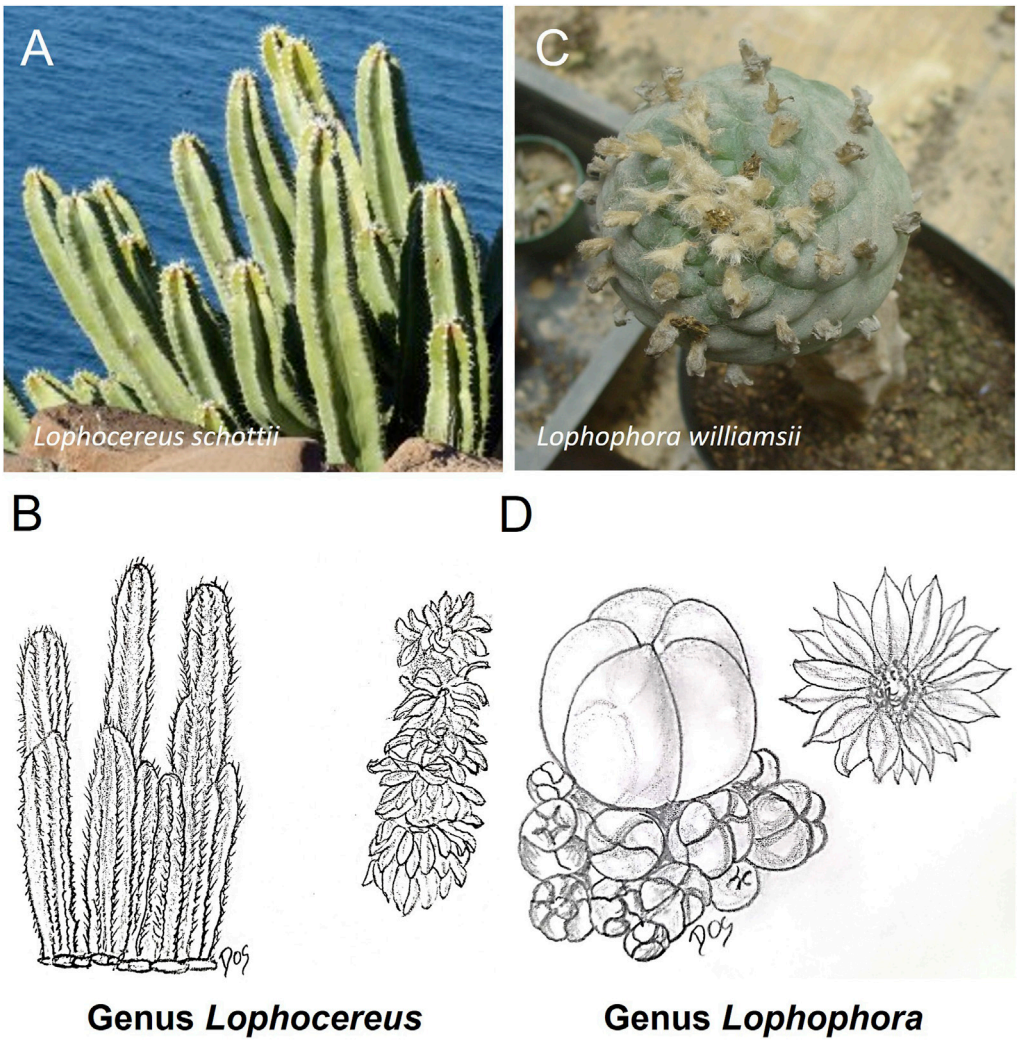

FIGURE 5
Schematic representative draw **(B, D)** and detailed picture **(A, C)** for the genus *Lophocereus* **(A, B)** and *Lophophora* **(C, D)** of cactaceae family. The most common representative of the Cactaceae from the genus *Lophocereus* is *Lophocereus schottii*, commonly known as the "senita" or "old man cactus". From the genus *Lophophora* is *Lophophora williamsii*, commonly known as "peyote."

most potent anticarcinogenic metabolites (Orozco-Barocio et al., 2022) (Supplementary Table S1).

2.6 Genus *Lophophora*

Lophophora, a genus of North American cacti, derives its name from the Greek words "lophos" (crest) and "phoros" (bearing), referring to the distinctive brushes of hair on their areoles (Figure 5D). These ornamental plants, which mainly consist of two species, *Lophophora williamsii* (Figure 5C) and *Lophophora diffusa*, grow close to the ground, often in clusters, with spherical, flattened stems known as "buttons" that are 2–7 cm high and 4–12 cm in diameter. Both species flower from March to September and reproduce clonally by forming new shoots from the lower areoles (Bravo Hollis and Sánchez-Mejorada, 1991). *Lophophora williamsii*, commonly known as peyote, has no spines, a blue-green coloration and reaches a diameter of 2–5 inches. The plant is native to central Mexico and is of great cultural and medicinal importance. It is traditionally used to treat various ailments such as infections, arthritis, asthma, influenza, intestinal disorders, diabetes, and snake and scorpion bite (Franco-Molina, et al., 2003). Peyote extracts are known for their effect on the central nervous system and regulate functions such as blood pressure, sleep, hunger and thirst (Shetty, et al., 2012).

Franco-Molina and colleagues (2003) conducted a study demonstrating that the viability of various tumor cell lines, in particular the murine lymphoma cell line L5178Y-R, U937, L929 and MCF7, was significantly reduced by the methanolic extract of *L. williamsii*. In particular, the MCF7 cell line exhibited the highest sensitivity to the cytotoxic effects of the peyote extract. At an extract concentration of 18 μg/mL, the viability of MCF7 was reduced by 1.3%. Simultaneously, the viability of L5178Y-R, U937, and L929 cell lines was reduced by

TABLE 1 In vivo evidence for anticancer properties of metabolites from cacti. Arranged alphabetically.

Genus/ specie	Part of cactus	Extract type	Metabolites	In vivo model	Dosis / Effect observed	Molecular / cellular effect	Reference
Lophocereus schottii	Stem	Ethanolic	Alkaloids (Lophocerine), Flavonoids, Phenols	Mouse lymphoma L5178Y	50-100 mg/kg body weight/day intratumoral route, and 100 mg/kg body weight/day intramuscularly. Survival rate increased significantly and reduced (56.37%) the tumor growth rate	ED50 = 7.8 μg/ml with 63% of cytotoxicity. Lymphoproliferation indice did not differ among the groups treated	Orozco-Barocio et al. (2013)
Lophophora williamsii		Methanolic		Wistar rats inoculated subcutaneously into the left flank with C6 cells	1.92 mg/ml of Lophophora williamsii. Reduce tumor growth rate by 42.8%	N.D.	Franco-Molina, et al. (2021)
Melocactus bellavistensis	Stem	Methanolic	Alkaloids	Rats with colon cancer induced with dimethylhydrazine (DMH)	1, 5 and 10 mg/kg body weight/day/orally	At concentrations of 10, 5, and 1 mg/kg no neoplasia was observed, and the percentage of dysplasia was 33.3%, 22.2%, and 33.3%, respectively	Ríos-León, et al. (2017)
Opuntia ficus-indica	Fruit (Prickly pear)	Aqueous		nu/nu BALB/c mice with SK-OV-3 cells	0.4 ml/day/ intraperitoneally. Reduce tumor growth rate by 42.8%, and had no significant effect on body weight loss	Increased annexin IV and decreased VEGF expression in animal tumor tissues	Zou et al. (2005)
	Fruit (Prickly pear)	Methanolic	Indicaxanthin	Mouse melanoma (B16-F10)	3.2 mg/kg orally three/ day for 14 days. Reduce tumor growth rate by 86%	Reduction of CXCL1 levels by 42%	Allegra et al. (2018)
	Stem (Cladodes)	Folin polyphenolic method	Phenols and Kaempferol	PC-3 - MCF-7	5 mg/kg body weight/ day intraperitoneally. Reduce tumor growth rate by 37.6%, also myeloperoxidase activity and total cholesterol level	Overexpression of cleaved Caspase-9, Hdac11, and Bai1 proteins	Antunes-Ricardo et al. (2021)
	Flour	Alkaline hydrolysis by NaOH	Glycoside isorhamnetin-3-O-glucosylrhamnoside	Tumor growth of HT29 RFP cells xenograft in immunosuppressed mice. Subcutaneous injection	Standard pellet form feed with either 1% (w/ w) or 3% (w/w) O.humifusa. Vertical epidermal thickness of the skin were significantly reduced (63.3% and 53.3%)	Increassed antioxidative capacity, superóxido dismutasa (SOD) actiivity and apoptosis. Decreased lipid peroxidation activity	Lee et al. (2012)
Opuntia humifusa	Fruit (Prickly pear)	Powder (1 - 3% w/w)		Carcinogenesis induced by 7, 12-dimethylbenz [a] anthracene (DMBA)	Standard pellet form feed with 3% (w/w) O.humifusa. Vertical epidermal thickness of the skin were significantly reduced (50%) and MPO level	COX-2 and iNOS expression were significantly reduced. TNF-a, IL-1b and IL-6 were significantly inhibited. PCNA and cyclin D1were significantly reduced. The level of NF-jB/p65 of the nuclear fraction was significantly decreased	Lee et al. (2013)
	Fruit (Prickly pear)	Ethyl acetate	Flavonoids	Tumor growth of HeLa cells xenograft in immunosuppressed mice. Subcutaneous injection	500 mg/kg body weight/day. Reduced (57.4%) the tumor growth rate, Tissue weights of the lungs, liver, kidneys, spleen, and uterus did not differ among the groups treated	Overexpression of p53 and p21 proteins; reduced the expression of Rb, Cdk4 and cyclinD1 proteins; and levels of Cdk2 and cyclin E did not change	Hahm et al. (2015)

(Continued on following page)

TABLE 1 (*Continued*) In vivo evidence for anticancer properties of metabolites from cacti. Arranged alphabetically.

Genus/ specie	Part of cactus	Extract type	Metabolites	In vivo model	Dosis / Effect observed	Molecular / cellular effect	Reference
Pachycereus marginatus	Stem	Hexane, chloroform, methanol, and methanol-aqueous partition	Lophenol, β-sitosterol, and palmitic acid	Mouse lymphoma L5178Y-R	0.5 mg/kg metOH-aqueous orally. caused 60% survival at 60 days without altering liver parenchyma and enzymes	N.D.	Gomez-Flores et al. (2019)

8%, 45%, and 60%, respectively (Franco-Molina et al., 2003) (Supplementary Table S1). Afterwards, Zhi-Yu and colleagues (2011) investigated the cytotoxic effects of methanolic, hydrochloric, and sodium hydroxide extracts of *L. williamsii* on HeLa cells (a human cervical carcinoma cell line). The *in vitro* MTT analysis revealed that the methanolic and HCl extracts strongly inhibited HeLa cell proliferation in a dose-dependent manner. Interestingly, the NaOH extract had only minimal effects on the HeLa cells. At concentrations of 100 and 150 μg/mL, the extracts induced cell death in over 90% of the cells. The effective concentrations 50 (EC50) for the methanolic and HCl extracts were 4.2 and 7.8 μg/mL, respectively (Zhi-Yu et al., 2011).

Recently, Franco-Molina´s group applied the methanolic extract of Lophophora williamsii (LW) together with the chemotherapeutic agents temozolomide (TMZ) and panobinostat (PAN) to the C6 glia cell line of the mouse brain to investigate the cytotoxic potential of each agent individually and in combination, and to investigate their role in the release of damage-associated molecular patterns (DAMP) by assessing the proteins HMGB1, HSP70 and HSP90, which are characteristic indicators of immunogenic cell death inducers (ICD). All treatments induced cell death: PAN extract and LW induced apoptosis, while TMZ induced both apoptosis and necrosis. The concentrations of HMGB1, HSP70 and HSP90 in the cell lysates did not differ significantly, except for a difference between the LW-treated and non-treated cells. Furthermore, in a rat glioma model, they observed that all treatments decreased tumor volume, but the mechanism of cell death *in vivo* was not ICD. Finally, they indicate that the combination of TMZ, PAN and LW has a cytotoxic effect on glioma cells but does not induce ICD (Franco-Molina, et al., 2021) (Table 1).

2.7 Genus *Mammilaria*

Mammillaria, a prominent genus within the cactus family (Cactaceae), comprises around 200 species and is characterized by a remarkable morphological diversity (Figure 6B). These species are mainly native to North and Central America, with a particular stronghold in Mexico. Notable representatives include *M. roseoalba, M. magnimamma, M. gaumeri, M. nivosa, M. prolifera,* and *M. uncinata*. *Mammillaria* cacti typically exhibit globular or occasionally columnar growth forms. Their surface is decorated with a system of tubercles from which a crown of small pink or white flowers emerges (Bravo Hollis and Sánchez-Mejorada, 1991). The juicy, often bright orange-red or purple berries protrude above the tubercles. These berries are 11–17 mm long and 4.5–5.6 mm wide and have a flavor reminiscent of strawberries. Some *Mammillaria* fruits, affectionately known as "chilitos", owe their name to their dainty shape, reminiscent of chili peppers. Fruit production occurs during two distinct seasons: from January to May and from August to September (Aparicio-Fernandez, et al., 2013).

While *Mammillaria* is a favored choice for ornamental gardens within the Cactaceae family, scientific studies on the bioactivity of its stem extracts remain relatively scarce. In particular, Elansary et al. (2019) delved into the polyphenol profiles of seven *Mammillaria* cacti. Employing a HPLC-DAD method, they qualitatively and quantitatively assessed chosen flavonoids and phenolic acids. Their study also included the evaluation of anticancer, antioxidant and antibacterial properties. The methanolic extracts from the strains of different *Mammillaria* species exhibited antiproliferative activity against several cancer cell lines, including MCF-7, HeLa, Jurkat and HT-29, as well as HEK-293 (normal human cells). The highest activity was observed against HeLa, MCF-7, and Jurkat cells. Among these, the treatments involving *M. rhodantha, M. spinosissima* and *M. muehlenpfordtii* demonstrated the strongest antiproliferative effects. Additionally, the extracts of *M. rhodantha* and *M. spinosissima* exhibited the only anti-cancer activity against HT-29, while the treatments with *M. rhodantha* and *M. spinosissima* were most effective against Jurkat cells (Elansary, et al., 2019) (Supplementary Table S1).

2.8 Genus *Melocactus*

Melocactus, a genus of cacti, grow either singly or in groups with short cylindrical, occasionally spherical or flattened stems. Typically, they have 9 to 20 straight ribs, rarely spiral. The flowers arise from a well-developed, large, cylindrical, apical cephalium that is narrower than the stem itself. This cephalium bears areoles and sessile spines. The flowers are nocturnal and have simplified structures and colors ranging from pink to other shades. They have a long floral tube, the lower part of which is located between the cephalium elements (Figure 6D). *Melocactus* species are distributed in the Antilles and the continent from Brazil to Mexico. They are commonly known as "Turk's head" and in Mexico they are also called "viznaga de dulce". These cacti are used as ornamental plants and, in some regions, they are used to prepare a delicious regional dessert (Bravo Hollis and Sánchez-Mejorada, 1991). The genus *Melocactus* is characterized by a complex mixture of essential oils, flavonoids, steroids, terpenoids, alkaloids, carbohydrates and amino acids (Aquino-Martins et al., 2019; da Silveira Agostini-Costa, 2020) Figure 6A. In Brazil, *Melocactus zehntneri* has long been used in traditional medicine.

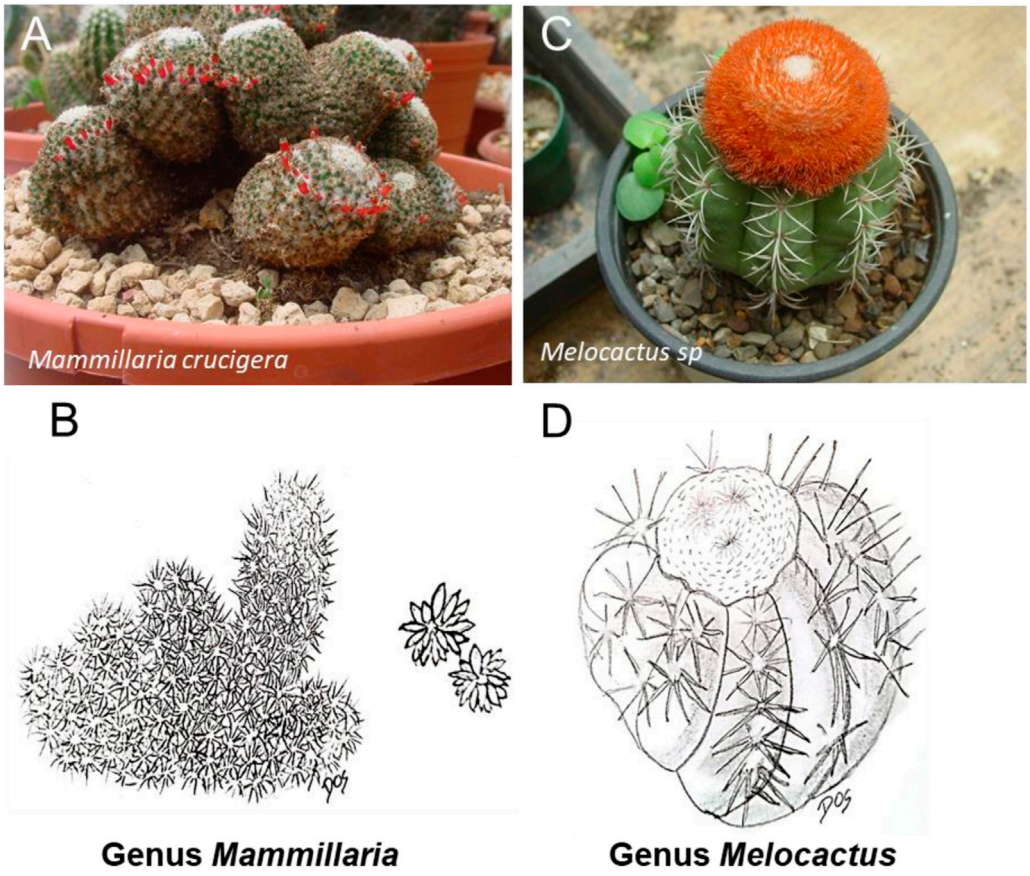

FIGURE 6
Schematic representative draw **(B, D)** and detailed picture **(A, C)** for the genus *Mammillaria* **(A, B)** and *Melocactus* **(C, D)** of cactaceae family. Mammillaria is the most popular cactus in cultivation. The most common representative of the Cactaceae from the genus *Mammillaria* is *Mammillaria crucigera* commonly called "Owl Eye Cactus". From the genus *Melocactus* is *Melocactus* zehntneri, also known as the Turk's cap cactus, or Pope's head cactus.

It has been employed to address respiratory conditions, influenza, physical fragility, throat inflammation, and whooping cough. Additionally, it is associated with "uterine cleaning" (Aquino-Martins et al., 2019; Brandão, et al., 2017; da Silveira Agostini-Costa, 2020).

Research on *Melocactus* has revealed intriguing properties. For instance, supercritical fluid extraction from young *M. zehntneri* plants yielded an alkaloid (or phenethylamine) fraction with activity against *Trichomonas vaginalis* and human vaginal malignant melanoma (HMVII) (Brandão, et al., 2017) (Supplementary Table S1). Various extracts from the pulp of *M. zehntneri* were tested for their effects on cell viability in the murine fibroblast cell line NIH/3T3. While hexane and final water extracts had no significant effect, ethanol, methanol and water extracts exhibited slight effects. Notably, the chloroform extract showed cytotoxicity and reduced cell viability to 68% (Aquino-Martins et al., 2019). *In vivo* evaluation of the alkaloid extract from *Melocactus bellavistensis* in Holtzmann rats with dimethylhydrazine (DMH)-induced revealed no neoplasms at doses of 1.5 and 10 mg/kg. These findings highlight the multifaceted nature of *Melocactus* and its potential applications in both traditional medicine and scientific research (Ríos-León, et al., 2017) (Table 1).

2.9 Genus *Myrtillocactus*

Native to central and northern Mexico, *Myrtillocactus* sp. is a stately, shrub-like cactus with impressive growth potential. It can reach a height of 4–5 m and has candelabra-like branches Figure 7B. The stems have a diameter of 6–12 cm and have five or six ribs. The flowers, which open in the morning, are white, pale yellow or cream-colored and are pollinated by bees and flies. This fast-growing cactus is resistant to adverse conditions and can tolerate poor soil, light frost and temperatures of up to 45°C. However, it does not thrive in salty soils or in heavy shade. The fruits of this species resemble a berry with a diameter of 1–2 cm. These fruits are commonly known as "garambullo" and are used in various culinary forms: eaten fresh, made into ice cream, popsicles, jam, jelly and even dried as raisins (Figure 7C). They are also mixed with "aguardiente" (a distilled alcoholic drink) to make spirits (Bravo-Hollis and Sánchez-Mejorada, 1978).

Salazar et al. (2011) investigated the sterols isolated from *M. geometrizans* (Figure 7A), in particular peniocerol and macdougallin. These metabolites showed cytotoxicity against several human cancer cell lines, including PC-3 prostate carcinoma, K-562 leukemia, U-251 central nervous system

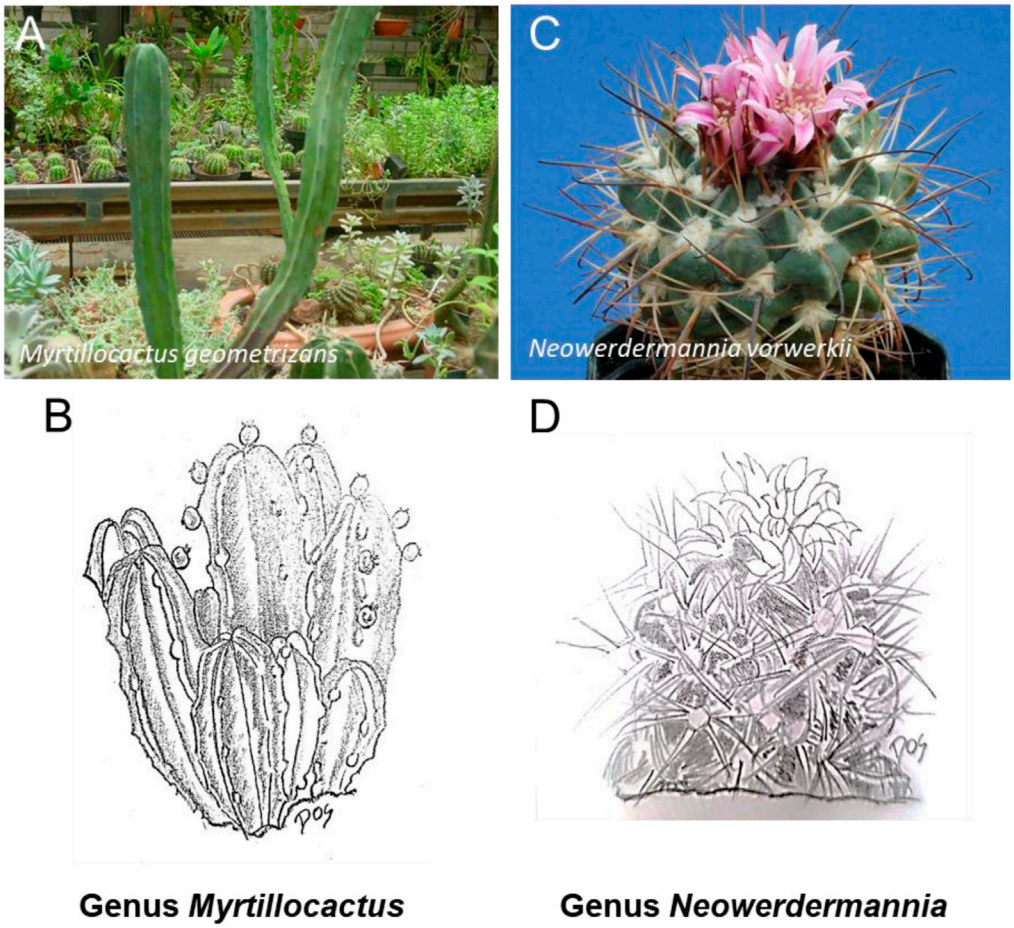

FIGURE 7
Schematic representative draw **(B, D)** and detailed picture **(A, C)** for the genus *Myrtillocactus* **(A, B)** and *Neowerdermannia* **(C, D)** of cactaceae family. The most common representative of the Cactaceae from the genus *Myrtillocactus* is *Myrtillocactus geometrizans* commonly called blue candle or blue myrtle-cactus. From the genus *Neowerdermannia* is *Neowerdermannia varwerkii*, also known as achakana (Aymara and Quechua).

glioma, MCF-7 breast carcinoma, and HCT-15 colon carcinoma. Both peniocerol and macdougallin showed moderate cytotoxic effects with IC50 values between 7.55 and 24.73 μM. Remarkably, peniocerol showed stronger activity than macdougallin in most cancer lines tested, with the exception of K-562 cells where macdougallin showed higher potency (Salazar, et al., 2011) (Supplementary Table S1). Dalla Via et al. (2014) also investigated the cytotoxic effect of peniocerol isolated from the root of *M. geometrizans* on three human tumor cell lines: HeLa (human cervical adenocarcinoma cells), HepG2 (hepatocellular carcinoma) and A-431 (epidermoid carcinoma). Peniocerol showed antiproliferative activity in all cell lines, with IC50 values in the micromolar range. In particular, it showed greater efficacy against HepG2 cells (26.0 μM) compared to HeLa cells (40.1 μM) and A-431 cells (34.6 μM) (Dalla Via et al., 2014) (Supplementary Table S1). In another study, Bolaños and colleagues (2015) administered peniocerol and macdougallin to colon and breast cancer cell lines. These bioactive metabolites showed a dose-dependent reduction in proliferation and apoptosis and effectively disrupted the cell cycle *in vitro* (Bolaños-Carrillo, et al., 2015) (Supplementary Table S1). Recently, Melchor Martínez et al. (2021) investigated the antioxidant capacity and bioactivity of clarified juices from different cactus fruits, including *M. geometrizans, Stenocereus* spp. and *Opuntia* spp. The clarified juice from *M. geometrizans* had the highest concentration of phenolic metabolites and induced cell death in liver and colon cancer cell lines and fibroblasts. The clarified juice from the yellow *Opuntia ficus-indica* fruit showed antioxidant activity and acted selectively on a liver cancer cell line without damaging fibroblasts (Melchor Martínez et al., 2021) (Supplementary Table S1).

2.10 Genus *Neowerdermannia*

Neowerdermannia vorwerkii Fric, is a solitary cactus native to the Andes region. These low-growing plants have a spherical to depressed spherical shape characterized by strong taproots. Approximately 16 indistinct ribs spiral around the cactus, dividing it deeply into tubercles. The areoles, which are often sunken, are located at the bases of the tips of these tubercles. Spines, some curved or even hook-shaped, adorn the cactus. The flowers, which are located at the tips of the tubercles, open during the day and are funnel-shaped. Their color ranges from white to lilac-pink. The spherical fruits open sideways and release the broadly

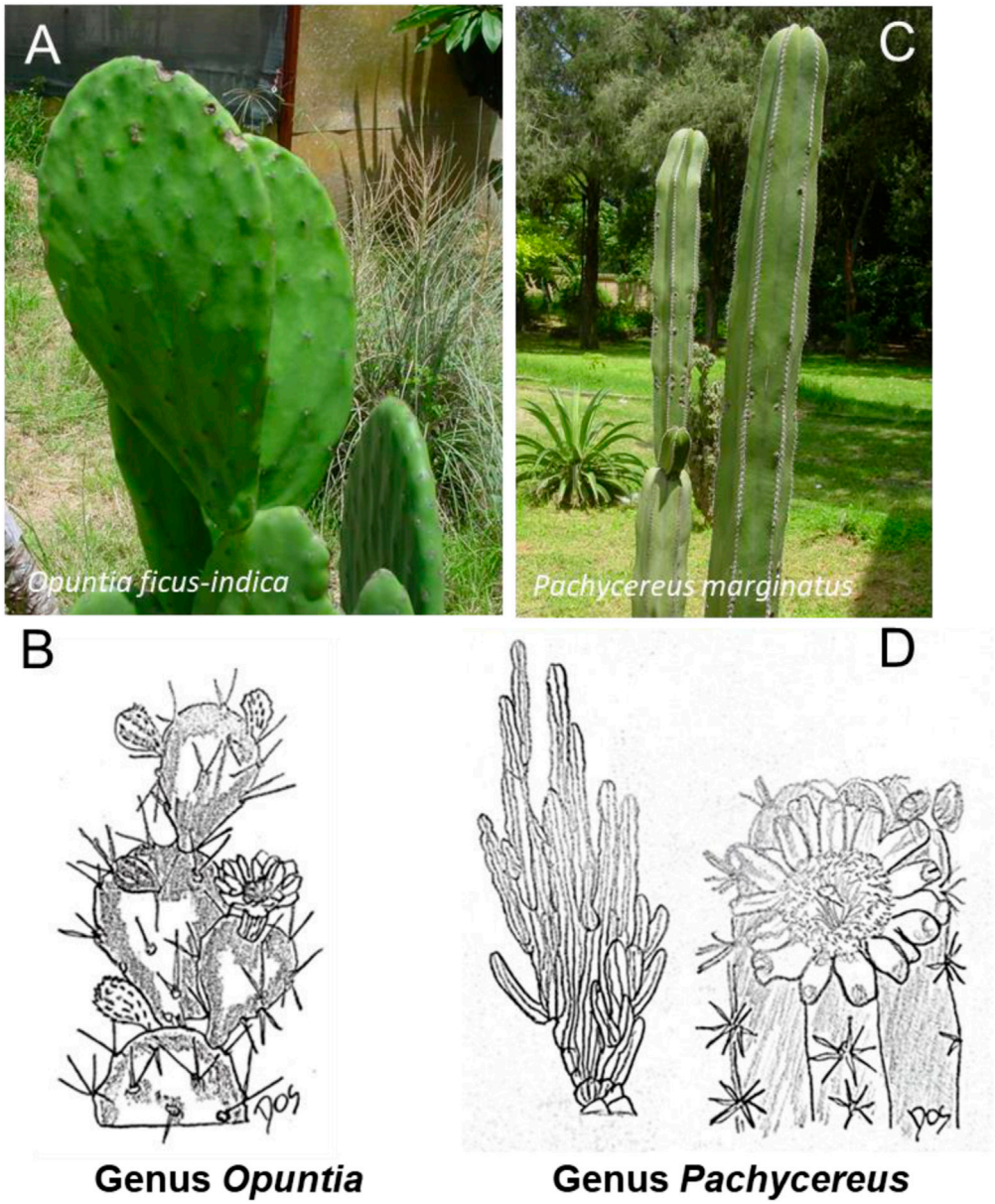

FIGURE 8
Schematic representative draw **(B, D)** and detailed picture **(A, C)** for the genus Opuntia **(A, B)** and Pachycereus **(C, D)** of cactaceae family. One of the most common and widely recognized representatives of the Cactaceae from the genus *Opuntia* is *Opuntia ficus-indica*, also known as the Prickly Pear Cactus. From the genus *Pachycereus* is *Pachycereus pringlei*, commonly known as the Cardón cactus or Mexican giant cardon.

oval seeds (Figures 7C, D). *Neowerdermannia vorwerkii,* known locally as 'Achacana', derives its name from the Quechua dialect, where 'achakahana' translates as 'woman of truth'. Traditionally, this cactus is used by communities in Bolivia, Peru, northern Argentina and northern Chile (Anderson, 2001). as a food (stew) and natural remedy (infusion) for various gastrointestinal complaints, kidney and liver diseases (Chuquimia, et al., 2008; Polini and Camaqui, 2018). In a recent study by Apaza Ticona et al. (2022), an aqueous extract of *N. vorwerkii* yielded three 1,2,4-oxadiazole-type metabolites derived from the n-hexane (HEX) sub-extract. These metabolites were subsequently fractionated with HEX and AcOEt mixtures. Spectroscopic and spectrometric analyzes identified three main metabolites that exhibited cytotoxicity in two tumor cell lines: SK-HEP-1 and Caco-2. Of note, one of them: 3-(pyridin-3-yl)-5-(tiophen-3-yl)-1,2,4-oxadiazole, showed a particularly high cytotoxic effect with CC50 values of 1.48 ± 0.40 μM (SK-HEP-1 cell line) and 1.35 ± 0.54 μM (Caco-2 cell line) (Apaza Ticona, et al., 2022) (Supplementary Table S1).

2.11 Genus *Opuntia*

Opuntia spp. commonly known as the prickly pear cactus belongs to the Cactaceae family, is one of the most aboundant

and widely distributed genera in America, Africa, Asia, Australia, and in the central Mediterranean area (Bravo-Hollis and Sánchez-Mejorada, 1978; Anderson, 2001; Madrigal-Santillán et al., 2022) (Figure 8B). However, the richest diversity of wild species occurs in Mexico, where at least 126 species have been observed (Bravo-Hollis and Sánchez-Mejorada, 1978; Santos Díaz et al., 2017; Daniloski, et al., 2022; Wang et al., 2023). Having several common names such as "tuna cardona", "tuna cascarona", "tuna chaveña", "tuna mansa", "tuna tapona and "xoconoxtle (cuaresmeño)" (Ramírez-Rodríguez, et al., 2020; Daniloski, et al., 2022; Madrigal-Santillán et al., 2022). *Opuntia* sp. plants produce edible cladodes, which are oval-shaped and flat shoot systems devoid of leaves. The pulp of their fruits, named as prickly pear, can exhibit various colors depending on ripeness (from white to purple, passing through yellow, green, orange and red). Recently, Jameel et al. (2023) investigated the insecticidal, antimicrobial and anticancer properties of cadmium sulfide nanoparticles (CdS NPs) synthesized from the fruit extract of *Opuntia* sp. They observed a reduction in the viability of pancreatic cancer cells, with viability at a concentration of 200 μg/mL being only 18 and 12 after 24 and 48 h of CdS NP exposure, respectively (Jameel, et al., 2023) (Supplementary Table S1).

Opuntia ficus-indica (*O.f-i*) is the most studied species of *cactis*. Their extracts and metabolites showed a plethora of antioxidant, antiviral, antifungal, antibacterial, and anticancer activities. Mainly, their antioxidant activity has been studied as part of their chemopreventive and antigenotoxic potential (Figure 8A). An aqueous acetone extract of Tunisian *O.f-i* syrup showed strong antioxidant potential and also antibacterial activity, being effective against the bacteria *Staphylococcus aureus* and *Staphylococcus epidermidis*, with a minimum bactericidal concentration of 1.3 mg phenols/mL (Dhaouadi, et al., 2013). In addition, acetone, aqueous and ethanol extracts of *Opuntia* stricta cladodes, showed antioxidant activity and anti-inflammatory effects. It is noteworthy that the total phenolic content in all extracts was higher than that of other phytochemicals (Izuegbuna, et al., 2019). The major fatty acid in the oil of *O.f-i* was linolenic acid, followed by oleic acid, linoleic acid, palmitic acid, and stearic acid. The oil also contained total tocopherol (α-, β-, γ-tocopherol) and phytosterols, with campesterol being the major phytosterol, followed by γ- and β-sitosterol and stigmasterol. Being the phenolic metabolites the ones that shows the strongest antioxidant activity (Alqurashi, et al., 2022).

The chemopreventive and antigenotoxic properties of *O.f-i* have been studied in *vitro* models by using different types of extracts, such as: hexane, ethyl acetate (EtOAc), acetone, methanol (MeOH) and aqueous ones, showing promising results (Madrigal-Santillán et al., 2022; Daniloski, et al., 2022). Studies have shown different cytotoxic effects of *O.f-i* extracts on different cancer cell lines. Dhaouadi et al. (2013) showed that an aqueous acetone extract at concentrations ranging from 41.38 to 186.25 μg polyphenols/mL reduced the viability of human SH-SY5Y neuroblastoma and 3T3 fibroblast cells *in vitro* in a dose- and time-dependent manner. While concentrations greater than 106.43 μg/mL resulting in a 50%–60% decrease in cancer cell viability within 1–3 h of treatment. Similarly, Kim et al. (2015) investigated various extracts from *O.f-i* strains for their chemopreventive activities against human SW480 colon and MCF7 breast cancer cells. They found significant cytotoxicity in most extracts, especially in SW480 cells, accompanied by inhibition of cyclooxygenase-2 and increased Bax/Bcl2 ratio (Supplementary Table S1). In addition, Kim et al. observed increased degradation of poly-(ADP-ribose) polymerase, which triggered apoptosis, especially in MCF7 cells treated with *O.f-i* stem extracts. In addition, Abdulazeem et al. (2018) and Das et al., 2021 found cytotoxic activity against MCF-7 and WRL-68 cell lines in alkaloid extracts from dried *Opuntia polyacantha* plants with inhibition rates of 52.7% and 91.89%, respectively, at a concentration of 400 μg/mL. In contrast, Izuegbuna et al. (2019) reported that only the acetone-dried extract showed activity against the U937 cell line at concentrations of 100 and 200 μg/mL. Posteriorly, Alqurashi, et al. showed a differential effect on the cytotoxic activity of the *O.f-i* oil over Vero cells (derived from the kidney of the African green monkey), the PC-3 cell line (prostate carcinoma), and the A2780 cell line (ovarian carcinoma). Revealing that oil exhibited highest cell viability (99.63% ± 0.45%) at 250 μg/mL of oil against Vero cells. In the case of A-2780 cell line, *O.f-i* oil showed weak inhibitory activity. Although in the case of PC-3 cell line, *O.f-i* oil showed significant inhibitory activity (70% and 83% at 250, and 500 μg/mL of oil respectively) (Alqurashi, et al., 2022). At that time, Ali et al. (2022) evaluated the cytotoxicity of different concentrations (1, 10, 100, 200, 300, 400 μg/mL) for cyclohexanone (OFICE), 70% (OFI70EE) and 100% (OFI100EE) ethanol of *O.f-i* peel (OFIPs) extracts, against MCF-7 cell line detected by sulforhodamine B (SRB) assay. They showed a high dose of OFICE, OFI70EE, and OFI100EE highly significantly ($p < 0.05$) reduced cell viability of MCF-7 cancer cells at a concentration of 400 μg/mL after 72 h. Respecting the effect of three *O.f-i* extracts on cells exhibits dose-dependent toxicity and that OFICE at different concentrations showed significantly reduced cell viability than the ethanol extracts and the former have a greater effect as antitumor (Ali, et al., 2022). These results underline the diverse anticancer potential of *Opuntia* extracts in different cell types and extraction methods.

Some works have been demonstrated anti-cancer properties from *O. ficus* extracts. Using an aqueous extract of prickly pear (*O.f-i*) on ovarian, cervical and bladder cancer cells and tumor growth in nu/nu Balb/C mice, Zou et al., have shown that after a 1-, 3-, and 5-day treatment of the cells, a dose-dependent and time-dependent inhibition of cell growth and an induction of apoptosis was observed. In addition, the extracts showed a significant suppression of tumor growth and an upregulation of annexin IV expression in the animals. Furthermore, they affected the cell cycle of cancer cells by promoting G1 phase arrest and decreasing G2 and S phases (Zou et al., 2005) (Supplementary Table S1, Table 1). On another studies, Feugang et al. (2010) showed that an aqueous extract of prickly pear cactus increases oxidative stress by increasing the concentration of reactive oxygen species (ROS) and inducing DNA methylation of p16 and RASSF-1A in bladder cancer cells (Feugang, et al., 2010). Additionally, it is capable of eliciting a significant degree of DNA fragmentation in ovarian cancer cells (OVCA420) (Feugang, M., et al., 2010). In both, changes in the expression of apoptosis-related genes, such as: Bax, Bad, caspase 3, Bcl2, p53 and p21, that respond to ROS were detected. Likewise, a decrease in NF-kappa B expression was observed in conjunction with an increase in p-AKT expression. Also, Serra and coworkers (2013) using an hydroalcoholic extracts of *O.f-i* and *Opuntia robusta* evaluated their antiproliferative effect in a human colon carcinoma

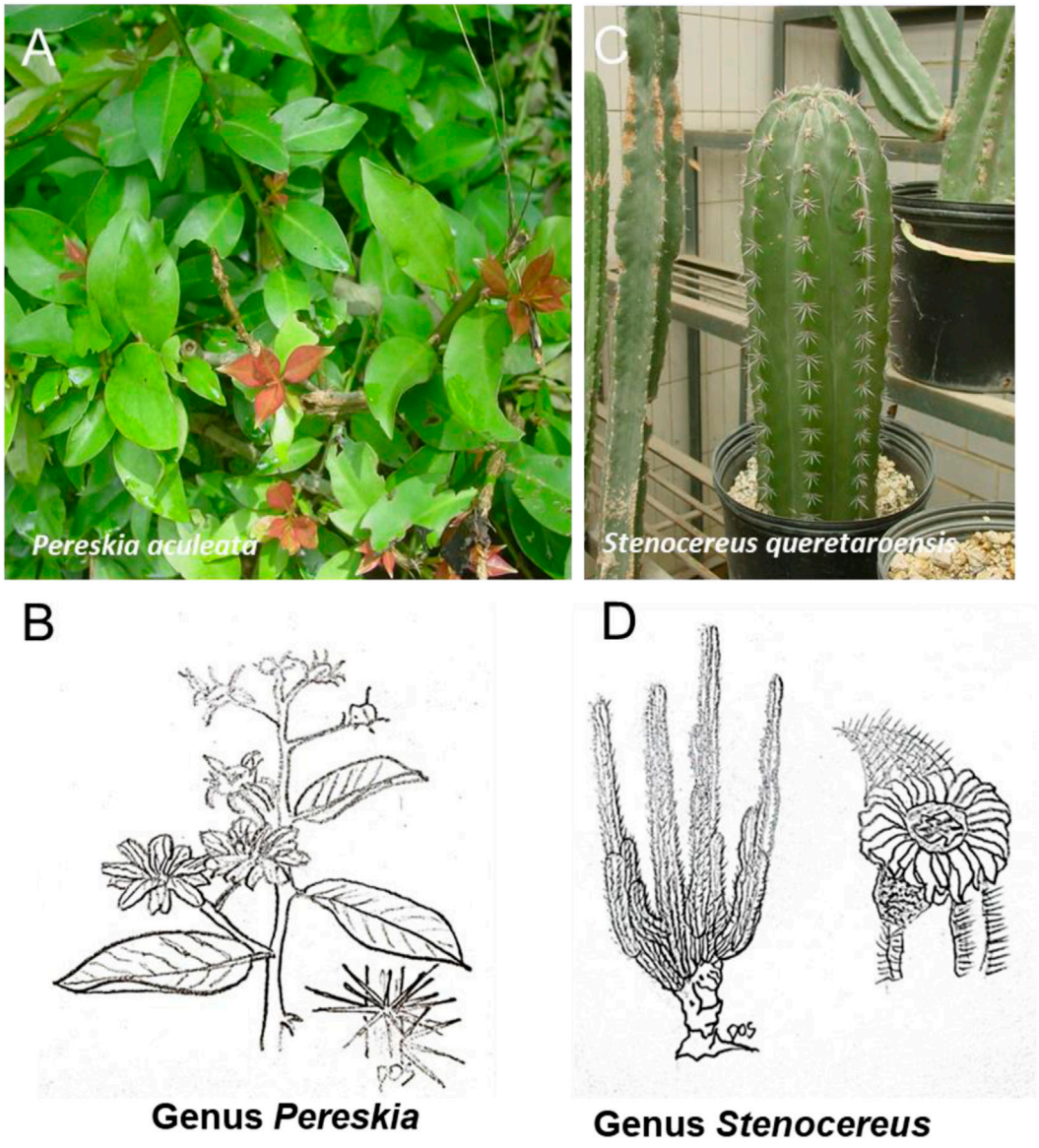

FIGURE 9
Schematic representative draw **(B, D)** and detailed picture **(A, C)** for the genus Pereskia **(A, B)** and Stenocereus **(C, D)** of cactaceae family. One of the most common representatives of the Cactaceae from the genus *Pereskia* is *Pereskia aculeata*. Also known as Barbados gooseberry or leaf cactus. From the genus *Stenocereus* is *Stenocereus pruinosus*, also known as the Organ Pipe Cactus.

HT29 cell line. The results showed that the natural extracts efficiently inhibited cancer cell growth and induced cell cycle arrest in different checkpoints G1, G2/M, and S. The phytochemical metabolites present in the samples, namely, betacyanins, flavonoids and phenolic acids were identified as the main responsible for the cell cycle arrest (Serra, et al., 2013). More recently, Ortíz-González et al. (2022) used the big data platform Cellulat to model the PI3K/Akt/mTOR signaling pathway, which is critical for cell cycle regulation and cancer cell survival. Their aim was to predict the targets affected by *O.joconostle* extract and determine the concentration range for *in vitro* experiments on breast cancer cells. *In silico* analysis revealed that the extract inhibits cell proliferation, regulates the cell cycle and induces apoptosis via the PI3K/Akt/mTOR pathway. *In vitro* experiments showed the antiproliferative effect of the aqueous crude extract by arresting the cell cycle in the G2/M phase (Ortíz-González, et al., 2022).

Different types of *O.f-i* seed oils exhibited apoptotic induction effects on colon adenocarcinoma cell lines. Becer et al. investigated, in a couple of studies, the fatty acid content and apoptotic effect of cactus pear seed oils (CPS) with and without spines of *O.f-i* on colon adenocarcinoma cell lines. They observed activity against Colo-320 and Colo-741 cells with CPS oil with spines, while CPS oil without spines significantly reduced the number of viable cells in both lines. Using the TUNEL assay, they found a significantly higher number of TUNEL-positive cells in Colo-320 cells treated with CPS without spines than in the control group. In another study, they investigated the antiproliferative effect of CPS oils on primary (Colo-320) and

metastatic (Colo-741) colon adenocarcinoma cell lines. They found a reduction in the proliferation of Colo-320 cells, together with a protective effect on molecular mechanisms related to angiogenesis. Interestingly, thornless CPS oil increased angiogenesis through signaling molecules, while spiky CPS oil decreased signaling molecules involved in angiogenesis. Both types of CPS oils increased TNF-α expression in Colo-320 cells, but not IL-6, indicating no significant changes in the tumor microenvironment (Becer et al., 2018; 2021). Meanwhile, Heikal et al. (2021) compared the anticancer effects of extracts from *O.f-i* cladodes cultured *in vitro* and *in vivo* on PC3 prostate cancer and Mcf7-7 breast cancer cell lines. They showed that *in vitro* micropropagated cladodes produced more phenols and kaempferol than those cultured *in vivo*, with a dose-dependent increase in cytotoxicity observed in both PC3 and Mcf7 cells (Heikal, et al., 2021) (Supplementary Table S1).

While betacyanins, flavonoids, and phenolic acids have been identified as responsible for the antiproliferative effects of the extracts (Serra et al., 2013; Aruwa et al., 2018), various studies have investigated the antitumor properties of phytochemicals extracted from different parts of *O.f-i*. Sreekanth et al. (2007) investigated the antiproliferative effect of betanin on chronic myeloid leukemia cells and found a reduction in cell proliferation and induction of apoptosis. Li et al. (2014) demonstrated the growth inhibition of lung squamous cell carcinoma cells by polysaccharides extracted from *Opuntia dillenii*, suggesting cell cycle inhibition and induction of apoptosis. Antunes-Ricardo et al. (2014) investigated the cytotoxicity of extracts and glycosides from *O.f-i* on colon cancer cells and emphasized their differential effects on cell viability and induction of apoptosis. In their subsequent studies, they elucidated the mechanisms involved in apoptosis induction by isorhamnetin-3-O-glucosylpentoside (IGP) and demonstrated caspase-dependent mitochondrial damage in colon cancer cells. In addition, they demonstrated the growth inhibitory effect of *O.f-i* extract and its glycoside isorhamnetin-3-O-glucosylrhamnoside (IGR) on colorectal adenocarcinoma cells and their effects on apoptosis induction and tumor growth reduction in a mouse model. Another compound, indicaxanthin, extracted from the fruit of *O.f-i*, showed significant inhibitory effects on human melanoma cells both *in vitro* and *in vivo* by inducing apoptosis and reducing cell invasiveness (Antunes-Ricardo et al., 2019; 2021) (Supplementary Table S1, Table 1). Meanwhile, Lefsih et al. (2018) discovered the cytotoxic activity of pectin extracts on LAN5 cancer cells without affecting normal cells, suggesting their potential as selective anticancer agents. Overall, these results highlight the promising anticancer properties of *O.f-i*-derived metabolites in various cancer types and provide insights into their mechanisms of action (Lefsih, et al., 2018) (Supplementary Table S1).

Summarizing, *O.f-i* and its various extracts and metabolites show promising potential in cancer prevention and treatment through multiple mechanisms, including antioxidant activity, apoptosis induction, and cell cycle arrest.

2.12 Genus *Pachycereus*

The genus *Pachycereus*, which belongs to the tribe *Pachycereae* in North America, comprises five species of tree-like cacti that are mainly found in western Mexico, with some extensions to Baja California, Sonora and Chiapas. *Pachycereus* is characterized by its columnar and candelabra-like shape and is known by various names such as cardón, organ or candelabra (Figure 8D). *Pachycereus weberi*, for example, can reach a height of up to 15 m, with trunks 60–200 cm tall and 30–60 cm in diameter, with gray bark and persistent spines. This species is native to southern and central Mexico, especially in the arid and semi-arid regions of Puebla, Guerrero, Morelos and Oaxaca. Its fruit, which is often referred to as "chico fruit" or "tuna de cardón"," is usually used to make refreshing fruit drinks by pureeing it and adding water (Arias and Terrazas, 2009).

Various extracts from the fruit juice of *Pachycereus marginatus* (Figure 8C) and *P. weberi* were tested for their cytotoxic effect against different cancer cell lines. Extracts from *P. marginatus* showed significant inhibition against murine lymphoma L5178Y-R and skin melanoma cells B16F10, with methanolic and hexane extracts exhibiting high cytotoxicity (Quintanilla-Licea, et al., 2016). The fruit juice of *P. weberi* showed cytotoxic activity against four different mammalian cancer cell lines: Mammary carcinoma (MCF-7), prostate (PC3), colon (CaCo-2) and liver (HepG2), specifically damaging colon CaCo2 and mammary carcinoma MCF-7 cells (Sandate-Flores, et al., 2020). In addition, studies with *P. marginatus* extract and isolated metabolites such as lophenol, β-sitosterol and palmitic acid showed promising cytotoxic effects against lymphoma cells *in vitro* and improved survival rates in tumor-bearing mice.

In a different work, the cytotoxic effect of different extracts and metabolites from *P. marginatus* on L5178Y-R lymphoma cells was investigated. Hexane, chloroform and methanol extracts showed varying degrees of cytotoxicity, with significant percentages ranging from 20% to 85%, 32%–84% and 32%–72%, respectively. The extract of methane and the aqueous partition also showed significant cytotoxicity, ranging from 65% to 78% (Hernández-Martínez, et al., 2016). Later on, another study investigated the effects of the *P. marginatus* extract and the isolated compounds, including lophenol, β-sitosterol and palmitic acid, on mice with tumors. Oral administration of the aqueous partition extract resulted in a 60% survival rate at 60 days, with no adverse effects on liver function or histopathology. In addition, the n-hexane extract and metabolites showed considerable cytotoxicity against L5178Y-R cells *in vitro*, indicating their potential as anticancer agents (Gomez-Flores, et al., 2019) (Table 1). Recently, Aispuro-Hernández and colleagues evaluated the nutritional quality and antiproliferative capacity of the fruit juice of *Pachycereus pecten-aboriginum* and *P. pringlei* in cervical cancer (HeLa) and breast cancer (MDA-MB-231, MCF-7 and T-47D) cell lines. The results showed that the fruit juices of both cacti are rich in polyphenols, flavonoids, betalains, vitamin C and myo-inositol and have antioxidant and anticancer potential by inhibiting the proliferation of all cell lines studied, with IC50 values ranging from 198 to 287 μg gallic acid equivalents/mL (Aispuro-Hernández, et al., 2023).

2.13 Genus *Pereskia*

The genus *Pereskia*, which comprises 17 species, is characterized within the cactus family (Cactaceae) by its unique features, which

include regular leaf development and green leaves. Native to regions between Brazil and Mexico, *Pereskia* plants have large leaves and thin stems, unlike typical cacti (Figure 9B). Historically, species such as *Pereskia aculeata* (Figure 9A) and *Pereskia grandifolia*, known as ora-pro-nobis, were used to decorate colonial-era churches in Brazil (da Silveira Agostini-Costa, 2020). In addition to its use as an ornamental plant, *Pereskia* also has a long history in traditional medicine. Various species are used to treat diseases such as cancer, high blood pressure, diabetes and stomach problems. In addition, the *pereskia* leaves, which are known for their high protein content, are consumed as leafy vegetables in Brazil. They contain essential amino acids and fulfill the nutritional requirements according to FAO standards. These leaves have various biological activities, including anti-inflammatory, antimicrobial and anti-cancer properties, which make them valuable in both traditional and modern medicine (Sharif, et al., 2013; da Silveira Agostini-Costa, 2020).

Some *Pereskia* species, including *P. grandifolia, P. aculeate, Pereskia sacharosa* and *Pereskia bleo*, have been studied for their cytotoxic effects on various cancer cell lines, with particular attention given to *P. bleo*. Studies by Malek NurestriAS. et al. (2009) revealed that the hexane extract of *P grandifolia* (Haw) exhibited the highest cytotoxicity on the KB cell line, while the ethyl acetate extract showed significant cytotoxic effects on both KB and MCF7 cell lines. In addition, seven isolated metabolites from *P. grandifolia* (Haw) were tested for cytotoxic activity, with 2,4-di-tret-butylphenol showing similar cytotoxicity to *P. bleo* (Kunth). Malek et al. performed similar studies with methanolic and fractionated extracts of *P. grandifolia* (Haw) on a human lung carcinoma cell line (A549). In addition, Pinto et al. (2012) investigated the cytotoxic activity of the crude methanolic extract and different fractions from the leaves of *P. aculeate* (Miller) on human promyelocytic leukemia (HL60) and human breast adenocarcinoma (MCF-7) cell lines and observed inhibition of the cancer cell line without affecting normal cells. In addition, Asmaa et al. (2014) demonstrated the proliferation inhibitory effect of ethanolic extract of *P. sacharosa* (Griseb) on two leukemic cell lines, MV4-11 and K562, by inducing intrinsic apoptotic pathways and cell cycle arrest with upregulation of apoptosis and cell cycle-related regulatory proteins (Asmaa, et al., 2014).

Research on *P. bleo* (Kunth) has demonstrated its potential cytotoxic effect on various cancer cell lines. Tan et al. (2005) found that the methanol extract of *P. bleo* (Kunth) showed a significant cytotoxic effect on human breast cancer cells T-47D, with saponin and complex glycosides being responsible for the high cytotoxicity. This study also illustrated the mechanism of cell apoptosis and concluded that the activation of caspase-3 and c-myc pathways was the route of cell death. Similarly, Er et al. (2007) applied both methanolic and aqueous extracts of *P. bleo* (Kunth) to mouse breast cancer cell line (4T 1) and a normal mouse fibroblast cell line (NIH/3T3), and observed significant anti-proliferative effects under different conditions. The result of this study also contradicted the study by Tan et al. The authors suggested two possible reasons for this contradiction: 1) The metabolites of the plant extract responsible for the cytotoxic activity may not be concentrated in the leaves, as the extracts of the first study were obtained from leaves and stems of the plants, while only leaves were used in the second study. 2) The content of fetal bovine serum was different in both tests (Er, et al., 2007).

Malek et al. (2008) investigated the cytotoxic activity of methanolic and fractionated extracts (hexane, ethyl acetate, and water) of *P. bleo* (Kunth) on different human carcinoma cell lines, with the ethyl acetate and methanol extracts showing significant cytotoxicity against the KB cell line (Malek, et al., 2008). On the other hand, five isolated bioactive metabolites (dihydroactinidiolide, 2,4-di-tret-butylphenol, α-tocopherol, β-sitosterol and phytol) from the ethyl acetate extract of *P. bleo* (Kunth) were individually evaluated against five cancer cell lines. But only 2,4-ditret-butylphenol showed remarkable inhibition and α-tocopherol was well cytotoxic against different cell lines Ethyl acetate and methanol extract of *P. bleo* (Kunth) showed the most significant cytotoxicity against only 1 cell line (KB), while the hexane fraction exerted a moderate cytotoxic effect on the same cell line. The interesting result was that the methanolic crude extract and all fractionated extracts had no damaging effect on the non-cancer cell line (MRC-5) (Malek, et al., 2009a). Conversely, Liew et al. (2012) found moderate cytotoxicity of crude methanol extracts of *P. grandifolia* (Haw) against hypoxic cancer cells through inhibition of HIF (Hypoxia Inhibition Factor) activity, while extracts of *P. bleo* (Kunth) showed no cytotoxic effect.

More recently, Siew et al. (2019) investigated the antiproliferative activity of *P. bleo* (Kunth) extracts in different cancer cell lines and obtained promising results. They used three different solvents (water, ethanol, and methanol) from seven medicinal plants, including *P. bleo*, over twelve human cancer cell lines. In addition, Mohd-Salleh et al. (2020a) in a couple of studies identify the phytochemicals present in the crude extracts of *P. bleo* (Kunth) leaves by using hexane, ethyl acetate, methanol, and aqueous via Gas chromatography mass spectrometry (GCMS) technique.

Mohd-Salleh et al. (2020b) conducted a study to identify phytochemicals present in crude extracts of *P. bleo* (Kunth) leaves and assesed their cytotoxic effect on selected cancer cell lines. The ethyl acetate extract demonstrates significant cytotoxicity against cervical and breast cancer cells. Furthermore, this extract induced cell cycle arrest and apoptosis in cervical cancer cells (Siew, et al., 2019). Specifically, the ethyl acetate extract of *P. bleo* leaves exhibited potent cytotoxic effect on HeLa cells at IC50 value of 17.51 ± 8.6 μg/mL, and showed activity against MDA-MB-231 cells at 19.39 ± 1.26 μg/mL. Meanwhile, it exhibited a moderate cytotoxic effect on SW480 cells with an IC50 value of 31.80 ± 16.1 μg/mL (Mohd-Salleh, et al., 2020a). In a separate study, the same researchers investigated the effects of the ethyl acetate extract of *P. bleo* leaves (PBEA) on inducing cell death through cell cycle arrest and apoptosis in cervical cancer cells (HeLa), using flow cytometry. Their findings reveal that PBEA caused cell cycle arrest at the G0/G1 phase and upregulated apoptotic protein levels of Bax and p53 while downregulating Bcl-2 in a time-dependent manner over a 72-h treatment period (Mohd-Salleh, et al., 2020b). Overall, these findings underscore the potential of *P. bleo* (Kunth) as a promising source of cytotoxic metabolites for cancer therapy.

2.14 Genus *Stenocereus*

Stenocereus (A. Berger) Riccob, commonly referred to as organ pipe cactus, is a columnar cactus species with a wide geographic distribution spanning from the southwest United States of America

to Venezuela in South America Figures 9B–D. It comprises 24 species, of which around 80% are found in various Mexican states such as Jalisco, Nayarit, Colima and Oaxaca (Bravo-Hollis and Sánchez-Mejorada, 1978; Ramírez-Rodríguez, et al., 2020; García-Cruz, et al., 2022). The plant has columnar branches that grow from the ground. The stems grow 15–20 cm thick and up to 5 m high and often have no branches, hence its nickname. It produces pink flowers and edible fruit known as "piraya". They are characterized by deciduous areoles that contain pulp in shades of red, yellow, orange, purple, white or pink, along with small black seeds (Bravo-Hollis and Sánchez-Mejorada, 1978; Harlev, et al., 2013; Ramírez-Rodríguez, et al., 2020). These fruits have a low acidity, a high soluble solids content and a sweet taste. Typically, the fruit is spherical to ovoid, 5–10 cm long and is harvested twice a year, from April to May and from September to October (Pimienta-Barrios and Nobel, 1994; Ramírez-Rodríguez, et al., 2020). Ethnobotanical studies indicate various traditional uses of piraya, including use against rheumatism, insect bites, snake bites, hemorrhages, gastrointestinal problems, diabetes mellitus, hypertension, and to protect the immune system (Ramírez-Rodríguez, et al., 2020). In particular, *S. thurberi,* a species from the genus *Stenocereus*, is known for its effectiveness in treating skin cancer and skin lesions and is often used for this purpose in traditional Mexican medicine (Harlev, et al., 2013).

Melchor Martínez et al. (2021) investigated the cytotoxic effect of five clarified juices extracted from different cactus fruits on four different mammalian cancer cell lines, including MCF-7, PC3, Caco-2 and HepG2, as well as on a normal cell line NIH/3T3. They observed a significant decrease of 70% in the viability of HepG2 and PC3 cells when treated with the juices from red and yellow pitayas of *S. spp*. respectively. However, these juices showed no antiproliferative effect on MCF-7 and Caco-2 cells. Conversely, the proliferation of normal NIH/3T3 cells was inhibited by 43% and 55% when exposed to the juice of yellow and red pitayas, respectively (Melchor Martínez et al., 2021) (Supplementary Table S1).

3 Conclusion and perspectives

The surge in life expectancy has led to an increased prevalence of chronic degenerative diseases, particularly cancer. This trend represents a significant challenge to public health, given the high mortality rates associated with these conditions. Chemotherapy, a critical component of cancer treatment, is hampered by the phenomenon of multidrug resistance, highlighting the need for alternative therapeutic strategies. Natural products, particularly those derived from plant sources such as cacti, are emerging as potential solutions to these challenges. Their historical medicinal use and burgeoning scientific research provide compelling evidence of their potential. The cacti family, a diverse group of succulent plants, harbors a wealth of bioactive metabolites that exhibit potential anti-cancer properties. However, research into the chemopreventive and anticancer capabilities of cacti is still in its early stages. Although there are more than 1,500 species, very few have been studied. In this review, we report on 14 genera and 36 species with which anticancer studies have been conducted. On the other hand, most of these studies have been conducted with crude extracts of fruits, leaves and stems, and very few pure metabolites have been determined, and even less studies have investigated the molecular or immunological mechanisms of action (Supplementary Table S1). This review, based on an extensive literature search, is intended to shed light on experimental efforts investigating the role of cacti as potential anticancer agents. It is important to point out that most of these studies have been conducted *in vitro* with tumor lines of human origin, where experimental conditions are tightly controlled, and many systemic physiological variables are not checked or measured. For example, it is not known whether the concentrations used *in vitro* can be reproduced *in vivo* or whether there are side effects in other systems, and only a very limited number have been extended to animal models. As we look towards the future, further research into the therapeutic potential of cacti and other natural products holds promise for the development of novel anti-cancer agents. These agents may have improved efficacy and reduced side effects compared to current treatments, which would represent a significant advance in the field of cancer therapeutics.

Author contributions

AO-B: Conceptualization, Supervision, Writing–original draft, Writing–review and editing, Data curation, Formal Analysis, Investigation, Methodology, Resources. MS-S: Investigation, Writing–review and editing, Data curation, Validation. AR-M: Investigation, Writing–review and editing, Project administration, Supervision, Visualization. MG-R: Data curation, Funding acquisition, Investigation, Resources, Validation, Writing–review and editing. MR-M: Data curation, Investigation, Validation, Writing–review and editing. DO-S: Conceptualization, Funding acquisition, Project administration, Supervision, Visualization, Writing–original draft, Writing–review and editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. CONACyT-Mexico Grant Ciencia de Frontera-2019-552265 to DO-S. Universidad de Guadalajara APPAC 2024 to MS-S, MG-R, MR-M and DO-S, also CUCBA for AR-B.

Acknowledgments

The authors would like to thank Enrique Sabes Sánchez Ibañez, a cactologist, who confirmed that the pictures of the different genera correspond to the nomenclature and scientific names.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2024.1421136/full#supplementary-material

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frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 30 October 2024
DOI 10.3389/fphar.2024.1471307

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OPEN ACCESS

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RECEIVED 27 July 2024
ACCEPTED 08 October 2024
PUBLISHED 30 October 2024

CITATION
Wang Z, Wang X, Mou X, Wang C, Sun Y and Wang J (2024) *Rehmannia glutinosa DC.-Lilium lancifolium Thunb.* in the treatment of depression: a comprehensive review and perspectives.
Front. Pharmacol. 15:1471307.
doi: 10.3389/fphar.2024.1471307

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Rehmannia glutinosa DC.-Lilium lancifolium Thunb. in the treatment of depression: a comprehensive review and perspectives

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Background: In recent years, the incidence of depression, recognized as a serious psychological disorder, has escalated rapidly. *Rehmannia glutinosa DC.* (Scrophulariaceae; Rehmanniae Radix, Crude drug) and *Lilium lancifolium Thunb.* (Liliaceae; Lilii bulbus, Crude drug) constitute a classic anti-depressant combination, exhibiting pharmacological effects that include anti-depressive, anti-anxiety, and anti-inflammatory properties. Current clinical studies have demonstrated that Baihe Dihuang Decoction, a traditional Chinese herbal compound, is effective in treating depression. However, the majority of scholars have predominantly examined *Rehmannia glutinos*a and *Lilium* in isolation, and a comprehensive elucidation of their principal active metabolites and pharmacological mechanisms remains lacking.

Methods: A comprehensive literature search was conducted as of 29 September 2024, utilizing databases such as PubMed, CNKI, Wanfang Data, Baidu Scholar, and Google Scholar. Additionally, classical texts on Chinese herbal medicine, the Chinese Pharmacopoeia, as well as doctoral and master's theses, were included in the collected materials. The search employed specific terms including "*R. glutinosa*," "*Lilium*," "Baihe Dihuang decoction," "application of Baihe Dihuang decoction," "pathogenesis of depression," and "pharmacological action and mechanism of depression.

Results: This paper reviewed the traditional applications and dosages of the *R. glutinosa-Lilium* as documented in Chinese medical classics, thereby establishing a foundation for the contemporary development and clinical application of the classical formula Baihe Dihuang Decoction. Additionally, recent years have seen a comprehensive review of the pharmacological effects and mechanisms of *R. glutinosa-Lilium* and its principal metabolites in the context of depression.

Conclusion: This paper has reviewed the active metabolites of *R. glutinosa-Lilium* and demonstrated its efficacy in the treatment of depression, as well as its role in

modulating the underlying mechanisms of the disorder. The findings aim to serve as a reference for further research into the mechanisms of depression, its clinical applications, and the development of novel therapeutic agents.

KEYWORDS

Lilium, *Rehmannia glutinosa*, depression, active metabolites, pharmacological mechanism

1 Introduction

Depression, being a prevalent mental disorder, is characterized by a range of clinical manifestations including but not limited to low mood, sluggish cognitive processing, sleep disturbances, social withdrawal, reduced motivation, and in severe cases, self-injurious behavior (Filatova et al., 2021). The COVID-19 pandemic resulted in a global increase of 28% in depression cases in 2020, with young people in particular suffering (Sljivo and Kulenovic, 2023; Blomqvist et al., 2023). At present, the etiology of depression remains incompletely comprehended, albeit the most notable hypotheses include an imbalance of monoamine neurotransmitter imbalance (Hirschfeld, 2000), decreased concentration of neurotrophins (Duman and Monteggia, 2006), inflammation and oxidative stress (Yang et al., 2020), disorder of Hypothalamus-Pituitary-Adrenal (HPA) axis (Yu et al., 2023), intestinal flora imbalance (Lach et al., 2018), mitochondrial dysfunction (Buttiker et al., 2022), etc. Despite the efficacy of selective serotonin reuptake inhibitors, tricyclic antidepressants, and other Western pharmacological interventions in treating depression, they are associated with certain drawbacks, including suboptimal therapeutic outcomes, prolonged duration of action, significant adverse effects, and high expenses (Kovich et al., 2023; Stachowicz and Sowa-Kucma, 2022). As a result, it is imperative to find new depression-treating drugs.

Traditional Chinese medicine (TCM) compound prescriptions offer numerous advantages in the treatment of depression, such as the incorporation of multiple metabolites, pathways, and targets, with a notable emphasis on drug compatibility (Xiong et al., 2022; Liu et al., 2023; Yang et al., 2022). Compared with the compound of TCM, drug pairing is a relatively fixed form of two-flavor drug in clinical use, which is more conducive to clarifying the interaction mechanism between drugs and the mechanism of action of drugs on the body (Tao et al., 2018; Song et al., 2017; Wang B. H. et al., 2021). *Rehmannia glutinosa-Lilium* is a classic antidepressant pair with pharmacological effects such as antidepressant, anxiolytic, anti-inflammatory, etc (Ma et al., 2019; Zhao et al., 2022; Chi et al., 2019). *Rehmannia glutinosa DC.*, a member of the Scrophulariaceae family, is a traditional Chinese medicinal botanical drug that possesses the ability to alleviate heat, promote blood cooling, and enhance yin and fluid nourishment (Geng, 2022). Contemporary pharmacological studies have demonstrated its antioxidant, anti-inflammatory, bacteriostatic, antidepressant, sedative, and hypnotic properties (Li et al., 2022; Yan et al., 2021; Liu C et al., 2017). *Lilium lancifolium Thunb.* is a dry, fleshy scale leaf of the Liliaceae family, which is a Chinese medicinal botanical drug that nourishes the lungs and clears the mind and calms the mind (He D et al., 2022). Modern pharmacology has found that it has antioxidant, anti-inflammatory, bacteriostatic, antidepressant, sedative, and hypnotic effects (Zhou et al., 2021; Pan et al., 2017; Sim et al., 2020). At present, there are many studies on *R. glutinosa* and *Lilium* single medicine, and clinical studies show that Baihe Dihuang Decoction as a Chinese medicine's compound prescriptions have a good effect on depression treatment, but its main active metabolites and pharmacological mechanism have not been described. This article reviews the antidepressant active metabolites and their mechanism of action in the combination of *R. glutinosa-Lilium*, for the purpose of providing references for research on depression's mechanism of action, clinical application, and new drug development.

2 Methods of data acquisition

To ensure a comprehensive and systematic review of the existing literature on Rehmannia glutinosa-Lilium, a meticulous search strategy was implemented. A comprehensive literature search was conducted as of 29 September 2024, utilizing databases such as PubMed, CNKI, Wanfang Data, Baidu Scholar, and Google Scholar. Additionally, classical texts on Chinese herbal medicine, the Chinese Pharmacopoeia, as well as doctoral and master's theses, were included in the collected materials.

The search terms were carefully selected to encompass the broad spectrum of research areas relevant to Rehmannia glutinosa-Lilium. The search employed specific terms including "*R. glutinosa*," "*Lilium*," "Baihe Dihuang decoction," "Traditional uses of *R. glutinosa-Lilium*," "Chemical composition of *R. glutinosa*," "chemical composition of Lilium" "neurotransmitters and depression," "Brain-derived neurotrophic factor and depression," "oxidative stress and depression," "glutamic acid and depression," "the hypothalamus-pituitary-adrenal and depression," "intestinal microorganisms and depression," "application of Baihe Dihuang decoction," "pathogenesis of depression," and "pharmacological action and mechanism of depression.

The inclusion criteria for the studies were as follows: 1) studies that report traditional uses of *R. glutinosa-Lilium*, 2) studies that report active metabolites in antidepressants of Rehmannia glutinosa-Lilium, 3) research the effect of the active metabolites in *R. glutinosa-Lilium* on depression. Studies not directly pertinent to these areas and *in vitro* experimental studies are excluded, and records are subsequently screened based on title and abstract to identify those meeting the inclusion criteria. The full articles are then obtained for further relevance assessment. Data extraction concentrates on the historical application of *R. glutinosa-Lilium*, its chemical composition, and its role in the treatment of depression. Finally, the extracted data are synthesized and prepared for comprehensive analysis in the review.

3 Traditional uses of *Rehmannia glutinosa-Lilium*

The synergistic effects of *R. glutinosa* and *Lilium* have been found to be efficacious in the treatment of a diverse range of ailments

TABLE 1 Traditional uses of *Rehmannia glutinosa-Lilium*.

Dynasty of ancient China	Classic medica books	Traditional application	Dose change	References
The Eastern Han Dynasty	*Jin Kui Yao Lun*	Lily disease	7 lilies, 1 L of raw *Rehmannia glutinosa*	He and He (2005)
The Tang Dynasty	*Bei Ji Qian Jin Yao Fang*	Lily disease, Irregular menstruation	7 lilies, 2 L of raw *Rehmannia glutinosa*	Gao and Shen (2008)
The Song Dynasty	*Shang Han Zong Bing Lun*	Lily disease	10 lilies, half a liter of raw *Rehmannia glutinosa*	Zou and Liu (1989)
The Yuan Dynasty	*Jin Kui Fang Lun Yan Yi*	Lily disease	7 lilies, 1 L of raw *Rehmannia glutinosa*	Zhou and Wang (1993)
The Ming Dynasty	*Ben Cao Hui Yan*	Lily disease	7 lilies, 1 L of raw *Rehmannia glutinosa*	Zheng (2005)
The Ming Dynasty	*Yi Zong Bi Du*	Lily disease	7 lilies, eight taels of raw *Rehmannia glutinosa*	Gu (2005)
The Ming Dynasty	*Zu Ji*	Lily disease	7 lilies, 1 L of raw *Rehmannia glutinosa*	Da (1987)
The Qing Dynasty	*Jin Kui Fang Ge Kuo*	Lily disease	7 lilies, 1 L of raw *Rehmannia glutinosa*	Chen and Chen (1963)
The Qing Dynasty	*Zhang Shi Yi Tong*	Lily disease	7 lilies, 1 L of raw *Rehmannia glutinosa*	Sun and Wang (2005)
The Qing Dynasty	*Wen Re Jing Wei*	Lily disease	7 lilies, 1 L of raw Rehmannia glutinosa	Lu (1997)

such as depression, climacteric syndrome, anxiety, insomnia, cancer, hypertension, and others (Qing et al., 2023; Zhu and Xie, 2022). The earliest recorded herbal literature of *R. glutinosa* and *Lilium* is *Shen Nong Ben Cao Jing* of the Eastern Han Dynasty (Wu, 1963). Nevertheless, the composition of the Baihe Dihuang Decoction, which includes both *R. glutinosa* and *Lilium*, was first recorded in *Jin Kui Yao Lun* authored by Zhang Zhongjing (He and He, 2005). The treatment and preparation methods of Baihe Dihuang Decoction, as documented by physicians in previous dynasties, have remained largely consistent, with the exception of variations in the quantities of *Lilium* and raw *R. glutinosa* juice utilized. During the Tang Dynasty, Sun Simiao, a physician, modified the dosage of *R. glutinosa* juice to 2 L in *Bei Ji Qian Jin Yao Fang*, a treatment for Lily disease and irregular menstruation (Gao and Shen, 2008). Lily disease is clinically manifested as anxiety and depression, and depression is the main clinical manifestation of Lily disease (Junjie et al., 2024). Similarly, in the Song Dynasty, Pang Anshi altered the dosage of *Lilium* to ten and the dosage of *R. glutinosa* juice to half a liter in the treatment of Lily disease, as documented in *Shang Han Zong Bing Lun* (Zou and Liu, 1989). The documentation pertaining to the primary administration, formulation, and application of Baihe Dihuang Decoction in *Jin Kui Fang Lun Yan Yi* during the Yuan Dynasty aligns with the principles outlined in Zhang ZhongJing's theory (Zhou and Wang, 1993). In *Ben Cao Hui Yan*, Ni Zhumu, a physician during the Ming Dynasty, introduced a modification to the administration of *R. glutinosa* by increasing the dosage to eight taels (Zheng, 2005). The effectiveness and preparation techniques documented in other medical texts from the Ming Dynasty, such as *Yi Zong Bi Du* (Gu, 2005) and *Zu Ji* (Da, 1987) as well as those from the Qing Dynasty, including *Jin Kui Fang Ge Kuo* (Chen and Chen, 1963), *Zhang Shi Yi Tong* (Sun and Wang, 2005), *Wen Re Jing Wei* (Lu, 1997) have not changed much compared with the *Jin Kui Yao Lun*. Presently, Baihe Dihuang Decoction has been included in the initial group of ancient traditional formulas and is predominantly employed in the management of depression (Ma et al., 2019) (Table 1).

4 *Rehmannia glutinosa-Lilium* active metabolites in antidepressants

The efficacy of TCM in treating depression has garnered the attention of scholars worldwide, prompting them to explore TCM's compound prescription. Recently, many Chinese proprietary medicines with good antidepressant properties have been discovered, including Chaihu Shugan Powder (Fan et al., 2023), Yueju Pill (Ren and Chen, 2017), Baihe Dihuang Decoction (Xue X. Y. et al., 2022), Kaixin Powder (Xu F. et al., 2023) and Sini Powder (He X et al., 2022), etc. With further in-depth study, it is found that the material basis of antidepressant effect in TCM compound prescription is the active metabolites of TCM (Zhang H. et al., 2021; Deng et al., 2022; Yang et al., 2023). *Rehmannia glutinosa and Lilium* are both medicinal and edible plants, as a commonly used antidepressant pair, has a good improvement effect on depression (Table 2). Baihe Dihuang Decoction was identified by liquid mass spectrometry (LP-MS) with 94 chemical metabolites, including 33 metabolites into blood and 9 metabolites into brain (Wu et al., 2021). The liquid chromatography-mass spectrometry (LC-MS) technique was employed to analyze the decoction of *Lilium*, *Rehmannia* and Baihe Dihuang Decoction, which revealed the presence of 36 novel compounds in the Baihe Dihuang Decoction that were not detected in the individual decoctions of *Lilium* and *Rehmannia*, and the antidepressant active metabolites verbascoside only existed in the co-decoction (Mao et al., 2024). The identified metabolites were correlated with depression, and it was determined that saponins, phenylpropanoids, iridoid terpenoids, flavonoids, alkaloids, and phenylethanol glycosidesmay constitute the

TABLE 2 Ameliorating effect of Baihe Dihuang Decoction on depression.

Extracts/ metabolites	Controls	Model	Animal/ cell	Dose range tested	Duration	Key indicators	References
Baihe Dihuang Decoction	Fluoxetine hydrochloride (18 mg/kg)	Solitary feeding and chronic unpredictable mild stress stimulation (CUMS)	Male SD rat	3.75, 7, 15 g/kg	28 days	Firmicutes↑,Bacteroidota↓, V (Zhao et al., 2021a) oteobacteria↓, Cyanobacteria↓	Feng et al. (2024)
Baihe Dihuang Decoction	Rolipram (0.1 mg/mL)	Chronic unpredictable stress (CUS)	Male ICR mice	0.3, 0.6, 1.2 g/mL	35 days	ACTH↓CORT↓, cAMP↑	Zhou et al. (2023)
Baihe Dihuang Decoction	venlafaxine (13.5 mg/kg)	Chronic restraint stress combined with subcutaneous injection of corticosterone	SD rats	4, 16 g/kg	21 days	IL-1β↓, IL-6↓, IL-18↓, NLRP3↑, ASC↑, Caspase-1↑	Zhao et al. (2021b)
Baihe Dihuang Decoction	Fluoxetine hydrochloride (20 mg/kg)	CUMS	Male SD rat	90 g/kg	28 days	IL-1β↓, IL-6↓, TNF-α↓, Glu↓, IL-10↑, 5-HT↑, DA↑, NE↑, GABA↑	Pan et al. (2023)

primary active metabolites in the therapeutic management of depression (Table 3).

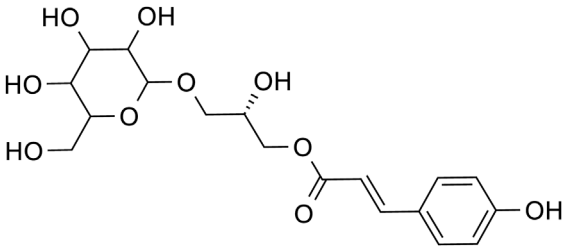
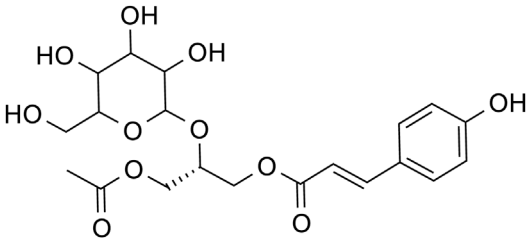
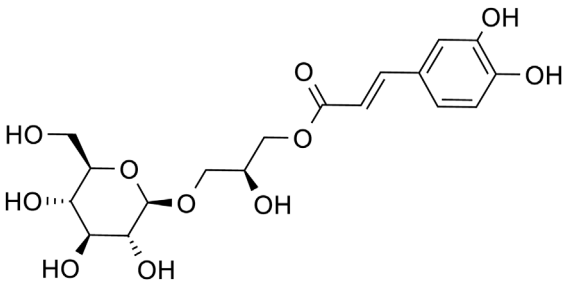
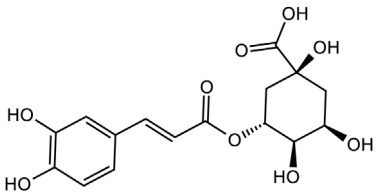
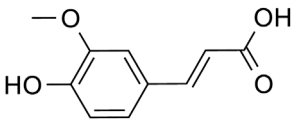
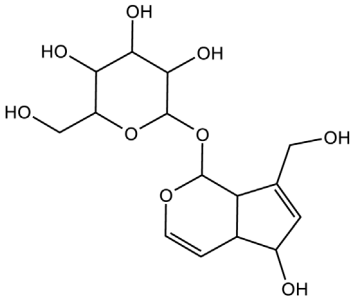
5 Antidepressant mechanism of *Rehmannia glutinosa-Lilium*

5.1 Effects of active metabolites of *Rehmannia glutinosa-Lilium* on the function of monoamine neurotransmitters

Neurotransmitters play a crucial role in facilitating signal transmission between neurons and effectors within the body (Hu and Wu, 2023). Monoamine neurotransmitters are central neurotransmitters, including catecholamine and indoleamine, the former is a neurotransmitter based on catecholamine, including norepinephrine (NE), epinephrine and dopamine (DA), while the latter is composed of indole and ethylamine, which mainly refers to 5-hydroxytryptamine (5-HT) (Wu, 2023). The occurrence of depression is associated with the modulation of monoamine neurotransmitter levels, and when a decrease in the levels of 5-HT, NE, and DA may have an impact on the emotional state of individuals (El et al., 2010; Perez-Caballero et al., 2019).

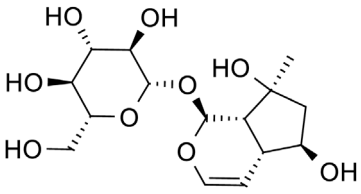
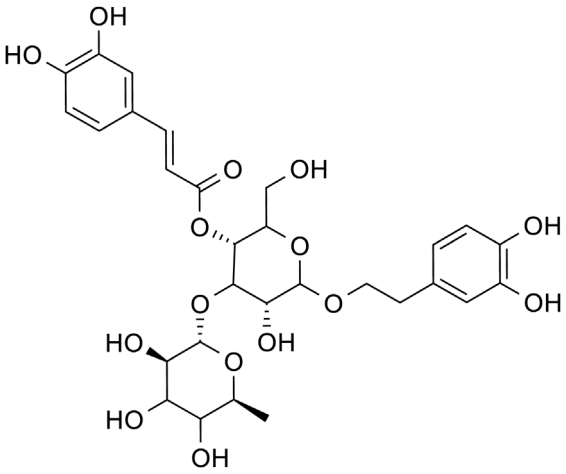
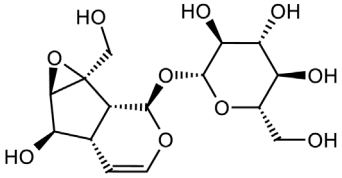
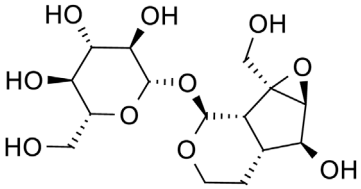
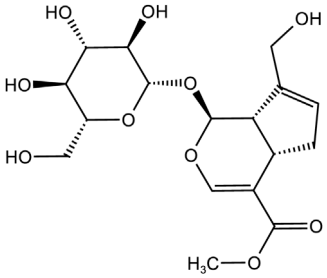
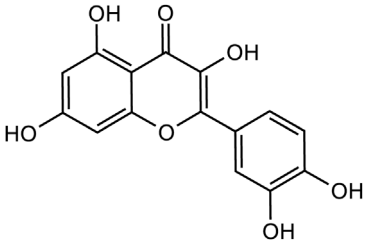
Catalpol, the chemical metabolites, found in *R. glutinosa*, possesses a significant concentration of iridoids and displays a multitude of pharmacological properties, including but not limited to antidepressant, cognitive-enhancing, and neuroprotective effects (Zhang and Liu, 2019). Treatment with catalpol (5, 10, or 20 mg/kg) for 14 days reduced mice's depressive-like behavior in a depression model, and it was found that catalpol increased the content of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) in mice's brains, while exhibiting minimal influence on the levels of NE and DA. This study to indicate that catalpol has an antidepressant-like effect and that its action may be mediated by the central serotonergic system (Wang et al., 2014). The phenylethanol glycoside acteoside, extracted from *Radix Rehmanniae*, exhibits many pharmacological properties, such as antidepressant properties, antitumor properties, anti-inflammatory properties, neuroprotective properties, etc (Ge et al., 2023). Current studies have have demonstrated that acteoside substantially elevates the serum concentrations of 5-HT, GABA, and DA in depressed mice, and the mechanism underlying the antidepressant effects of acteoside is believed to involve the augmentation of monoamine neurotransmitters, the attenuation of pro-inflammatory cytokines, and the restoration of neurotransmitter levels (Xue X. et al., 2022). Recent studies have revealed that the primary metabolites of *Lilium* are saponins, which exhibit antidepressant, antioxidant, anti-inflammatory, antibacterial, and regulatory properties on the cerebral and gut axis (Sun et al., 2022). Administration of intragastric *Lilium* saponins to mice with depression resulted in a reduction of depressive-like behavior and a decrease in body temperature (Wang, 2014). Moreover, the administration of *Lilium* extracts resulted in elevated levels of DA and 5-HT, thereby restoring the function of monoamine neurotransmitters in rats with depression (Guo et al., 2009). Gallic acid, a ubiquitous phenolic acid in nature, is a crucial bioactive metabolites of *Lilium*, exhibiting anti-aging, antioxidant, anti-inflammatory, and other therapeutic properties (Zhang and Ma, 2020; Wang P. et al., 2023). Administration of 60 mg/kg gallic acid reduced depressive behavior in depressive model mice, and its antidepressant action is mediated by increasing not only 5-HT levels in the synaptic space, but also catecholamine levels in the brain (Can et al., 2017). Berberine, the principal bioactive metabolites of *Lilium* alkaloids, exhibits a range of pharmacological effects including antibacterial, anti-inflammatory, antiviral, lipid-modulating, hypoglycemic, antiarrhythmic, antihypertensive, immunomodulatory, and antitumor properties (Chen et al., 2023; Zhao L. et al., 2023; Tan et al., 2023). Berberine has been observed to significantly decrease the resting time of TST and FST in mice with depression, while concurrently elevating the levels of NE and 5-HT in the hippocampus and prefrontal cortex, and the mechanism of action of berberine is believed to be linked to the regulation of monoamine neurotransmitters in the brain (Peng et al., 2007) (Figure 1; Table 4).5-HT may contribute to the pathophysiology of depression via the cAMP/PKA/CREB signaling pathway, which is mediated by the 5-HT1A receptor (Brites and Fernandes, 2015). The elevated concentration of the psamine transporter (DAT) enhances

TABLE 3 The main antidepressant metabolites of *Rehmannia glutinosa-Lilium*.

Classify	Metabolites	Structural formula	Chemical formula	CAS	Source
Saponins	Regaloside A		$C_{18}H_{24}O_{10}$	114,420-66-5	*Lilium*
	Regaloside B		$C_{18}H_{24}O_{11}$	114,420-67-6	*Lilium*
	Regaloside C		$C_{18}H_{24}O_{11}$	117,591-85-2	*Lilium*
Phenylpropanoids	Chlorogenic acid		$C_{16}H_{28}O_9$	1,049,703-62-9	*Lilium*
	Ferulic acid		$C_{10}H_{10}O_4$	1,135-24-6	*Lilium*
Iridoid terpenoids	Aucubin		$C_{15}H_{22}O_9$	479-98-1	*Rehmannia glutinosa*

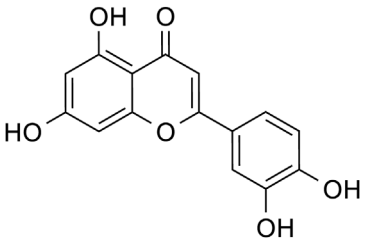
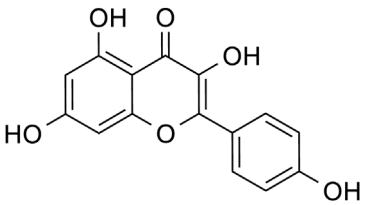
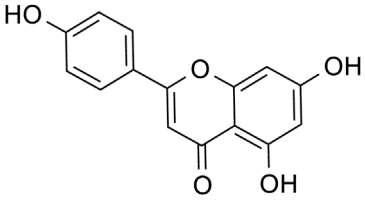
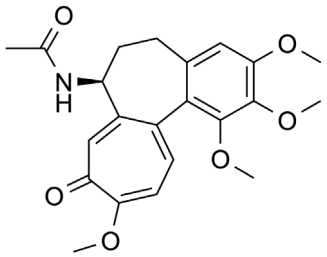
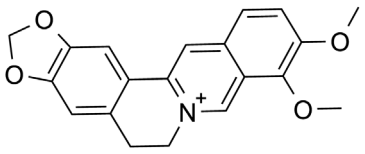
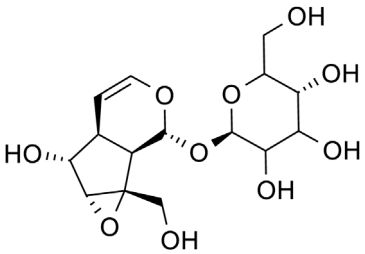
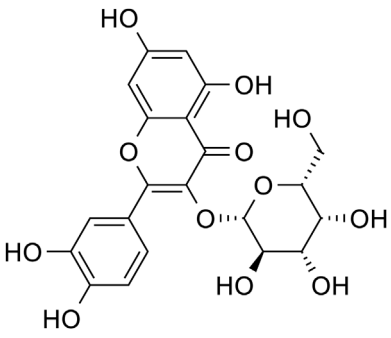
(Continued on following page)

TABLE 3 (*Continued*) The main antidepressant metabolites of *Rehmannia glutinosa-Lilium*.

Classify	Metabolites	Structural formula	Chemical formula	CAS	Source
	Ajugol		$C_{15}H_{24}O_9$	52,949-83-4	*Rehmannia glutinosa*
	Acteoside		$C_{29}H_{36}O_{15}$	61,276-17-3	*Rehmannia glutinosa*
	Catalpol		$C_{15}H_{22}O_{10}$	2,415-24-9	*Rehmannia glutinosa*
	Dihydrocatalpol		$C_{15}H_{24}O_{10}$	6,736-86-3	*Rehmannia glutinosa*
	Geniposide		$C_{17}H_{24}O_{10}$	24,512-63-8	*Rehmannia glutinosa*
Flavonoids	Quercetin		$C_{15}H_{10}O_7$	117-39-5	*Lilium*

(Continued on following page)

TABLE 3 (*Continued*) The main antidepressant metabolites of *Rehmannia glutinosa-Lilium.*

Classify	Metabolites	Structural formula	Chemical formula	CAS	Source
	Luteolin		$C_{15}H_{10}O_6$	491-70-3	*Lilium*
	Kaempferol		$C_{15}H_{10}O_6$	520-18-3	*Lilium*
	Apigenin		$C_{15}H_{10}O_5$	520-36-5	*Lilium*
Alkaloid	Colchicine		$C_{22}H_{25}NO_6$	64-86-8	*Lilium*
	Berberine		$C_{17}H_{17}N$	2086-83-1	*Lilium*
Phenylethanol Glycosides	Rhmannioside D		$C_{27}H_{42}O_{20}$	81,720-08-3	*Rehmannia glutinosa*
	Hyperoside		$C_{21}H_{20}O_{12}$	482-36-0	*Rehmannia glutinosa*

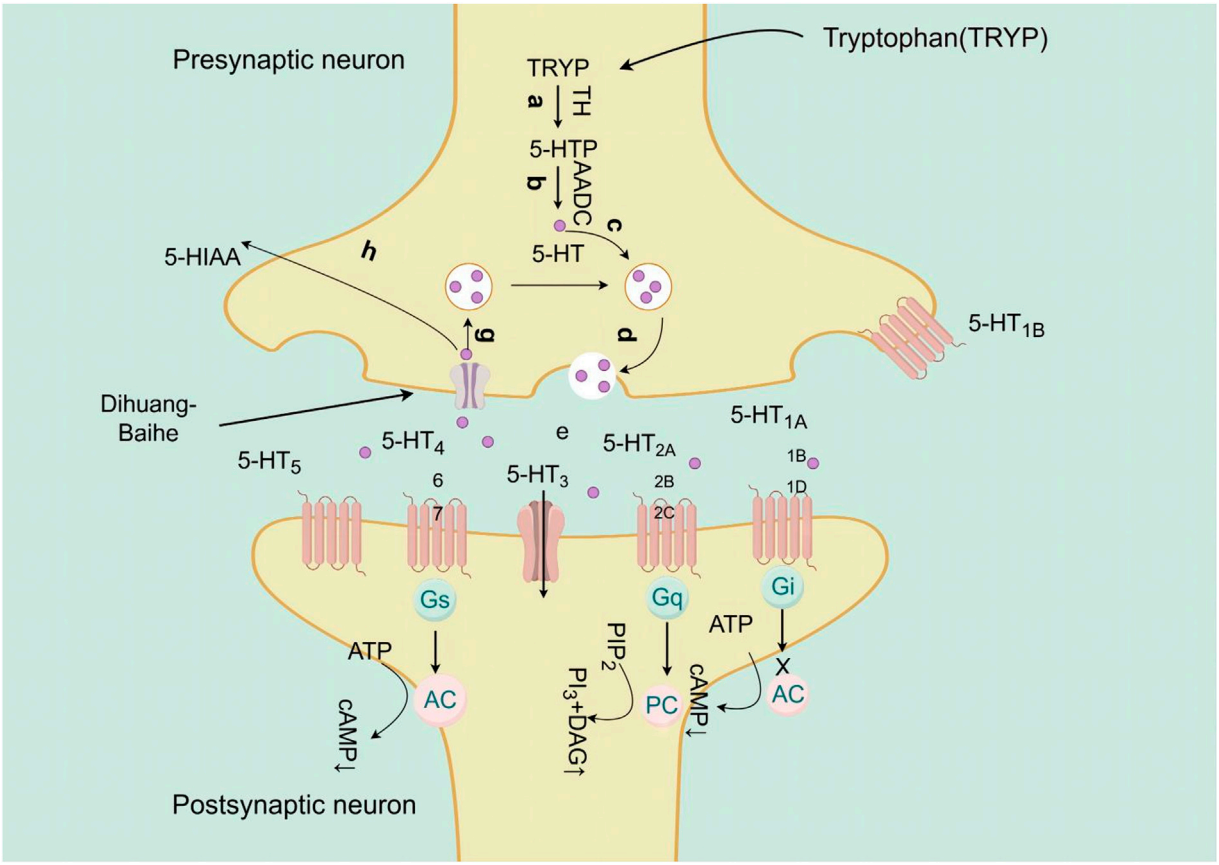


FIGURE 1
The active substances in *Rehmannia glutinosa-Lilium* inhibits 5-HT reuptake in nerve endings and cell bodies.

TABLE 4 Active substances regulating monoamine neurotransmitters in *Rehmannia glutinosa-Lilium*.

Extracts/ metabolites	Controls	Model	Animal/cell	Dose range tested	Duration	Key indicators	References
Catalpol	Fluoxetine hydrochloride (10 mg/mL)	Reserpine	Male Kunming mice	5, 10, 20 mg/kg	14 days	5-HT↑, 5-HIAA↑	Wang et al. (2014)
Acteoside	Fluoxetine (20 mg/mL)	CUMS	Male C57BL/ 6 mice	60 mg/kg	21 days	5-HT↑, GABA↑, DA↑	Xue X. Y. et al. (2022)
Lilium saponins	Fluoxetine (0.04 g/kg)	Reserpine	Female, male Kunming mice	25, 50, 100 mg/kg	7 days	Reduce the change of body temperature in mice	Wang (2014)
	Fluoxetine (2 mg/mL)	CUMS	Male SD rat	12, 24, 48 mg/kg	21 days	DA↑, 5-HT↑	Guo et al. (2009)
Gallic acid	Fluoxetine (30 mg/mL)	Depression	Male BALB/c mice	30, 60 mg/kg	4 days	5-HT↑, DA↑	Can et al. (2017)
Berberine	Fluoxetine (20 mg/mL)	Depression	Male ICR albino mice	10, 20 mg/kg	30 min	NE↑, 5-HT↑	Peng et al. (2007)

Note: 5-HT, 5-hydroxytryptamine; CUMS, chronic unpredictable mild stimulation; 5-HIAA, 5-hydroxyindole acetic acid; GABA, γ-aminobutyric acid; DA, Dopamine.

the reuptake rate of DA at synaptic terminals, resulting in a reduction of DA levels in the synaptic cleft and subsequently contributing to depressive symptoms (Zaaijer et al., 2015). 5-HT and NE interact with G protein-coupled receptors (GPCRs) to facilitate neural transmission and generate electrical signals that modulate emotional responses (Yang et al., 2018). Consequently, *R. glutinosa-Lilium* may modulate depressive symptoms by enhancing the secretion and synthesis of neurotransmitters such as 5-HT, DA, and NE.

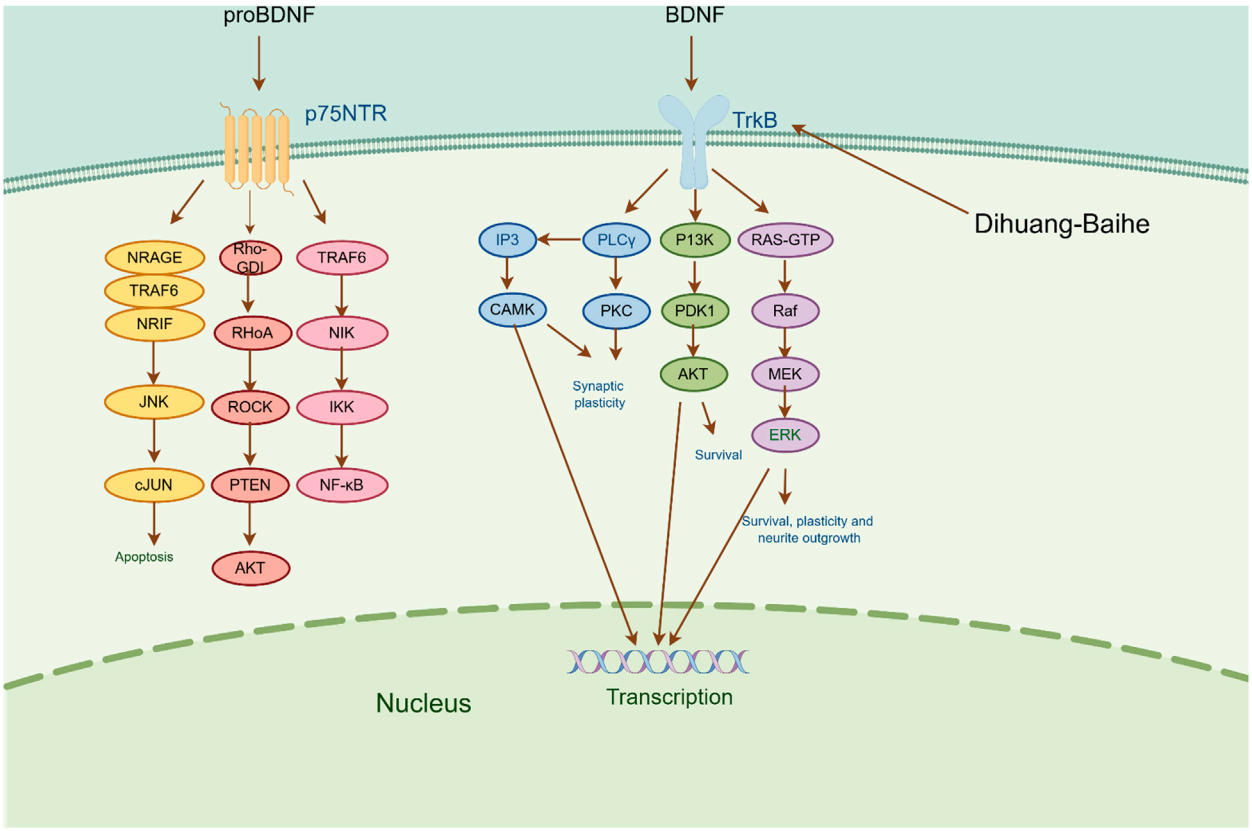


FIGURE 2
The active metabolites of *Rehmannia glutinosa-Lilium* can regulate brain-derived neurotrophic factor. (BDNF, Brain-derived neurotrophic factor; TrkB, Tyrosine kinase receptor B; P75NRT, Neurotrophin P75 receptor; PLCγ, Phospholipase Cγ; PI3K, Phosphatidylinositol 3 kinase; Raf, Raf kinas).

TABLE 5 An active metabolites in *Rehmannia glutinosa-Lilium* that regulates the level of a brain-derived neurotrophic factor.

Extracts/ metabolites	Controls	Model	Animal/ cell	Dose range tested	Duration	Key indicators	References
Catalpol	Fluoxetine hydrochloride (10 mg/mL)	CUMS	Male SD rats	10 mg/kg	35 days	PI3K↑, Akt↑, Nrf2↑, HO-1↑, TrkB↑, BDNF↑	Wang H. H. et al. (2021)
Regaloside A	Mock	CORT	SH-SY5Y cells	5, 15, 25, 50, 75, 100 μmol/L	24 h	Akt↑, BDNF↑, TrkB↑, PI3K↑	Yuan et al. (2021)
Rehmannia Glycoside D	Fluoxetine (0.3 μmol/L)	CORT	PC-12 cells	5, 10, 20 μmol/L	24 h	BDNF↑, TrkB↑	Zhang et al. (2022)
Chlorogenic acid	0.5% sodium carboxymethyl cellulose (20, 50, 100 mg/kg)	Aβ	Male Kunming mice	20, 50, 100 mg/kg	21 days	BDNF↑, NGF↑, 5-HT↑, 5-HIAA↑, iNOS↓, IL-6↓, TNF-α↓, NLRP3↓, IL-1β↓	Liu et al. (2021)

Note: PI3K, Phosphatidylinositol-3-kinase; CUMS, chronic unpredictable mild stimulation; Nrf2, Nuclear factor E2-related factor 2; HO-1, Heme oxygenase 1; CORT, cortisol; TrkB, Tyrosine kinase receptor B; BDNF, Brain-derived neurotrophic factor; Akt, Protein kinase B.

5.2 Brain-derived neurotrophic factor levels in response to *Rehmannia glutinosa-Lilium* active metabolites

Brain-derived neurotrophic factor (BDNF) is a crucial neurotrophic factor in the brain that plays a pivotal role in the growth, survival, and synapse formation of neurons that are linked to emotional and cognitive functions (Lima et al., 2019). According to a study of depression patients, the level of BDNF decreased with increasing severity of the disease, and the more serious the condition, the lower the level of BDNF (Du et al., 2023; Lee et al., 2007). There are two receptors for BDNF, one high-affinity

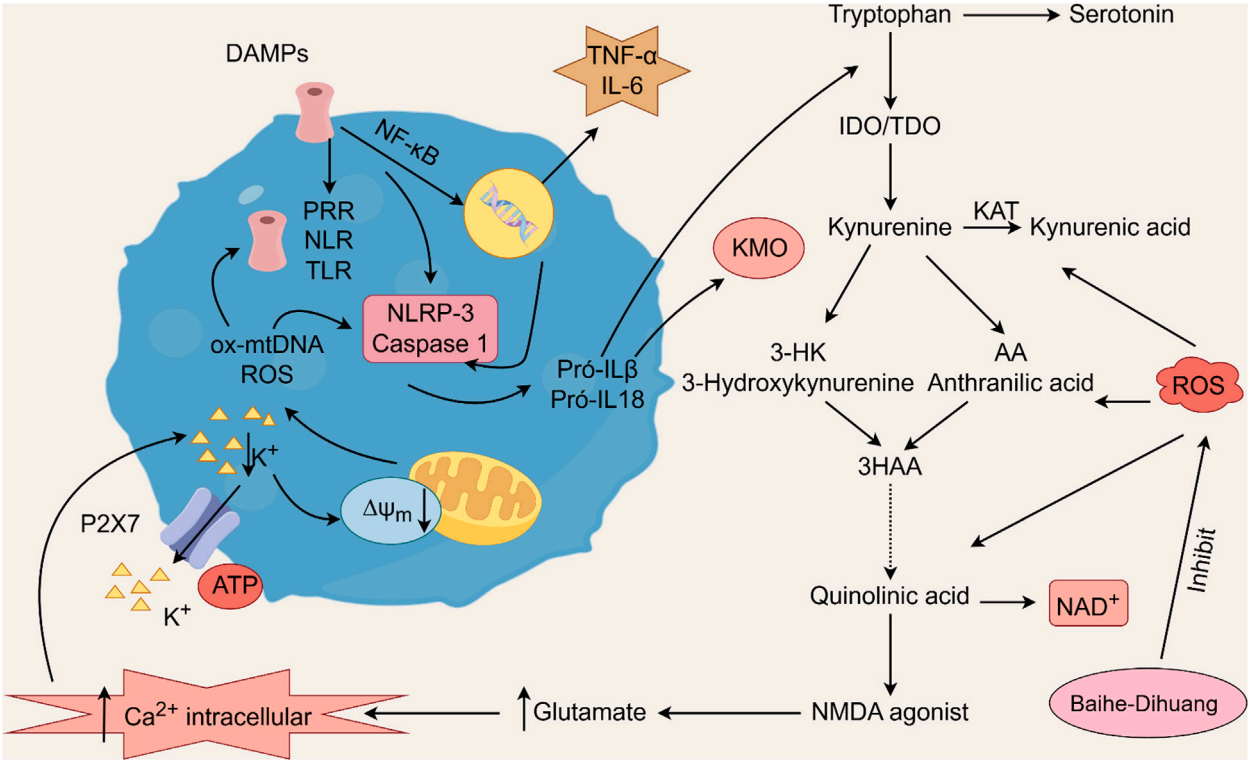


FIGURE 3
The activation of pattern recognition receptors (PRRs) triggers the NLRP3 inflammasome and caspase-1, leading to interleukin-1 beta (IL-1β) and interleukin-18 (IL-18) activation. Oxidized mitochondrial DNA (ox-mtDNA) and mitochondrial reactive oxygen species (ROS) also activate the inflammasome. Additionally, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) promotes the production of tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6). The proinflammatory cytokines IL-1β and IL-18 further activate the enzymes indoleamine 2,3-dioxygenase (IDO) and tryptophan 2,3-dioxygenase (TDO) within the kynurenine pathway, facilitating the degradation of tryptophan into kynurenine. These two cytokines further activate kynurenine 3-monooxygenase (KMO), the enzyme responsible for directing the degradation of kynurenine into 3-hydroxykynurenine (3HK) and quinolinic acid, both of which are neurotoxic agents, rather than into kynurenic acid, a neuroprotective agent. Kynurenic acid functions as an NMDA receptor agonist and enhances glutamate levels, subsequently increasing intracellular calcium concentrations. This process results in the excessive production of reactive oxygen species (ROS) via the kynurenine pathway.

receptor that binds tyrosine kinase receptor B and another low-affinity receptor that binds neurotrophic factor (p75 NTR). BDNF participates in the pathophysiological process of depression mainly through the induction of intracellular tyrosine residue autophosphorylation and receptor dimerization by binding to TrkB (Liu and Wang, 2015). The primary routes through which phosphorylated TrkB initiates downstream signaling cascades predominantly encompass the PI3K/AKT pathway, MAPK pathway, and PLCγ/PKC pathway. These pathways facilitate enhanced synaptic plasticity, improved neuronal growth and survival, and ultimately provide neuroprotection and nutritional support to the nerves (Wang et al., 2020; Mosiolek et al., 2021).

According to the experiment, catalpol significantly increased PI3K, Akt, Nrf2, HO-1, TrkB, BNDF, and other gene and protein expression in rats modeled by CUMS, and confirmed that PI3K/Akt/Nrf2/HO-1 signaling pathways were upregulated by catalpol's antidepressant mechanism on depression, improving hippocampal neuroprotection and antioxidant levels (Wang J et al., 2021). Phenolic acids are one of the main active metabolites of lilies, in which Regaloside A in *Lilium* saponins plays a role in various antidepressant compound prescriptions (Luo et al., 2017). Following treatment with Regaloside A, there was an increase in the cell survival rate and phosphorylation levels of BDNF, TrkB, PI3K, and Akt, which is postulated that Regaloside A exerts antidepressant effects via the BDNF-TrkB pathway (Yuan et al., 2021). Contemporary pharmacological research has determined that Rehmanin D possesses the capability to mitigate PC-12 cell impairment caused by elevated levels of cortisol, and the effect is attributed to its potential to augment BDNF expression and elicit anti-apoptotic responses via the BDNF-TrkB pathway, ultimately safeguarding nerve cells and manifesting antidepressant properties (Zhang et al., 2022). After chlorogenic acid treatment, the nerve damage score and brain water content of mice decreased, BDNF, NGF, 5-HT, and 5-HIAA proteins were upregulated, while pro-inflammatory cytokines iNOS, IL-6, TNF-α, NLRP3, and IL-1β were significantly downregulated (Liu et al.,

TABLE 6 The active substances in *Rehmannia glutinosa-Lilium* that regulate inflammation and oxidative stress.

Extracts/ metabolites	Controls	Model	Animal/cell	Dose range tested	Duration	Key indicators	References
Geniposide	Fluoxetine hydrochloride (20 mg/mL)	RRS	Male ICR mice	50, 100 mg/kg	15 days	TNF-α↓, IL-1β↓	Zhao et al. (2018)
Ethanol extract of *Lilium*	Mock	LPS	RAW264.7 cells	0–300 μg/mL	24 h	COX-2↓, TNF-α↓, iNOS↓, NF-κB↓	Han et al. (2018)
Quercetin	0.3% carboxymethyl cellulose	CUS	Swiss albino mice	30 mg/kg	26 days	IL-6↓, TNF-α↓, IL-1β↓, COX-2↓	Mehta et al. (2017)
Luteolin	Mock	CUMS	Male Kunming mice	20, 40, 60 mg/kg	21 days	SOD↑, GSH-Px↑, MDA↓	Liu et al. (2013)
Kaempferol	Fluoxetine hydrochloride (10 mg/mL)	CSDS	Male CD1 and C57 mice	10, 20 mg/kg	35 days	IL-6↓, iNOS↓, IL-1β↓, COX-2↓, SOD↑, CAT↑, GSH-Px↑, GST↑, MDA↓	Gao et al. (2019)
Rehmannia glycoside D	Mino (0.1 μmol/L)	LPS	N9 cells	5, 10, 20 μmol/L	24 h	iNOS↓, IL-6↓, IL-1β↓	Wang J et al. (2021)
Catalpol	Fluoxetine hydrochloride (10 mg/mL)	CUMS	Male SD rat	10 mg/kg	35 days	SOD↑, CAT↑, GSH-Px↑, GST↑, GSH↑, MDA↓	Wang Y. L et al. (2021)
	Mitochondrion-targeted antioxidant peptide SS31 (5 mg/kg)	CUMS	Male C57BL/6 mice	20 mg/kg	35 days	IL-1β↓, TNF-α↓, iNOS↓	Wang Y. T. et al. (2021)

Note: SOD, superoxide dismutase; MDA, malondialdehyde; GSH-Px, Glutathione peroxidase; CAT, catalase; GST, Glutathione S-transferase; COX-2, Cyclooxygenase-2; TNF-α, Tumor necrosis factor-α; RRS, repeated restraint stress; INOS, inducible nitric oxide synthase; IL-6, Interleukin-6; CSDS, chronic social defeat stress; IL-1β, Interleukin-1β; CUMS, chronic unpredictable mild stimulation; LPS, lipopolysaccharides; CUS, Chronic unpredicate stress.

2021) (Figure 2; Table 5). BDNF specifically binds to the TrkB receptor, thereby activating downstream signaling pathways such as PI3K/Akt, MAPK, and cAMP, among others (Fries et al., 2023). This interaction enhances the release of presynaptic neurotransmitters, facilitating nerve signal transmission (Li et al., 2024). Consequently, *R. glutinosa-Lilium* has been shown to elevate BDNF levels, thereby supporting normal neuronal function and promoting emotional recovery as well as the improvement of cognitive function.

5.3 *Rehmannia glutinosa-Lilium* metabolites' effects on inflammation and oxidative stress

Oxidative stress refers to the state of cellular imbalance resulting from the overproduction of reactive oxygen species (ROS) and the insufficient antioxidant capacity of cells (Sies, 2015). This condition can lead to various pathological processes, including but not limited to inflammation, neurodegeneration, tissue damage, and cell death, when ROS production exceeds the antioxidant response (Bhatt et al., 2020; Redza-Dutordoir and Averill-Bates, 2016; Xing et al., 2024a). The pathophysiology of depression is strongly influenced by oxidative stress and inflammation, the body is stimulated by stress, the redox balance is broken, and the body's antioxidant enzyme function changes abnormally, producing excess ROS, pro-inflammatory factors are released, and the inflammatory response is activated, ultimately leading to disturbances in the structure and function of biological macromolecules and proteins in nerve cells, culminating in the manifestation of depression (Kohler et al., 2016; Pandey et al., 2018; Vavakova et al., 2015; Zhang, 2018). Clinical studies have provided empirical evidence indicating that individuals suffering from inflammatory diseases are more likely to experience depression (Beurel et al., 2020). Furthermore, an elevation in the levels of pro-inflammatory cytokines, specifically IL-1β, IL-6, and TNF-α, is positively correlated with the severity of depressive symptoms (Neupane et al., 2022; Boucas et al., 2022). Moreover, inhibiting ROS and malondialdehyde (MDA) and increasing antioxidant enzymes like superoxide dismutase and catalase (CAT) can alleviate depression symptoms (Lindqvist et al., 2017).

Geniposide, an iridoid discovered in *R. glutinosa*, also has demonstrated antidiabetic, antioxidant, antidepressant, and neuroprotective properties (He et al., 2023; Kimura et al., 2023; Li et al., 2020). The current investigation provides evidence that the regulation of GLP-1R/AKT by geniposide effectively mitigates depressive behavior induced by repeated inhibitory stress (RRS) and hippocampal neuronal apoptosis in mice, concomitantly decreasing the content of pro-inflammatory cytokines IL-1β and TNF-α (Zhao et al., 2018). The ethanol extract derived from *Lilium* exhibits a specific inhibitory effect on the nuclear factor κ B (NF- κ B) signal pathway, which is induced by inhibitor kappa B kinase β (IKK β), thereby exerting an anti-inflammatory effect and the main bioactive metabolites in the *Lilium* alcohol extract were identified as quercetin, luteolin, and kaempferol through high performance liquid chromatography (HPLC) (Han et al., 2018). This observation indicates that lilies may mitigate oxidative stress by modulating glutamate metabolism, which subsequently activates the Nrf-2 signaling pathway (Xing et al., 2024b). Quercetin, luteolin, and kaempferol are flavonoids in Lilium, which have potent antioxidant activity (Xu et al., 2022). The administration of Quercetin has been observed to yield a significant reduction in anxiety and depression in mice that have been subjected to chronic unpredicted stress (CUS)-induced depression and quercetin has been observed to decrease the

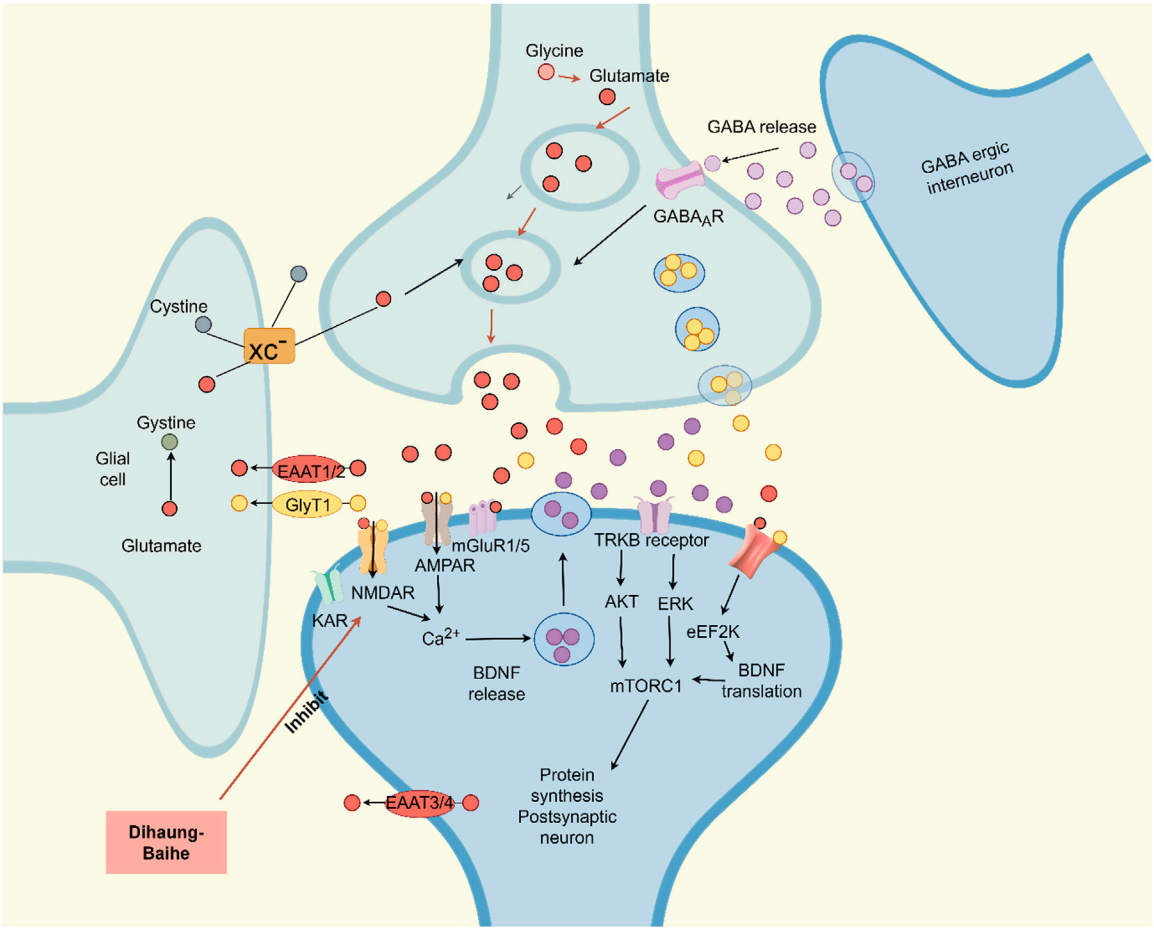


FIGURE 4
Upon depolarization of presynaptic glutamatergic neurons, gamma-aminobutyric acid (GABA) receptors inhibit the fusion of glutamate-containing vesicles with the presynaptic membrane. Additionally, Group II metabotropic glutamate receptors (mGluRs) modulate glutamate release by inhibiting adenylyl cyclase activity, thereby indirectly influencing synaptic plasticity and long-term potentiation (LTP). In a postsynaptic glutamatergic neuron, the activation of N-methyl-D-aspartate receptors (NMDARs) via brain-derived neurotrophic factor (BDNF) can be associated with the initiation of neurotrophic or apoptotic pathways. Subsequently, BDNF-TrkB signaling enhances the activation of extracellular signal-regulated kinase (ERK),serine/threonine-specific protein kinase (Akt), and the mechanistic target of rapamycin complex 1 (mTORC1) signaling pathways.

TABLE 7 Active substances regulating glutamate in *Rehmannia glutinosa-Lilium.*

Extracts/ metabolites	Controls	Model	Animal/ cell	Dose range tested	Duration	Key indicators	References
Catalpol	Mock	L-Glu	PC-12	1, 10, 100 μmol/L	23 h	M receptor↑	Wang et al. (2008)
Aucubin	Fluoxetine (0.3 μmol/L)	GLU	PC-12 cells	1, 5, 10, 20, 40 μmol/L	24 h	ROS↓, SOD↑, NMDAR1↓, LDH↓	Lu et al. (2022)
Echinacoside glycoside	Fluoxetine (0.3 μmol/L)	GLU	PC-12 cells	2, 5, 10 μmol/L	24 h	ROS↓, SOD↑, NMDAR1↓, LDH	Lu et al. (2021)
Berberine	0.5% carboxymethyl cellulose	STZ	Male ICR mice	50 mg/kg	42 days	ROS↓, SOD↑, Caspase-3↓, Bax/Bcl-2↓	Xue (2021)

Note: SOD, superoxide dismutase; ROS, reactive oxygen species; NMDAR, N-methyl-D-aspartate receptor; LDH, lactate dehydrogenase; Glu, Glutamate.

expression of oxidative stress markers and pro-inflammatory cytokines in hippocampal neurons, thereby conferring protection to the mouse brain against oxidative and inflammatory stress (Mehta et al., 2017). The administration of luteolin to CUMS mice leads to a significant increase in the activation of SOD and GSH-Px in brain tissue, a reduction in MDA levels, and inhibition of

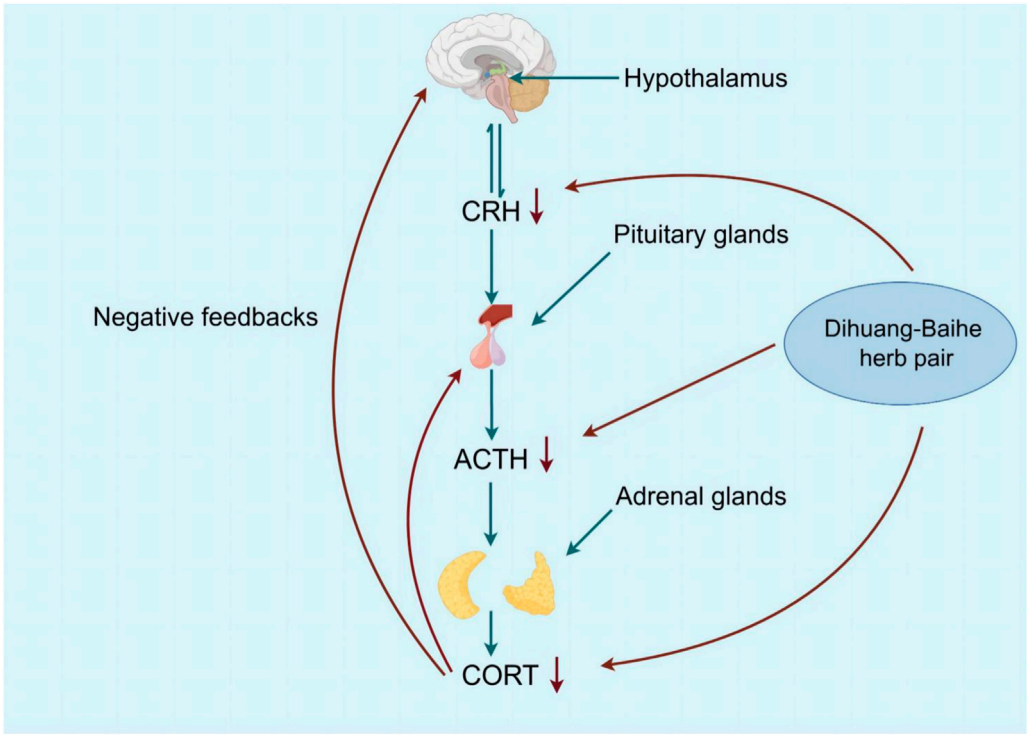


FIGURE 5
The active metabolites of *Rehmannia glutinosa-Lilium* can reduces COR levels and inhibits further release of ACTH and CRH, and depressive symptoms disappear.

TABLE 8 Active substances regulating hypothalamus-pituitary-adrenal axis in *Rehmannia glutinosa-Lilium*.

Extracts/ metabolites	Controls	Model	Animal/ cell	Dose range tested	Duration	Key indicators	References
Geniposide	Fluoxetine hydrochloride (10 mg/mL)	CUMS	Male SD rats	20, 50, 100 mg/kg	21 days	CORT↓, ACTH↓, CRH mRNA↓	Cai et al. (2015)
Lilium saponins	Fluoxetine hydrochloride (2 mg/mL)	CUMS	Male SD rats	12, 24, 48 mg/kg	21 days	CORT↓, ACTH↓, DA↑, 5-HT↑	Guo et al. (2010)
Catalpol	Fluoxetine hydrochloride (20 mg/mL	CORT	Male Kunming mice	20 mg/kg	35 days	Nrf2↑, NF-κB↓, IL-1β↓, TNF-α↓, iNOS↓, NO↓, GSH-Px↑, GST↑, SOD↑, MDA↓	Song et al. (2021)
Berberine	Diazepam (2.25 mg/kg)	PCPA	Female Kunming mice	75 mg/kg	5 days	NE↑, 5-HT↑, HPA↓	Zhou et al. (2014)

Note: CORT, cortisol; ATCH, adrenocorticotropin; DA, dopamine; 5-HT, 5-hydroxytryptamine; NF-κB, Nuclear factor kappa-B; iNOS, inducible nitric oxide synthase; Nrf2, Nuclear factor E2-related factor 2; IL-1β, Interleukin-1β; NO, nitric oxide; TNF- α, Tumor necrosis factor-α; GSH-Px, Glutathione peroxidase; SOD, superoxide dismutase; GST, Glutathione S-transferase; CUMS, chronic unpredictable mild stimulation; MDA, Malondialdehyde.

neuronal oxidative stress (Liu et al., 2013). Kaempferol activates the AKT/catenin cascade in the prefrontal cortex of CSDS mice, thereby augmenting its antioxidant and anti-inflammatory properties (Gao et al., 2019). Notably, a neuroinflammatory response is triggered by the activation of microglia and subsequent release of pro-inflammatory cytokines (Zhang et al., 2016). Under normal circumstances, microglia (M0) in the central nervous system are in a quiescent state and play the role of "immune surveillance," while microglia in the pathological state come to life and release a series of cytokines, which participate in the occurrence and development of neuroinflammation (Xu et al., 2020). The continuous activation of classical activated microglia (M1) will produce excessive inflammatory factors and oxidative stress, causing damage to nerve cells and leading to aggravation of the disease, while alternative activated microglia (M2) can promote tissue repair and regeneration and play a neuroprotective role (Kwon and Koh, 2020; Wolf et al., 2017; Chen et al., 2024). Thus, the inhibition of M1 microglia proliferation can lead to an

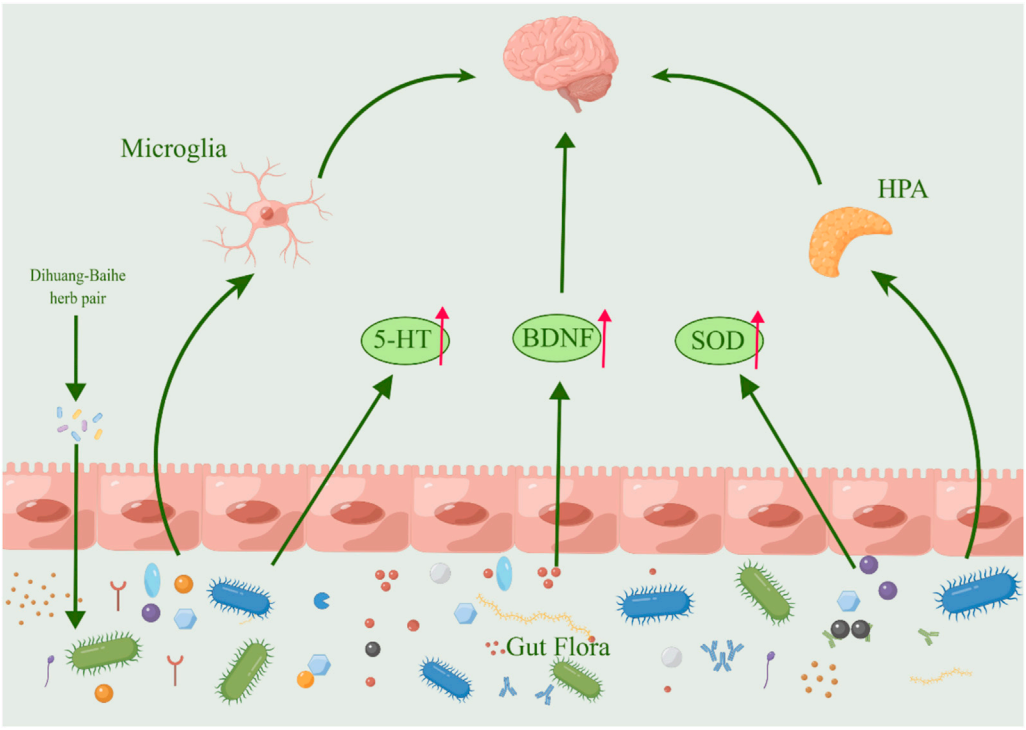


FIGURE 6
The active metabolites in *Rehmannia glutinosa-Lilium* can regulate intestinal microorganisms. (HPA, Hypothalamus-pituitary-adrenal; 5-HT, 5-hydroxytryptamine; BDNF, Brain-derived neurotrophic factor).

TABLE 9 Active substances regulating intestinal microorganisms in *Rehmannia glutinosa-Lilium*.

Extracts/ metabolites	Controls	Model	Animal/ cell	Dose range tested	Duration	Key indicators	References
Lilium polysaccharides	lizhu intestine (10 mg/kg)	Lincomycin hydrochloride	Male Kunming mice	50, 100, 200 mg/kg	21 days	LPS↓, IL-6↓, TNF-α↓	Zhao et al. (2020)
Rehmannia glutinosa stachyose	Metformin (400 mg/kg)	STZ	Male Kunming mice	300 mg/kg	49 days	*Lactobacillus*↓, *Bacteroides*↓	Wang (2013)
Kaempferol	Mock	UC	Female C57BL/6J mice	50 mg/kg	14 days	Firmicutes/*Bacteroides*↑, Proteobacteria↓	Qu (2021)
Berberine	Mock	NAFLD	SD rat	150 mg/kg	112 days	*Clostridium* ↓, Lactic acid bacteria↑, IL-6↓, TNF-α↓	Huang D. X. et al. (2023)
Chlorogenic acid	Mock	NAFLD	Male C57BL/6 mice	60 mg/kg	84 days	GLP-1↑, *Escherichia coli*↓, IL-6↓, TNF-α↓	Shi et al. (2021)

Note: LPS, lipopolysaccharide; IL-6, Interleukin-6; TNF-α, Tumor necrosis factor-α; STZ, streptozotocin; UC, Ulcerative colitis.

improvement in depressive symptoms and a prevention of neuroinflammation. Compared with the model group, the Rehmannia glycoside D-group exhibited a reduction in the levels of pro-inflammatory cytokines IL-6 and IL-1β released by M1 microglia, and an increase in the levels of anti-inflammatory cytokines IL-4 and IL-10 released by M2 microglia, which may be attributed to the inhibition of microglial transformation from M2 to M1 (Wang H. H. et al., 2021). As measured by CUMS, Catalpol not only increased hippocampal SOD, CAT, GSH-Px, GST, GST and GSH levels in rats, but also inhibit microglial polarization of the M1 phenotype and reduce the expression of IL-1β, TNF-α and iNOS (Wang Y. T. et al., 2021) (Figure 3; Table 6). Oxidative stress can enhance the activity of the rate-limiting enzyme in tryptophan metabolism by promoting neuroinflammation, leading to increased production of quinolinic acid and stimulating microglia to express kynurenine-3-monooxygenase (KMO, resulting in the conversion of kynurenine to the neurotoxic metabolite quinolinic acid (Vaglio-Garro et al., 2024; Qin and Zhang, 2020; Sipahi et al., 2023). Empirical studies have demonstrated that the activation of NF-κB and the presence of pro-inflammatory cytokines such as IL-

TABLE 10 The main antidepressant active metabolites in *Rehmannia glutinosa-Lilium*.

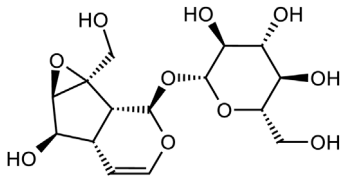
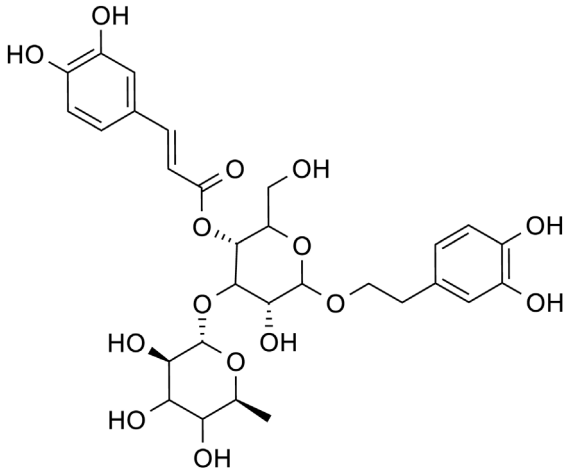
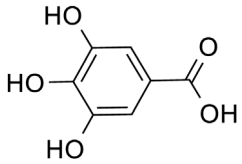
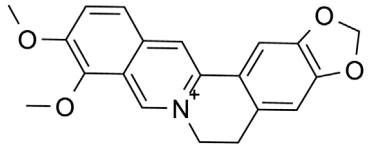
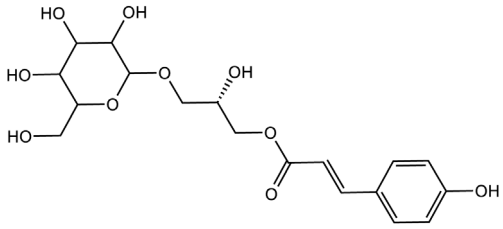
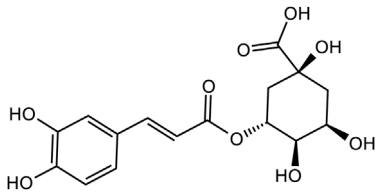
Metabolites	Structural formula	Source	References
Catalpol		*Rehmannia glutinosa*	Wang et al. (2014), Wang Y. L et al. (2021), Wang et al. (2008), Wang Y. T. et al. (2021), and Song et al. (2021)
Acteoside		*Rehmannia glutinosa*	Xue X. et al. (2022)
Gallic acid		*Lilium*	Can et al. (2017)
Berberine		*Lilium*	Peng et al. (2007), Xue (2021), Zhou et al. (2014), and Huang Y et al. (2023)
Regaloside A		*Lilium*	Yuan et al. (2021)
Chlorogenic acid		*Lilium*	Liu et al. (2021) and Shi et al. (2021)

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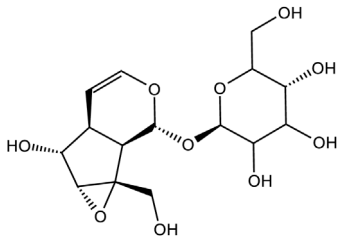
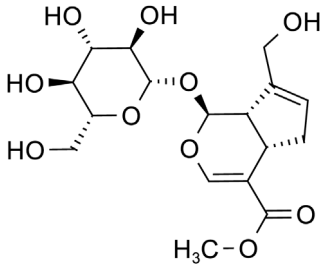
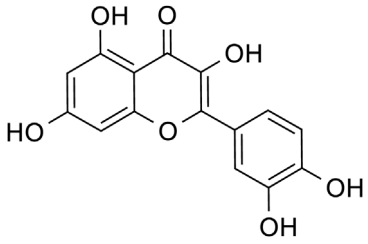
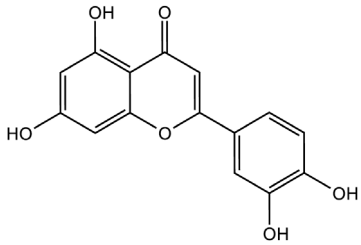
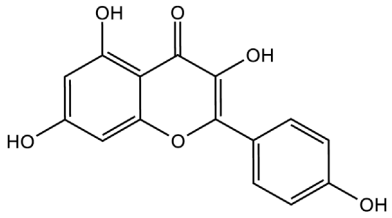
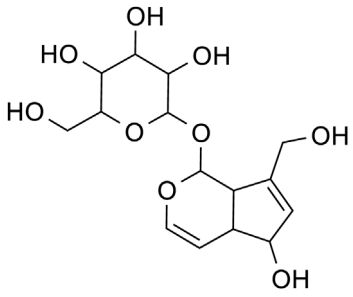
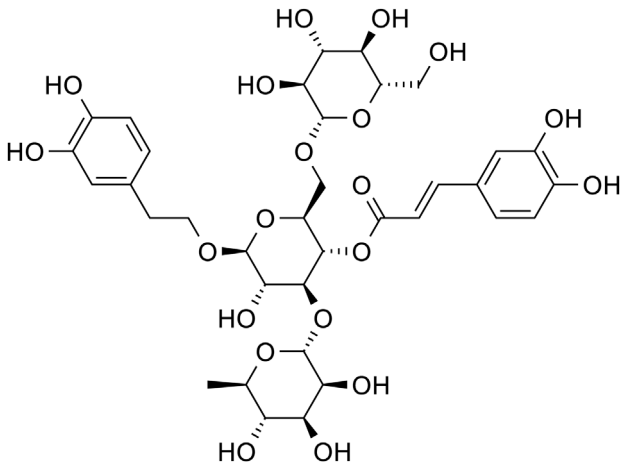
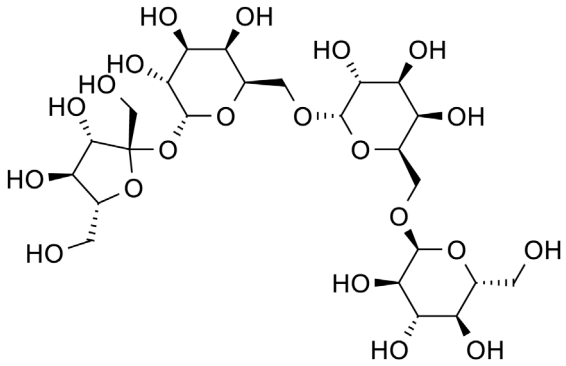
TABLE 10 (*Continued*) The main antidepressant active metabolites in *Rehmannia glutinosa-Lilium*.

Metabolites	Structural formula	Source	References
Rhmannioside D		*Rehmannia glutinosa*	Zhang et al. (2022) and Wang Y. L et al. (2021)
Geniposide		*Rehmannia glutinosa*	Zhao et al. (2018) and Cai et al. (2015)
Quercetin		*Lilium*	Mehta et al. (2017)
Luteolin		*Lilium*	Liu et al. (2013)
Kaempferol		*Lilium*	Gao et al. (2019) and Qu (2021)
Aucubin		*Rehmannia glutinosa*	Lu et al. (2022)

(Continued on following page)

TABLE 10 (*Continued*) The main antidepressant active metabolites in *Rehmannia glutinosa-Lilium*.

Metabolites	Structural formula	Source	References
Echinacoside		*Rehmannia glutinosa*	Lu et al. (2021)
Lupeose		*Rehmannia glutinosa*	Wang (2013)

1β and TNF-α can enhance the expression and activity of iNOS, promotes the production of nitric oxide (NO), induces the release of glutamate-containing vesicles from astrocytes, inhibits the reuptake of glutamate, and consequently elevates extracellular glutamate concentrations (Ida et al., 2008; Olivenza et al., 2000; Du et al., 2022). Consequently, oxidative stress interacts with inflammation to exacerbate depressive symptoms and *R. glutinosa-Lilium* may exert an antidepressant effect by reducing the levels of pro-inflammatory cytokines IL-1β and TNF-α and modulating the tryptophan-kynurenine pathway (Guo et al., 2024).

5.4 Effect of active metabolites of *Rehmannia glutinosa-Lilium* on glutamic acid

Neuroimaging and autopsy studies found that depression patients' plasma, cerebrospinal fluid, and brain glutamate (Glu) concentrations were higher, and serum Glu levels were positively correlated with MDD severity (Zhang et al., 2013; Yan, 2022). With ketamine, a glutamate receptor (NMDAR) antagonist, as a quick-acting antidepressant, the role of glutamatergic nervous system in depression has received widespread attention (Murrough et al., 2017). Glu homeostasis is maintained by the glutamate-glutamine cycle in the central nervous system, and neurons and astrocytes provide a strong guarantee of neuronal activity (Eid et al., 2016; Mahmoud et al., 2019). A high concentration of Glu damages nerve cells and overstimulates glutamate receptors (NMDAR, etc.), which may contribute to depression (Wu et al., 2014). In the rat model of depression induced by CUMS, the levels of NMDAR phosphorylation and subunit NR1/NR2B protein increased significantly, the abnormal concentration of Glu in synaptic space led to the overactivation of extra synaptic NMDAR, and a large amount of Ca^{2+} influx led to intracellular Ca^{2+} overload, resulting in nerve cell death (Li et al., 2018; Beneyto et al., 2007).

Modern studies have found that catalpol in *R. glutinosa* can significantly reverse the decrease of cell survival rate and muscarinic receptor density induced by L-Glu, suggesting that catalpol may have neuroprotective effects by regulating the cholinergic nervous system (Wang et al., 2008). Acubin inhibits glutamate receptor NMDAR1 and oxidative stress, thereby improving Glu excitotoxicity, and improving PC-12 cell damage induced by Glu, which has potential activity in the treatment of depression (Lu et al., 2022). The phenylethanol glycoside compound echinacoside exhibits neuroprotective, antiinflammatory, antioxidant, antiviral, cardiac activity, and many other biological properties (Liu et al., 2018). Moreover, echinacoside crosses the blood-brain barrier, suggesting potential clinical application for neurological diseases (Zhu et al., 2013). Echinacea glycoside may improve Glu-induced PC-12 cell damage by reducing intracellular Ca^{2+} accumulation, inhibiting NMDAR1 protein expression and antioxidation (Lu et al., 2021). The consumption of berberine significantly decreased ROS production, lipid peroxidation, and DNA fragmentation in glutamate-damaged hippocampal cells, increasing glutathione

content and SOD activity, and the anti-apoptotic effect of berberine was demonstrated by reducing the overexpression of Caspase-3 and Bax/Bcl-2 induced by glutamate (Xue, 2021) (Figure 4; Table 7). *In vivo*, elevated glutamate levels lead to the over-activation of NMDA receptors, causing a substantial influx of Ca2+ and subsequent Ca2+ overload (Zhou et al., 2024). This cascade triggers downstream pathways that result in the production of reactive oxygen species and mitochondrial dysfunction, ultimately leading to neuronal damage (Wang Y. L et al., 2021). *Rehmannia glutinosa-Lilium* may exert an antidepressant effect by mitigating Ca2+ accumulation within cells and inhibiting the expression of glutamate receptors.

5.5 The hypothalamus-pituitary-adrenal (HPA) axis is influenced by active metabolites of *Rehmannia glutinosa-Lilium*

Depression can be caused by excessive excitation of the HPA neurohormone, which regulates stress states in the body (Juruena et al., 2018; Tang et al., 2019). The hypothalamus begins with adrenocorticotropic hormone-releasing hormone (CRH) secreted by the paraventricular nucleus and then stimulates the pituitary gland, where the anterior lobe releases adrenocorticotropic hormone (ACTH), which in turn induces adrenal gland secretion of CORT. In turn, CORT regulates the stress response by reducing its own secretion by sending feedback signals to the hypothalamus and pituitary to reduce the production of CRH and ACTH (Sarno et al., 2021; Plotsky et al., 1998). It was found that the secretion and response of CORT, the level of CRH in cerebrospinal fluid and inflammation increased in patients with severe depression (Zunszain et al., 2011; Amasi-Hartoonian et al., 2022; Horowitz et al., 2020). The increase of CORT level caused by imbalance of HPA axis was directly related to depressive symptoms (Wang et al., 2022). Behavioral test results of mice after injection of CORT suggested depression-like behavior, and serum levels of CORT, ACTH, and CRH increased dose-dependently and over time (Chen et al., 2021; Mikulska et al., 2021; Lok et al., 2012; Joseph and Golden, 2017).

Geneniposide was found to restore the negative feedback between the CRH expression and HPA axis injured by CUMS, which inhibited its high activity, and a significant reduction in CORT serum levels, as well as CRH mRNA expression, was also observed, but ACTH levels were not significantly affected (Cai et al., 2015). *Lilium* saponins have been found to exhibit an antidepressant effect by suppressing the hyperactivity of the HPA axis, leading to a reduction in circulating levels of COR, ACTH and CRF mRNA in rats (Guo et al., 2010). In mice, the antidepressant effect of catalpol is attributed to its ability to regulate both NF-κB and Nrf2, thereby inhibiting HPA axis hyperactivity, central inflammation, oxidative damage, and depression-like behavior induced by CORT (Song et al., 2021). Berberine has the potential to induce a calming and hypnotic effect through the inhibition of the HPA axis and the augmentation of the levels of 5-HT and NE in the hypothalamus of a mouse model of insomnia induced by PCPA (Zhou et al., 2014) (Figure 5; Table 8). When 5-HT levels in the brain are low, ACTH secretion of the pituitary gland increases, resulting in increased secretion of peripheral cortisol, which suggest that the 5-HT system in the brain exerts an inhibitory effect on ACTH secretion (Zhao et al., 2021a). Following the interaction between cortisol and glucocorticoid receptors, there is an activation of tyrosine aminotransferase and tryptophan pyrroliase, which reduces the synthesis of the 5-HT and NE precursors, tyrosine and tryptophan, resulting in a decrease in the content of monoamine transmitters in the brain and worsening anxiety and depression (Arborelius and Eklund, 2007; Greenstein and Hunt, 2023). Therefore, *R. glutinosa-Lilium* regulates the HPA axis by reducing the content of hormones such as CORT, ACTH and CRH, thus playing an antidepressant role.

5.6 Effects of active metabolites of *Rehmannia glutinosa-Lilium* on intestinal microorganisms

The gut-brain axis, which refers to the complex interaction between the gastrointestinal tract and the brain, has been demonstrated to exert a significant impact on emotional regulation, cognitive processes, and the central nervous system, ultimately contributing to the pathogenesis of depression (Tian et al., 2022; Kim et al., 2023; Xie et al., 2023). Recent research has demonstrated notable alterations in the intestinal microbiota of both depressed individuals (Huang Y et al., 2023) and animal models of depression (Xie et al., 2023), indicating a strong association between gut flora and depression (Zhang M. et al., 2021; Zhao N. et al., 2023). By regulating coding RNA, non-coding RNA and various signal pathways, intestinal flora can regulate not only the function of hippocampal and microglia, but also the expression level of BDNF and immune inflammatory response related to depression, which ultimately affect depression's occurrence and development, suggesting that a potential target for treating depression could be inhibition of intestinal flora (Chen et al., 2022; Xu M. et al., 2023).

The anti-fatigue, antidepressant, antibacterial, and other effects of *Lilium* polysaccharides have been demonstrated in modern pharmacological studies (Gao et al., 2015). *Lilium* polysaccharides regulate intestinal flora imbalance by inhibiting the increase of LPS, IL-6, and TNF- α, increasing the content of secretory immunoglobulin A (SIgA) and regulating intestinal flora imbalance by cultivating beneficial bacteria and inhibiting harmful bacteria (Zhao et al., 2020). In STZ-induced diabetic mice, oral administration of 300 mg/kg *R. glutinosa* stachyose can significantly lower blood glucose levels, restore the number of *Lactobacillus* and some normal bacteria reduced by disease to a certain extent, which has the dual effect of regulating blood sugar and intestinal flora (Wang, 2013). Following the administration of kaempferol, the intestinal microbiota of mice with ulcerative colitis exhibited an increase in richness and the relative ratio of Firmicutes and *Bacteroides* was observed to increase, while the relative abundance of pathogenic species decreased and the abundance of probiotics increased (Qu, 2021). Berberine can significantly reduce the levels of both Trichobacterium and *Clostridium* diffrium in rats, increase the levels of Rumen and Lactic Acid Bacteria, and inhibit pro-inflammatory cytokines, thus inhibiting the overactivated inflammatory response by regulating rat intestinal flora (Huang D. X. et al., 2023). In mice with non-alcoholic fatty liver disease (NAFLD), the administration of chlorogenic acid resulted in an

increase in intestinal flora and the secretion of glucagon-like peptide-1 (GLP-1), which is known to regulate inflammation (Shi et al., 2021) (Figure 6; Table 9). Turicibacter is an intestinal bacterium that expresses a sodium transporter-related protein exhibiting sequence and structural homology to mammalian neurotransmitters, among which DA, NE, acetylcholine, and GABA are all neurotransmitters closely associated with depression, thus causing depressive mood and behavior (Fung et al., 2019; Feng et al., 2024). We hypothesize that *R. glutinosa-Lilium* may influence the synthesis and transport of neurotransmitters by effectively preventing intestinal microorganisms from entering systemic circulation, inhibiting the body's immune response, and regulating the abundance of intestinal flora, thereby achieving therapeutic effects in the treatment of depression.

6 Toxicity studies

The extract of *Rehmannia* was evaluated for acute toxicity in mice using the LD50 and MTD methods, with no fatalities observed. Concurrently, a subchronic toxicity study was conducted on 80 Sprague-Dawley rats, which were allocated into four groups: low (1,670 mg/kg), medium (8,330 mg/kg), high (16,700 mg/kg) doses of *Rehmannia* extract, and a control group. After 30 days of continuous intragastrical administration of the extract, there were results no significant differences in body weight, blood biochemical parameters, organ coefficients, or visceral histopathology between the treatment groups and the control group (Liu J et al., 2017). According to the acute toxicity classification standard of the World Health Organization (WHO) and the results from both acute and subchronic toxicity tests, *Rehmannia* extract is deemed safe and non-toxic at clinical doses. The cytotoxicity of the water extract of *Lilium*, both before and after sulfur fumigation, was assessed. The cytotoxicity of the aqueous extract of *Lilium*, both prior to and following sulfur fumigation, was assessed. Concentrations ranging from 0 to 800 mg/L demonstrated that the post-fumigation aqueous extract of *Lilium* exhibited no significant impact on the viability of human liver LO2 cells, human renal proximal tubule HK-2 cells, and rat adrenal pheochromocytoma PC-12 cells. Furthermore, no significant differences were observed when compared to the pre-fumigation aqueous extract. These findings suggest that *Lilium* concentrations between 0 and 800 mg/L do not induce cytotoxic effects (Zhang et al., 2023). *Rehmannia* and *Lilium* are not only recognized in the Chinese Pharmacopoeia, but are also listed among Chinese medicinal materials utilized for both therapeutic and dietary purposes, with a long-standing history of consumption. However, certain metabolites within *Rehmannia* and *Lilium* may exhibit toxic side effects.

Although colchicine in lilies is relatively less toxic, its metabolism in the liver through deacetylation results in the formation of the more toxic compound dicolchicine. This metabolite repeatedly interacts with the gastrointestinal mucosa during enterohepatic circulation, leading to symptoms of poisoning such as nausea, vomiting, and abdominal pain. These interactions can further result in damage to liver and kidney function and may lead to metabolic acidosis, as well as respiratory and circulatory failure (Liu et al., 2024). Furthermore, colchicine exhibits significant cardiotoxicity, with severe cases potentially resulting in mortality due to circulatory failure and fatal arrhythmias (Mullins et al., 2000). Liver biopsy specimens from mice treated with AU did not reveal any abnormal histological findings. Following a single intraperitoneal injection of 1–100 mg/kg AU, all Wistar rats survived, but administration of 100 mg/kg AU led to paralysis (Xue et al., 2012). Acute toxicity assessments conducted on mice with gavage doses of 10, 20, and 40 g/kg AU indicated that mice receiving 40 g/kg AU experienced a slight reduction in free movement and food intake, along with the presence of fatty or soft stools. Nevertheless, these phenomena gradually normalized by days 2–3 and no animals exhibited symptoms of poisoning or mortality within 14 days post-treatment (Li, 2011). Consequently, while medicinal and edible plants are generally considered safe, they are not devoid of potential adverse effects, including side effects and toxicity, which may be dose-dependent, particularly in long-term studies.

7 Conclusion and future perspectives

Globally, depression affects hundreds of millions of people, but because depression affects many systems of the body, the treatment of depression is a difficult problem for both modern medicine and TCM. The treatment of depression is currently limited to a single target or a single signal pathway, target-signal pathway interactions are not sufficiently discussed in depth, and the drugs used in clinics still cannot fully cure a variety of depression-related diseases.

The pathogenesis of depression is interconnected, with no single factor acting independently. For instance, an imbalance in monoamine neurotransmitters can lead to increased inflammation, while the inflammatory response can exacerbate the reduction of 5-HT levels, collectively contributing to the development of depression (Guo et al., 2024). Glutamic acid can elevate NO levels, and NO, in turn, can regulate the release of neurotransmitters such as 5-HT and DA (Wang R. et al., 2023). The hyperactivation of the HPA axis and the subsequent excessive secretion of corticosterone lead to the compromise of the blood-brain barrier, which in turn results in neuronal damage and contributes to the pathophysiology of depression (Zhao et al., 2021b). Additionally, the gut microbiota plays a significant role in modulating depressive states by influencing inflammatory pathways and altering the synthesis of neurotransmitters (Feng et al., 2024). Combinations of TCM compounds have multiple advantages, such as multi-metabolites, multi-pathway, and multi-target treatment. Chinese traditional medicine's active metabolites is a monomer compound extracted and purified from TCM, which is the TCM's main metabolites and its compound preparations to exert its pharmacological effects, and its target, signaling pathway and mechanism for treating diseases are relatively clear (Xing et al., 2024c). In this review, we reviewed for the first time that *R. glutinosa-Lilium* has an active ingredient in antidepressant. We found that catalpol, geniposide, *Lilium* saponins, gallic acid and berberine can relieve depression by enhancing the levels of monoamine neurotransmitters in the brain, such as 5-HT, DA, and NE. Catalpol, Regaloside A, Rhmannioside D and chlorogenic acid on depression can be attributable to its ability to upregulate the expression of BDNF and TrkB receptors. Geniposide, ethanol extract of *Lilium*, quercetin, luteolin, kaempferol, Rhmannioside D and catalpol inhibit the occurrence of depression by improving oxidative stress and inflammation. Catalpol, aucubin,

echinacosid and berberine reduces depression symptoms by reducing Glu levels and NMDAR expression. Geniposide, *Lilium* Saponins, catalpol and berberine inhibits the release of hormones such as ACTH and CRH, thereby reducing depression symptoms. *Lilium* Polysaccharides, stachyose, kaempferol, berberine and gallic acid regulates intestinal flora by inhibiting harmful bacteria, therefore reducing depression symptoms.

According to this review, we found that catalpol, acteoside, gallic acid, berberine, Regaloside A, chlorogenic acid, Rhmannioside D, geniposide, quercetin, luteolin, kaempferol, aucubin, echinacoside, stachyose and other main active metabolites were found in the *R. glutinosa-Lilium* (Table 10). Although the therapeutic effect of *R. glutinosa-Lilium* on depression has been substantiated, its application in the development of antidepressant agents remains relatively underexplored. Active metabolites such as catalpol, ralinosin A, and genipine present significant potential for development as lead compounds to enhance pharmacological efficacy, which provides a reliable basis for the development of antidepressant drugs. Secondly, leveraging the traditional prescription of Baihe Dihuang Decoction, advanced methodologies such as network pharmacology, bioinformatics, and systems biology were employed to optimize the formulation, enhance therapeutic efficacy, and refine the compatibility, which aim to harness the multi-metabolites and multi-target treatment characteristics inherent in traditional Chinese medicine, thereby augmenting its antidepressant effects. Therefore, the future research direction should focus on using new technology to systematically describe the antidepressant tool of TCM from many aspects, multi-targets, and multi-levels, and simultaneously explore new antidepressant targets and develop fast, effective, and specific antidepressant drugs to provide a new direction for clinical trials of depression.

Author contributions

ZW: Writing–original draft, Writing–review and editing. XW: Formal analysis, Writing–review and editing. XM: Formal analysis, Writing–review and editing. CW: Data curation, Writing–review and editing. YS: Data curation, Writing–review and editing. JW: Supervision, Writing–review and editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This study received financial backing from the Key Project of the Natural Science Foundation of Shandong Province (No. ZR2020ZD17), the Natural Science Foundation of Shandong Province (No. ZR2021MH125), Jinan City-School Integration Development Strategic Project (No. JNSX2023055).

Acknowledgments

Thanks to Shandong University of TCM's Research and Innovation team for providing the experimental platform and conditions.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2024.1471307/full#supplementary-material

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