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Optimization of anthocyanin extraction from mango peel wastes using ultrasound and microwave-assisted natural deep eutectic solvents

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This study investigates the efficient extraction of anthocyanin from mango peel wastes using ultrasound and microwave-assisted hydrophilic natural deep eutectic solvents (NADES). Among the various NADES combinations tested, choline chloride: lactic acid (1:2) exhibited the highest extraction yield. Process variables such as sonication duration (15–30 min), microwave intensity (30.11–60.22 W/g), and microwave duration (20–60 s) were optimized using the Box–Behnken experimental design. Under optimized conditions, the anthocyanin yield significantly increased to 44.77 mg/g dry weight for ChCl: lactic acid, compared to 4 ± 0.02 mg/g dry weight for acidified ethanol, demonstrating the synergistic effect of ultrasound and microwave-assisted NADES in enhancing extraction efficiency. Spectrophotometric and stability analyses confirmed the integrity and storage behavior of the extracted anthocyanin under different environmental conditions. Additionally, antioxidant activity (DPPH) and ferric reducing power of the ChCl: lactic acid extract were recorded as 39 ± 0.1 and 40 ± 0.82 μmol AAE/g dry weight, respectively, which were markedly higher than those obtained using the conventional method (17.11 ± 0.16 and 20.29 ± 0.87 μmol AAE/g dry weight). The total phenolic content was higher in ChCl: lactic acid (2.82 ± 0.72 mg GAE/g dry weight) than in acidified ethanol (1.21 ± 0.17 mg GAE/g dry weight). Color analysis also showed greater color intensity for ChCl: lactic acid (1.211 ± 0.23) compared to acidified ethanol (1.093 ± 0.10). The findings support the use of green solvents and non-conventional extraction techniques for the valorization of agro-industrial waste into valuable bioactive compounds.

KEYWORDS

anthocyanins, antioxidants, fruits, optimization, sustainability, valorisation

1 Introduction

Anthocyanin, a subclass of flavonoids, is responsible for the red, purple, and blue colors in many fruits and vegetables (Tzanova et al., 2024; Sportiello et al., 2025). In addition to the coloring ability, it has significant pharmacological benefits, such as anti-inflammatory, anti-cancer, cardioprotective, and neuroprotective activities (da Silva et al., 2020). Anthocyanin has a sugar molecule linked to the anthocyanidin (aglycone) by an α or β-glycosidic bond. It has a flavylium cation structure, which can be hydroxylated at different positions (C3, C5, C6, C7, and C3′, C4′, C5′) to produce various anthocyanidins. Although they contain a positively charged oxygen (oxonium group) on the C ring, the basic flavonoid ring structure remains the same. The sugars usually attach at the 3-OH position, forming 3-O-β-glucosides, which are the most common natural forms. The main monomeric anthocyanin found in nature is a glycoside of cyanidin, delphinidin, malvidin, and pelargonidin (Castañeda-Ovando et al., 2009; Liu et al., 2018; de Pascual-Teresa and Sánchez-Ballesta, 2008). The extraction and stabilization of anthocyanin is challenging due to its sensitivity to various factors such as pH, light, temperature, and oxygen (Tzanova et al., 2024; Prajapati and Jadeja, 2022).

The stability of anthocyanin is strongly dependent on pH, and it is most stable as vibrantly colored flavylium cations in an acidic environment. The increase in pH to neutral and basic conditions converts anthocyanin into less stable uncharged quinones. Under neutral conditions, it gets converted into uncharged quinones and degrades through different pathways, leading to a loss of color (Roberto et al., 2020). Anthocyanin is widely used as natural food colorants (E163) in many countries, like Europe, Japan, and the United States. They are used in products such as soft drinks, syrups, jams, jellies, sweets, baked goods, and dairy products. Anthocyanin provides antioxidant protection to foods and enhance their potential health benefits to consumers (Patras et al., 2010; Tiwari et al., 2009). The selection of a suitable extraction method and solvents plays an important role in preserving its bioactivity and yield. Conventional extraction methods rely on harmful organic solvents, which are deleterious to both human health and the environment (Kähkönen et al., 2021; Kalia et al., 2008). In order to mitigate this issue, researchers have recently explored green technologies like ultrasound and microwave-assisted extraction methods that are more efficient and less harmful to the environment (Sportiello et al., 2025).

Ultrasound (typically between 20 and 100 kHz) generates cavitation bubbles in the extraction solvent, and the implosion of these bubbles produces localized high-pressure and temperature spots that disrupt the cell walls and facilitate mass transfer. Ultrasound-assisted extraction is recognized for its simplicity in operation, lower energy consumption, shorter extraction duration, and ability to recover heat-sensitive compounds with minimal degradation (Jovanovic et al., 2022; Cravotto et al., 2018; Cravotto and Cintas, 2007; Cravotto et al., 2008). Microwave-assisted extraction uses microwave energy to heat the sample-solvent mixture by dipole rotation and ionic conduction, resulting in the rapid cell rupture and solubilization of target compounds. Microwave-assisted extraction offers several advantages, such as reduced solvent consumption and enhanced recovery of bioactive compounds (Veggi et al., 2012; Mandal et al., 2007; Leonelli et al., 2012). Ultrasound and microwave-assisted extraction of anthocyanin from blueberry peels resulted in a higher yield than conventional acidified hydroalcoholic extraction (Grillo et al., 2020).

Mango (*Mangifera indica* L.) peels, a by-product of mango processing industries, constitute about 15–20% of the total fruit mass. These are often discarded as waste despite being rich in pectin, phenolic compounds, carotenoids, and anthocyanin, which are known for their potent antioxidant and health-promoting properties (del Pilar Sánchez-Camargo et al., 2019; Ranganath et al., 2018). The increase in demand for natural bioactive compounds in food, pharmaceutical, and cosmetics-based industries resulted in the development of sustainable sources and eco-friendly extraction methods (Saravanan and Jeevitha, 2024). Natural Deep Eutectic Solvents (NADES) have been reported as new generation green solvents for the extraction of bioactive compounds (Popovic et al., 2022). The NADES are stable eutectic mixtures that have a lower melting point than that of individual constituents (sugars, organic acids, amino acids, polyols). They are formed by mixing two or more biocompatible and biodegradable natural components that interact with one another through strong hydrogen bonds (Jauregi et al., 2024; Dai et al., 2016). NADES offer various advantages over conventional solvents, as they are non-toxic, non-volatile, biodegradable, and customizable for applications (Choi et al., 2011).

Although both ultrasound and microwave are effective green extraction methods, a sequential approach was selected for this study. The extraction process begins with the application of ultrasound to disrupt the cell walls and improve solvent penetration, followed by microwave treatment, which provides rapid heating of the sample to accelerate the release of anthocyanin. The combination of ultrasound and microwave-assisted NADES-based extraction is anticipated to work synergistically, resulting in a superior anthocyanin yield and minimal environmental impact. The addition of a small volume of water to the NADES formulations can reduce their viscosity, improve mass transfer, and extraction kinetics (Saravanan and Jeevitha, 2024; Grillo et al., 2020). Chanioti and Tzia (2018) suggested that NADES, composed of choline chloride and lactic acid, significantly enhanced the recovery of polyphenols from olive pomace, compared to the ethanol-based extraction. Jovanović et al. also reported that a higher anthocyanin yield was obtained using NADES composed of choline chloride and lactic acid. There are a few reports available on NADES-based extraction of anthocyanin from berries and jamun, while their application to mango peel for the extraction of anthocyanin has not yet been explored. The Box–Behnken design is a response surface method that uses three levels of variables to develop a quadratic model. Compared to the central composite design, the Box–Behnken design requires fewer experiments and avoids extreme conditions, making it more economical. Therefore, Box–Behnken design is suitable for optimizing extraction processes, especially for heat-sensitive bioactive compounds (Saravanan and Jeevitha, 2024). Therefore, this study aims to optimize the extraction of anthocyanin from mango peels using ultrasound and microwave-assisted NADES, while also evaluating the extraction efficiency and stability of anthocyanin.

2 Materials and methods

2.1 Chemicals

Choline chloride (>98%) and DPPH (2,2-Diphenyl-1-picrylhydrazyl, >97%) were purchased from Tokyo Chemical Industry, Tokyo, Japan. Lactic acid (88%) was purchased from Finar Chemicals Ltd., Maharashtra, India. Maleic acid (99%), malonic acid (99%), L-proline (98%), sodium acetate (98%), potassium chloride (98%), sodium carbonate (98%), and hydrochloric acid (32%) were purchased from Avra Synthesis Pvt. Ltd., Hyderabad, Telangana, India. Citric acid, 1,2-propanediol were purchased from SD Fine-chemicals Ltd., Maharashtra, India. Betaine (≥98%) was purchased from Sisco Research Laboratory Pvt. Ltd., Taloja, Maharashtra, India. Folin–Ciocalteu reagent (98%) was purchased from Himedia, Thane, Maharashtra, India. Ethanol (>99%) was purchased from Alsucrose Corporation, Pune, Maharashtra, India. Ascorbic acid and gallic acid (97.5%) were purchased from Merck, Shanghai, China.

2.2 Materials

Mango peels were procured from a nearby food processing industry and dried in a hot air oven at 45 °C for 4 h until constant weight was achieved. The dried peels were then finely ground using a blender, sieved to ensure a particle size of less than 1,000 μm, and stored at 4 °C for further use. The moisture content of the dried peels was 8.3 ± 0.07%.

2.3 Preparation and characterization of NADES

The hydrophilic NADES were prepared according to the method outlined (Riyamol and Jeevitha, 2024). A total of eight different NADES were formulated using various molar ratios of hydrogen bond acceptors (HBA) and hydrogen bond donors (HBD), with 30% distilled water added to each mixture (Grillo et al., 2020). The viscosities of the prepared NADES were measured at 32 °C using a viscometer (Ametek Brookfield Viscometer Dv2t, Hyderabad, India) with a zero spindle, and the pH values were determined using a pH meter (Systronic pH system 361, India) (Zannou and Koca, 2022).

2.4 Single-factor experiments

A total of 300 mg of dried mango peel powder was combined with 9 mL of hydrophilic NADES (Table 1) and stirred at 420 rpm for 30 min. To improve anthocyanin yield, both ultrasound and microwave-assisted extraction techniques were employed. Ultrasound-assisted extraction was conducted in a water bath sonicator (USB 3.5 L, PCi Analytics, India) operating at a frequency of 40 kHz, at a constant temperature of 40 °C (Dai et al., 2016) for 10–40 min. Microwave-assisted extraction was carried out using a microwave oven (MS2043DB, LG Electronics India Private Limited). Microwave extraction was performed at different intensities (30.11–60.22 W/g) and exposure duration (20–60 s). The microwave intensity range was selected based on the work reported (Grillo et al., 2020), which indicated that intensities below 30 W/g resulted in insufficient extraction, while higher intensities (>60 W/g) cause overheating and possible anthocyanin degradation. Therefore, the chosen range ensured efficient extraction while minimizing the risk of compound loss. Furthermore, the impact of varying solid-to-liquid ratios (0.3:6–0.3:9 and 0.5:12–0.5:20) on anthocyanin yield was also studied (Table 2).

2.5 Sequential extraction process optimization of Box–Behnken design

The hydrophilic NADES, which has resulted in a higher extraction yield of anthocyanin, was selected for further optimization. A Box–Behnken design with three levels and three factors, along with the desirability function method, was applied to optimize the processing variables: sonication time (X1, 15–30 min), microwave intensity (X2, 30.11–60.22 W/g), and microwave time (X3, 20–60 s). The objective was to maximize the anthocyanin yield (Y1, %), and the optimization process was carried out using Design-Expert 13 software (Stat-Ease Inc., MN, USA). A total of 17 experimental runs were generated and executed in random order. The data were analyzed using a second-order polynomial model to determine the relationship between the independent variables and the response. The accuracy of the model was confirmed by comparing the predicted values with the experimental results (Popovic et al., 2022; Zannou et al., 2025).

2.6 Conventional method

300 mg of mango peel powder was mixed with 9 mL of acidified ethanol and stirred for 30 min using a magnetic stirrer at 420 rpm.

TABLE 2 Effect of solid–liquid ratio on anthocyanin extraction using ChCl: LA.

Solid–liquid ratio	Anthocyanin (mg/g dry weight)
0.3:6	4.5 ± 0.31[a]
0.3:9	7.3 ± 0.32[b]
0.3:10	4.5 ± 0.11[a]
0.5:12	6.6 ± 0.21[c]
0.5:15	2.6 ± 0.05[d]
0.5:20	1.3 ± 0.02[e]

Values with different small case superscript letters (a–e) in the same column for each factor indicate significant differences as estimated by Tukey's test ($p < 0.05$).

TABLE 1 NADES compositions, properties, and anthocyanin content.

Natural deep eutectic solvents	Molar ratio	pH	Viscosity (cP)	Anthocyanin content (mg/g dry weight)
Choline chloride: Lactic acid	1:2	2.13 ± 0.01[a]	0.96 ± 0.01[a]	8.68 ± 0.14[a]
Choline chloride: Citric acid	2:1	2.28 ± 0.02[b]	1.05 ± 0.02[b]	6.8 ± 0.24[b]
Choline chloride: Glycerol	1:2	3.94 ± 0.03[c]	1.02 ± 0.01[c]	3.67 ± 0.25[c]
Choline chloride: 1,2-propanediol	1:2	3.06 ± 0.03[d]	0.96 ± 0.01[a]	2.83 ± 0.15[d]
Choline chloride: Proline	1:1	5.85 ± 0.06[e]	1.03 ± 0.02[b]	3.3 ± 0.54[e]
Choline chloride: Maleic acid	1:1	2.52 ± 0.01[f]	0.97 ± 0.01[a]	2.67 ± 0.08[f]
Choline chloride: Malonic acid	1:1	2.53 ± 0.01[f]	0.98 ± 0.01[a]	2.54 ± 0.03[g]
Betain: Lactic acid	1:2	3 ± 0.02[g]	0.99 ± 0.01[a]	5 ± 0.34[h]

Values with different small case superscript letters (a–g), (a–c), (a–h) in the same column indicate significant differences as estimated by Tukey's test ($p < 0.05$).

The extract was filtered using Whatman filter paper and stored at −20 °C for further analysis.

2.7 Determination of total monomeric anthocyanin content

To determine the total monomeric anthocyanin content (TMAC), the standard AOAC Method 37,168 (200,502) (Zannou et al., 2020; Lee et al., 2005; Zannou et al., 2025) was employed. This method relies on spectrophotometric measurement, utilizing the pH-dependent structural changes of anthocyanin. Each extract was combined with two different buffer solutions: 0.025 M potassium chloride at pH 1.0 and 0.4 M sodium acetate at pH 4.5, resulting in two separate sample extracts. Absorbance was measured at wavelengths of 520 nm and 700 nm, and the anthocyanin concentration was calculated using Equation 1.

$$\text{Total monomeric anthocyanin content}\left(\frac{\text{mg}}{\text{g dry weight}}\right)=\frac{A*\text{Mw}*\text{Df}*1{,}000*V}{\varepsilon*l*m} \quad (1)$$

where A = (Absorbance$_{520}$ − Absorbance$_{700}$) pH 1.0 − (Absorbance$_{520}$ − Absorbance$_{700}$) pH 4.5; MW (molecular weight) = 449.4 gmol^{-1} for C3G, Df = dilution factor, V = volume of extract in L, ε = 26,900 molar extinction coefficient for cyanidin-3-O-glucoside, in L mol^{-1} cm^{-1}, l = pathlength in cm and m = dry mass of sample used for extraction (g). The anthocyanin content was expressed as mg/g dry weight.

2.8 Determination of antioxidant activity

2.8.1 Antiradical activity

The antiradical activity of the extracts was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay (Terlidis et al., 2023). About 50 µL of the extract was mixed with 950 µL of water and shaken vigorously for 30 s. Then, 25 µL of the resulting mixture was added to 975 µL of a 100 µM DPPH solution. The reaction mixture was incubated in the dark for 30 min, after which the absorbance was recorded at 515 nm. The antiradical activity was expressed as micromoles of ascorbic acid equivalents (AAE) per gram (dry weight), based on a calibration curve prepared with ascorbic acid concentrations ranging from 50 to 500 µg/mL. The calibration curve followed the regression equation $y = 0.7533x + 0.0427$ ($R^2 = 0.9861$). The antiradical activity was calculated according to Equation 2.

$$\text{Antiradical activity}\left(\mu\text{mol AAE / g dry weight}\right)=\frac{\text{CAA}\times V}{w} \quad (2)$$

where CAA is the concentration of ascorbic acid from the calibration curve, V is the volume of the extraction medium (L), and w is the weight of the sample (g).

2.8.2 Ferric reducing antioxidant power assay

To evaluate ferric-reducing antioxidant power, 50 µL of the sample was mixed with 950 µL of water and shaken vigorously for 30 s. Next, 50 µL of this mixture was added to 50 µL of $FeCl_3$ solution (4 mM in 0.05 M HCl) and incubated at 37 °C for 30 min. After incubation, 900 µL of TPTZ reagent (1 mM in 0.05 M HCl) was added, and the absorbance was recorded at 620 nm after 5 min (Terlidis et al., 2023). A standard curve was prepared using ascorbic acid (50–500 µg/mL). The calibration curve followed the regression equation $y = 0.7646x + 0.04$ ($R^2 = 0.9821$), and the ferric-reducing power was calculated using Equation 3, expressed as micromoles of ascorbic acid equivalents (AAE) per gram dry weight.

$$\text{Ferric reducing antioxidant power}\left(\mu\text{mol AAE / g dry weight}\right)=\frac{\text{CAA}\times V}{w} \quad (3)$$

where CAA is the concentration of ascorbic acid from the calibration curve, V is the volume of the extraction medium (L), and w is the weight of the sample (g).

2.9 Total phenolic content

Total phenolic content was quantified using the Folin–Ciocalteu method, following the procedure described (Zannou et al., 2020) with minor modifications. A 200 µL portion of the extract was mixed with Folin–Ciocalteu reagent at room temperature. Subsequently, 7% sodium carbonate (Na_2CO_3) and water were added. The mixture was incubated in the dark at room temperature for 1 h. After incubation, the absorbance was measured at 765 nm using a UV–Visible spectrophotometer. The total phenolic content was calculated and expressed as milligrams of gallic acid equivalents (50–500 mg/g dry weight, GAE).

2.10 Color analysis

2.10.1 Absorbance method

A 200 µL aliquot of the sample was mixed with 800 µL of water and shaken thoroughly for 30 s. The absorbance of the solution was recorded at 420, 520, and 620 nm. These absorbance values were then used to determine the color intensity and the distribution of yellow, red, and blue components, calculated using Equations 4–8.

$$\text{Color intensity} = A_{420} + A_{520} + A_{620} \quad (4)$$

$$H = \frac{A_{420}}{A_{520}} \quad (5)$$

$$\text{Yellow}\left(\%\right)=\frac{A_{420}}{\text{CI}}\times 100 \quad (6)$$

$$\text{Red}\left(\%\right)=\frac{A_{520}}{\text{CI}}\times 100 \quad (7)$$

$$\text{Blue}\left(\%\right)=\frac{A_{620}}{\text{CI}}\times 100 \quad (8)$$

where A_{420}, A_{520}, and A_{620} are the absorbance values at 420, 520, and 620 nm, respectively (Ranganath et al., 2018).

2.10.2 Colorimeter method

The color properties of the extracts were evaluated using a Hunter Lab, Color Flex EZ Imaging Colorimeter (45/0 USA), which operates in the CIE Lab color space (L*, a*, b*). In this system, L* indicates the brightness factor while a* and b* relate to the red and yellow components of the color, respectively (Terlidis et al., 2023). Additionally, these color coordinates were used to determine chroma (C^*_{ab}), signifying color saturation, and hue angle ($h°_{Ab}$), denoting color tone, by means of Equations 9, 10, respectively.

$$C_{ab} = \sqrt{\left(a^*\right)^2 + \left(b^*\right)^2} \tag{9}$$

$$h°_{Ab} = \tan^{-1}\left(\frac{b^*}{a^*}\right) \tag{10}$$

2.11 FTIR

FTIR spectra of anthocyanin extracts obtained using both NADES and the conventional method were recorded using an IRAffinity-1 spectrometer (Shimadzu, Japan). The analysis was conducted using the ATR technique, scanning over the wavenumber range of 4,000 to 400 cm^{-1} with a resolution of 4 cm^{-1}, averaged over 30 scans.

2.12 Stability test

Anthocyanin extracted using both the conventional method and NADES was stored under various conditions, including exposure to light, darkness, room temperature, and −20 °C for various durations (3, 6, 10, 13, 15, 18, and 30 days). Their stability was evaluated following the procedure outlined in Section 2.7. The percentage of degradation was calculated using Equation 11.

$$\text{Degradation}\,(\%) = C_0 - \frac{C_f}{C_0} * 100 \tag{11}$$

where C_0 is the initial anthocyanin concentration and C_f is the final concentration after storage.

3 Results and discussion

3.1 Selection of NADES

The pH and viscosity of eight different hydrophilic NADES used to extract anthocyanin from mango peels were studied (Table 1). All combinations contained 30% distilled water to control viscosity and enhance mass transfer during the extraction. Choline chloride: lactic acid (ChCl: LA) at a 1:2 molar ratio had the maximum extraction efficiency of anthocyanin (8.68 ± 0.14 mg/g dry weight); in addition, this NADES had a low pH (2.13 ± 0.01) and viscosity (0.96 ± 0.01 cP). The low viscosity of NADES facilitated anthocyanin solubilization, due to the better solvent penetration into plant material, leading to the increased mass transfer to the solids. The acidic environment of ChCl: LA also assisted anthocyanin stability (maintaining the flavylium cation form) while the low viscosity helped with solvent penetration and mass transfer. A similar trend was reported (Zannou and Koca, 2022), in which a low pH environment of ChCl: LA systems resulted in efficient polyphenol extraction. Choline chloride: citric acid (2:1) resulted in the next highest anthocyanin yield (6.8 ± 0.24 mg/g dry weight), but it had slightly higher pH (2.28 ± 0.02) and viscosity (1.05 ± 0.02 cP) compared to ChCl: LA. Although citric acid had a low pH, the higher viscosity potentially limited its extraction ability. Choline chloride: betaine (1:2) with a pH of 3.00 ± 0.02 and viscosity of 0.99 ± 0.01 cP resulted in a moderate yield of 5 ± 0.34 mg/g dry weight. On the other hand, NADES such as 1,2-propanediol, ChCl: maleic acid, and ChCl: malonic acid had lower anthocyanin yields (ranging from 2.54 to 2.83 mg/g dry weight). Jovanovic et al. (2022) observed similar levels of anthocyanin yield from bilberry by using NADES composed of 1,2-propanediol and malic acid. ChCl: proline (1:1) had the highest pH (5.85 ± 0.06), which may have negatively affected the anthocyanin stability, ultimately resulting in lower yield (3.3 ± 0.54 mg/g dry weight). Overall, the combination of low pH and low viscosity, with an appropriate hydrogen bonding environment for ChCl: LA, contributed to its higher extraction ability. Hence, ChCl: LA (1:2) was chosen for the following optimization studies on anthocyanin extraction.

3.2 Single-factor experiments

The different process variables, such as ultrasound duration, microwave duration, microwave intensity, and solid–liquid ratio for optimal anthocyanin extraction, were studied using single-factor experiments (Tables 2, 3). The anthocyanin extraction efficiency increased with an increase in ultrasound processing time up to 30 min (17 ± 0.80 mg/g dry weight). At 40 min, the extractability of anthocyanin decreased significantly (4 ± 0.77 mg/g dry weight at 40 min), likely due to degradation as a result of prolonged cavitation. The microwave processing duration of 60 s resulted in the anthocyanin extraction of 16 ± 0.31 mg/g dry weight. At 80 s of microwave treatment duration, the anthocyanin extraction was found to be lower (2.6 ± 0.20 mg/g dry weight), which in turn indicates the thermal degradation of anthocyanin. The increase in microwave intensity also positively impacted anthocyanin extraction, and the highest value noted was at 60.22 W/g (14.76 ± 0.73 mg/g weight). Higher microwave intensities disrupt the cell wall and promote anthocyanin dissolution in the extraction medium. The solid–liquid ratio was also observed to be an important variable affecting the anthocyanin extractability, while the highest yield was observed at 0.3:9 (7.3 ± 0.32 mg/g dry weight) (Panić et al., 2019). The lower (0.3:6) and higher (0.5:20) solid–liquid ratios resulted in lower yield due to poor solute/solvent interactions and extreme dilution, respectively (Hikmawanti et al., 2021; Saravanan and Jeevitha, 2024). The ranges for the optimization study were determined from the results obtained in the single-factor experiment, and are as follows: sonication duration of 15–30 min, microwave intensity of 30.11–60.22 W/g, and microwave duration of 20–60 s.

The conventional method using acidified ethanol resulted in significantly lower anthocyanin yield (4 ± 0.02 mg/g dry weight) than the ultrasound and microwave-assisted NADES. Among different NADES, ChCl: LA resulted in the highest anthocyanin yield of

TABLE 3 Effects of single-factor experiments on the extraction of anthocyanin by using ChCl: LA-based NADES.

Factors	Conditions	Anthocyanin (mg/g dry weight)
Sonication duration (min)	10	2 ± 0.12[a]
	20	5.3 ± 0.56[b]
	30	17 ± 0.80[c]
	40	4 ± 0.77[d]
Microwave duration (s)	20	5 ± 0.25[a]
	30	7.03 ± 0.45[b]
	40	11.3 ± 0.67[c]
	50	14.03 ± 0.89[d]
	60	16 ± 0.31[e]
	70	6 ± 0.76[f]
	80	2.6 ± 0.31[g]
Microwave intensity (W/g)	30.11	10 ± 0.55[a]
	45.165	12.45 ± 0.60[b]
	60.22	14.76 ± 0.73[c]

Values with different small case superscript letters (a–g) in the same column for each factor indicate significant differences as estimated by Tukey's test ($p < 0.05$).

44.77 mg/g dry weight. This is consistent with other studies (Popovic et al., 2022) found that microwave-assisted NADES enhanced anthocyanin yield compared to acidified ethanol. Likewise, Nemanja et al. (2024) reported that ultrasound-assisted NADES with ChCl: LA resulted in higher anthocyanin yield (7.48 mg/g) compared to conventional solvent extraction (6.26 mg/g). Likewise, Mariacaterina and Fabiana (2024) observed higher anthocyanin yield from grape pomace using microwave (99.6 mg/g) and ultrasound-assisted (75.2 mg/g) NADES than acidified ethanol (47.1 mg/g).

3.3 Response surface methodology

Response Surface Methodology (RSM) was used to optimize the ultrasound- and microwave-assisted extraction of anthocyanin from mango peel powder using hydrophilic NADES. In optimization, independent variables used included sonication time (X_1), microwave treatment time (X_2), and microwave intensity (X_3), as reported in Table 2. A Box–Behnken design (BBD) was used to determine the interaction effect of sequential ultrasound and microwave treatment for anthocyanin extraction. The experimental runs, along with the predicted values from the model, are provided in Table 4. Analysis of Variance (ANOVA) was performed on the fitted quadratic model to determine the significance of the model and each variable (Table 5) (Jovanovic et al., 2022). The experimental values correlated closely with the predicted values, which indicated good fit, reliability, and accuracy of the constructed model. The anthocyanin content extracted using hydrophilic NADES (ChCl: LA) varied significantly depending on the extraction parameters, ranging from 5.28 to 44.77 mg/g dry weight. The highest yield was obtained under optimized conditions of $X_1 = 30$ min (sonication duration), $X_2 = 45.165$ W/g (microwave intensity), and $X_3 = 20$ s (microwave duration). A quadratic model with a highly significant fit ($p < 0.0001$) was successfully developed to describe the relationship between the extraction variables and anthocyanin content (Table 5). This model effectively predicts the anthocyanin yield under varying conditions of ultrasound and microwave-assisted extraction.

A quadratic regression Equation 12 was generated using multiple regression analysis to model the anthocyanin content extracted with ChCl: LA, based on the experimental results.

$$\text{Anthocyanin content}\left(\frac{\text{mg}}{\text{g}}\text{dry weight}\right) = 18.002 + 16.18\,X_1 + 1.51\,X_2 - 1.0225\,X_3 + 1.565\,X_1X_2 - 0.615\,X_1X_3 - 0.855\,X_2X_3 + 07.304\,X_1^2 - 3.856\,X_2^2 + 1.569\,X_3^2 \quad (12)$$

The coefficient of determination ($R^2 = 0.999$) also indicated an excellent fit of the developed quadratic model to predict the anthocyanin extraction from mango peel using ChCl: LA. The low coefficient of variation (CV = 1.71%) and adequate precision (ADP) value of 147.978 indicated the very good accuracy and precision of the model. The coefficient of variation is designed to measure the extent of variability in the experimental results. This low value demonstrated that there is low dispersion, which resulted in high consistency. The ADP value, which is the signal-to-noise ratio, was greater than 4, indicating this model had a solid and reliable signal greater than noise. The model captured the complete range of the experiment, which is shown by how closely predicted values match the observed values. The normal probability plot of residuals (Figure 1a) also supported normality, as the residual points were close to a straight line. Similarly, the internally studentized residuals (Figure 1b) depicted a near Gaussian distribution, with only minor violations, thereby confirming the adequacy and robustness of the model. Furthermore, the F-value (2,225.91) was statistically significant, which in turn indicated the model's significance. The variables X_1 (sonication duration), X_2 (microwave intensity), X_3 (microwave duration), their interaction terms (X_1X_2, X_2X_3), and their quadratic terms (X_1^2, X_2^2, X_3^2) were all found to be significant contributors to the model.

These results validate the appropriateness of the quadratic model derived from the Box–Behnken design to optimize anthocyanin extraction from mango peel powder. The p-values for X_1, X_2, X_3, X_1X_2, X_2X_3, X_1^2, X_2^2, X_3^2 were below 0.05, indicating that each factor presented a significant effect on the anthocyanin yield. To assess the interactions between variables and their effects on the response, three-dimensional response surface plots were plotted (Figure 2). Ultrasound treatment has been shown to enhance plant tissue swelling and hydration, thereby enhancing solvent accessibility to the compounds. The collapse of bubbles formed through cavitation generates micro-jets that fracture cell walls, allowing increased release of intracellular materials, including anthocyanin compounds. Likewise, increased microwave intensity increases energy absorption by the mango peel matrix, significantly supporting the penetration and diffusion of the NADES solvent within the matrix. The two combined effects of localized heating and internal pressure greatly rupture the tissue, allowing for the dissolution of anthocyanin into the surrounding solvent (Gómez et al., 2019). In this study, ultrasound (30 min) combined with microwave (20 s) extraction system proved

TABLE 4 Box–Behnken design with the experimental and predicted values of anthocyanin.

Run	Independent variables			Response variable (anthocyanin mg/g dry weight)	
	X_1 (sonication duration, min)	X_2 (microwave intensity, W/g)	X_3 (microwave treatment duration, s)	Experimental values ChCl: LA	Predicted values
1	15	30.11	40	5.41	5.33
2	22.5	30.11	60	17.23	18
3	22.5	45.165	40	15.36	15.34
4	15	45.165	20	18	18
5	22.5	45.165	40	41.49	41.42
6	22.5	45.165	40	18.2	18
7	30	45.165	60	44.77	44.69
8	22.5	30.11	20	11.03	11.1
9	15	45.165	60	34.49	34.55
10	15	60.22	40	14.36	14.37
11	30	60.22	40	5.28	5.21
12	22.5	60.22	20	18.29	18
13	22.5	60.22	60	14.03	14.03
14	22.5	45.165	40	19.11	19.1
15	30	30.11	40	10.21	10.28
16	30	45.165	20	40.62	40.7
17	22.5	45.165	40	18.29	18

TABLE 5 Analysis of variance (ANOVA) for the quadratic model for optimization of extraction parameters of ChCl: LA.

Source	Sum of squares	df	Mean square	*F*-value	*p*-value
Model	2,423.82	9	269.313	2,225.91	<0.0001
A-sonication	2,094.34	1	2,094.34	17,310	<0.0001
B-MW intensity	18.2408	1	18.2408	150.763	<0.0001
C-MW duration	8.36405	1	8.36405	69.1301	<0.0001
X1X2	9.7969	1	9.7969	80.9728	<0.0001
X1X3	1.5129	1	1.5129	12.5043	0.0095
X2X3	2.9241	1	2.9241	24.1681	0.0017
$X1^2$	224.625	1	224.625	1856.56	<0.0001
$X2^2$	62.6052	1	62.6052	517.441	<0.0001
$X3^2$	10.3653	1	10.3653	85.6708	<0.0001
Residual	0.84693	7	0.12099		
Lack of Fit	0.04585	3	0.01528	0.07631	0.9695
Pure Error	0.80108	4	0.20027		
Cor Total	2,424.66	16			

df: Degree of freedom.

to significantly increase anthocyanin yield and provide potential benefits in terms of time and energy. The optimal extraction parameters for maximum anthocyanin yield (44.77 mg/g dry weight) using ChCl: LA were: ultrasound duration of 30 min, microwave intensity of 45.165 W/g, microwave duration of 20 s. These experimental values were reasonably close to the model prediction value of 44.69 mg/g dry weight, a virtual confirmation of the reliability and predictability of the developed quadratic model.

3.4 Validation process

To validate the positive effects of the RSM-predicted model for anthocyanin extraction with ChCl: LA, a validation experiment was performed based on the optimized parameters (sonication duration of 30 min, microwave intensity of 45.165 W/g, microwave duration of 20 s, and a liquid–solid ratio of 0.3:9). The experimental anthocyanin content of 44.77 mg/g dry weight was in line with the prediction of 44.69 mg/g

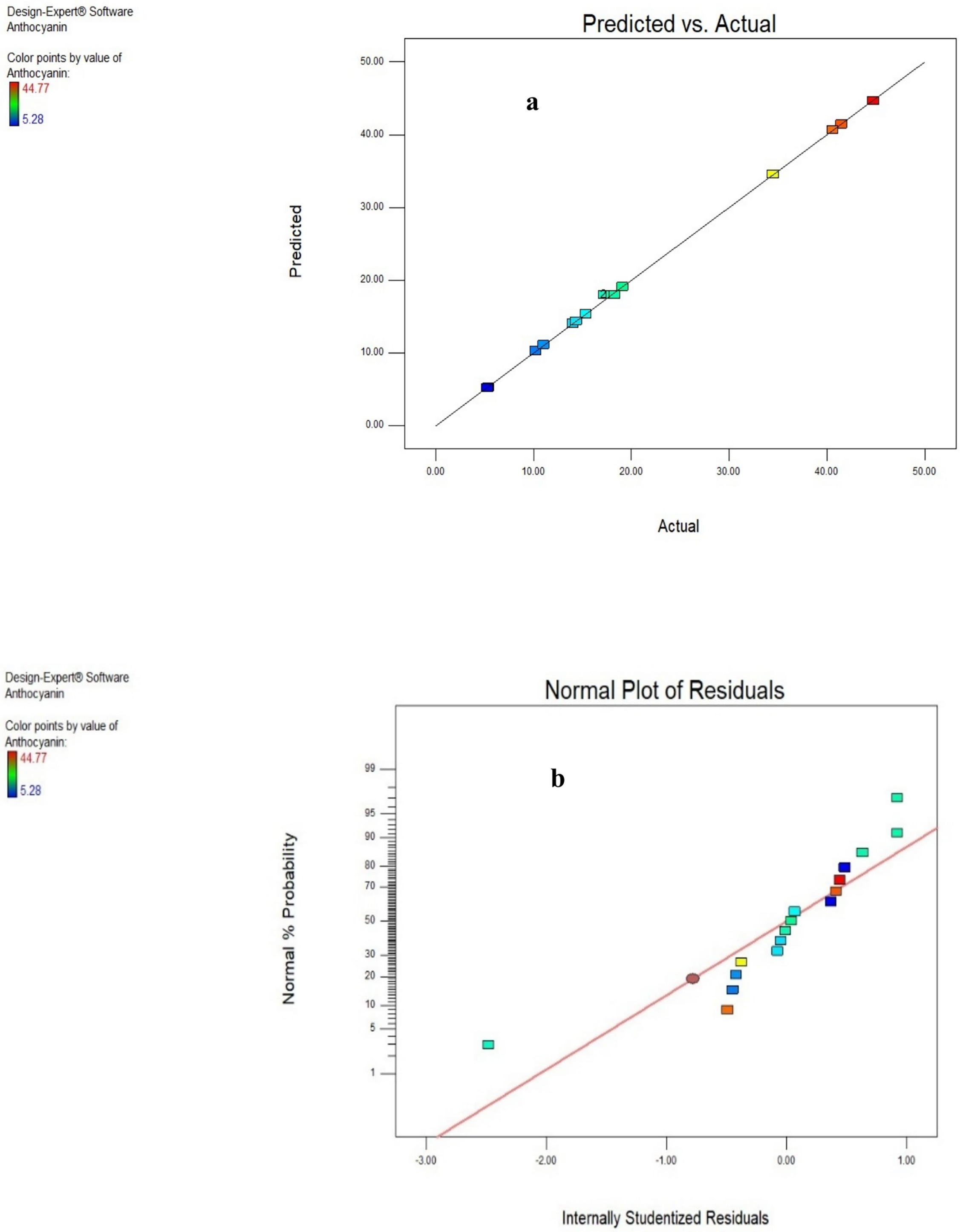


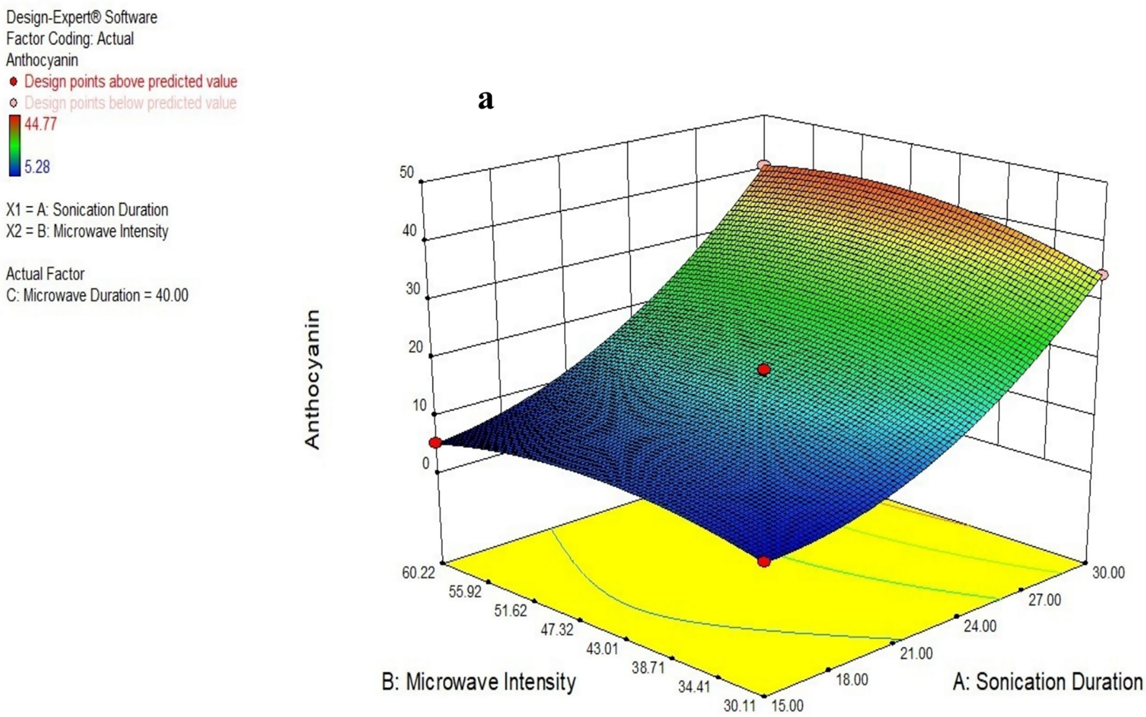
FIGURE 1
(a) Comparison between predicted and actual values of ChCl: LA obtained by anthocyanin content obtained by sequential microwave and ultrasound-assisted hydrophilic-based natural deep eutectic solvents extraction **(b)** normal probability of internally studentized residuals.

dry weight as can be seen in Table 6. The experimental anthocyanin results also fell within the 95% confidence interval of the response, which solidifies the workability of the model. The successful validation of the model demonstrates that the conditions provided by the RSM are indeed operational and realistic in order to maximize anthocyanin extraction from mango peel powder (Saravanan and Jeevitha, 2024).

3.5 Antioxidant activity

3.5.1 Antiradical activity

The antiradical activity (AAR) of the extracts obtained using different solvents showed a significant variation. The extract obtained using ChCl: LA-based NADES had an AAR of 39 ± 0.1 μmol AAE/g



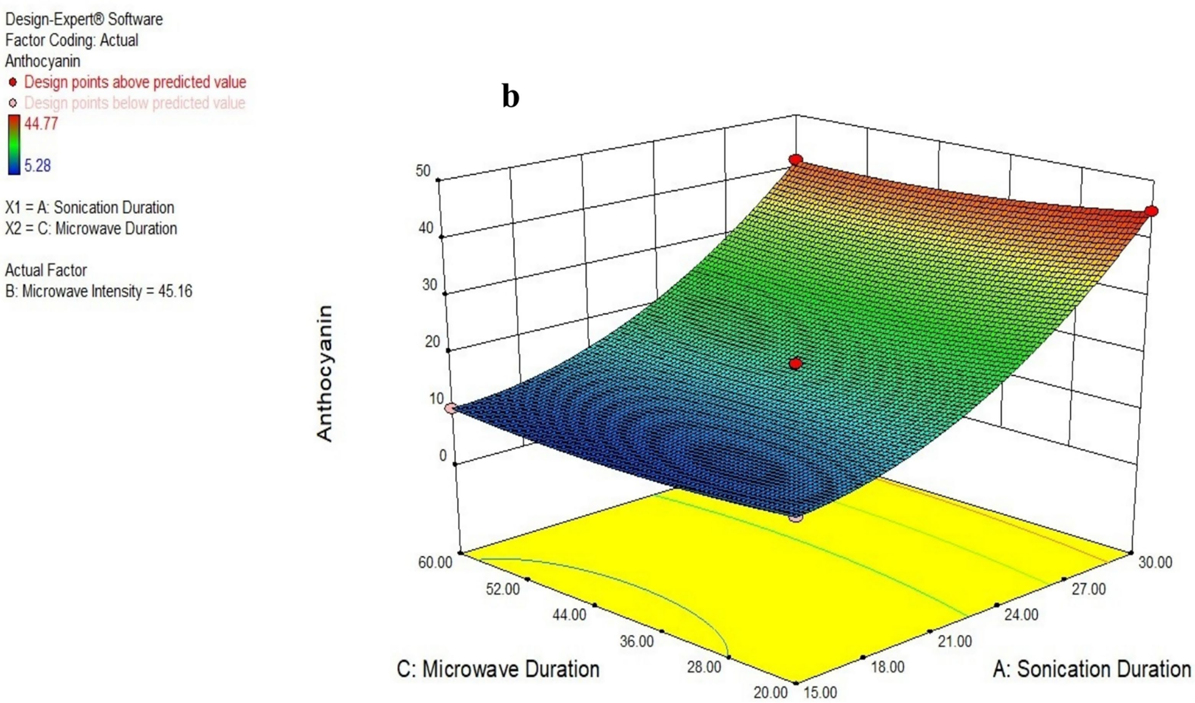


FIGURE 2 (Continued)

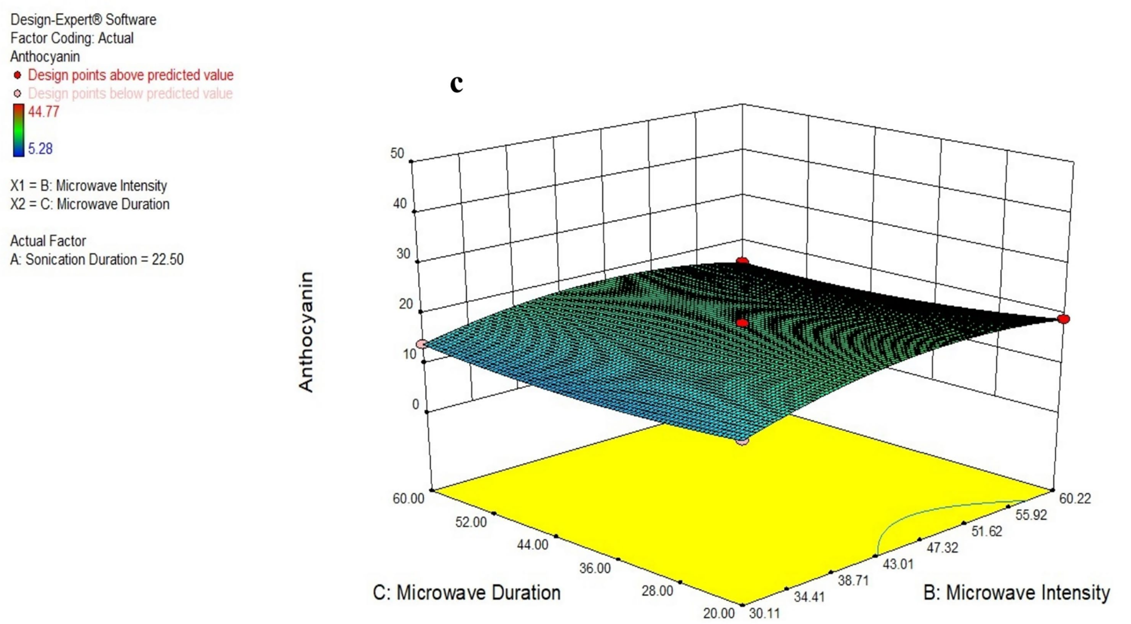


FIGURE 2
Three-dimensional response surface plots of anthocyanin content of ChCl: LA with three factors: **(a)** microwave intensity vs. sonication duration, **(b)** microwave duration vs. sonication duration, and **(c)** microwave duration vs. microwave intensity.

TABLE 6 Point prediction for the validation of RSM.

Response	95% confidence interval (CI)		95% prediction interval (PI)		95% tolerance interval (TI)		Validation
	Low	High	Low	High	Low	High	Experimental value
ChCl: LA	43.80	45.72	43.50	46.03	42.32	47.21	44.77

TABLE 7 Anthocyanin content, antioxidant properties and phenolic content of the extract obtained with solvent at optimum extraction conditions.

Solvent	Anthocyanin content (mg/g dry weight)	Antiradical activity (μmol AAE/g dry weight)	Reducing power (μmol AAE/g dry weight)	Total phenolic content (mg GAE/g dry weight)
Chcl: LA acid	44.77 ± 0.34[a]	39 ± 0.14[a]	40 ± 0.82[a]	2.82 ± 0.72[a]
Acidified ethanol	4 ± 0.02[b]	17.11 ± 0.16[b]	20.29 ± 0.87[b]	1.21 ± 0.17[b]

Values with different small case superscript letters (a–b) in the same column for each factor indicate significant differences as estimated by Tukey's test ($p < 0.05$).

dry weight, while the acidified ethanol method had a significantly lower AAR of 17.11 ± 0.16 μmol AAE/g dry weight (Table 7). This suggests that ChCl: LA is more effective at preserving or extracting compounds with free radical scavenging abilities, such as anthocyanin and other polyphenols. The enhanced AAR is attributed to the stronger hydrogen bonding and mild polarity of ChCl: LA, which helps stabilize antioxidant compounds. Similar observations were reported Zannou and Koca (2022) and Sportiello et al. (2025), where NADES extracts consistently exhibited higher antiradical activity compared to conventional organic solvents in phenolics-rich plant matrices.

3.5.2 Ferric reducing antioxidant power assay

The ferric reducing antioxidant power, which represents the electron-donating capacity of the extract and thus its antioxidant capacity, also showed a large variation between the two extraction methods. The extract obtained with ChCl: LA had an FRAP of 40 ± 0.82 μmol AAE/g dry weight, significantly more than acidified ethanol (20.29 ± 0.87 μmol AAE/g dry weight) shown in Table 7. This result reinforces the superior ability of ChCl: LA to extract and preserve antioxidant components, which contribute to reducing power. The increased values may be associated with a higher presence of anthocyanin and phenolic compounds in the NADES extract. Benvenutti et al. (2022) also reported similar findings, noting that NADES-based extractions often enhance reducing power due to better solubilization of redox-active compounds from plant matrices.

3.6 Total phenolic content

The total phenolic content is an important measure of the antioxidant capacity and biological potential of the extract. The total phenolic content of the ChCl: LA extract was 2.82 ± 0.72 mg GAE/g dry weight, more than double the value of the acidified ethanol extract (1.21 ± 0.17 mg GAE/g dry weight), as shown in Table 7. This shows that ChCl: LA-based NADES extracted more phenolic compounds from the mango peel powder. This is due to the increased number of hydroxyl groups in the components of NADES (lactic acid and choline chloride), improved hydrogen bonding, and better interaction with polyphenols and other compounds to increase their solubility. Similar enhancements in total phenolic content have been reported in several studies where hydrophilic NADES showed superior phenolic extraction efficiency from fruit waste compared to conventional methods (Zannou et al., 2020; Kantar et al., 2019).

3.7 Color analysis

The color properties of the anthocyanin extracts showed significant variation according to the solvent used. The extracts obtained using ChCl: LA exhibited higher color intensity (CI = 1.21 ± 0.23) compared to acidified ethanol (CI = 1.093 ± 0.10), as shown in Table 8. This suggests that ChCl: LA produced a purer anthocyanin extract, whereas ethanol likely extracted a broader spectrum of pigments. Likewise, the hue angle was lower for ChCl: LA (0.35 ± 0.42) than ethanol (1.39 ± 0.05), suggesting a stronger red pigment with ChCl: LA, since stable anthocyanin at acidic pH are typically found in the red color, flavylium cation. In terms of RGB composition of the extracts, the ChCl: LA extract exhibited the highest % red component at 72.89 ± 0.05%, which is substantially greater than the ethanol extract, which was 40.89 ± 0.19%, demonstrating the ability of ChCl: LA to stabilize the intact form of anthocyanin in the red form. Conversely, ethanol extraction resulted in a higher % yellow (56.99 ± 0.24%) than ChCl: LA (26.19 ± 0.13%), which may be attributed to degradation of some anthocyanin and or the co-extraction of some yellowish flavonoids or chalcones; thus, ChCl: LA extracts are more likely to contain pure forms of anthocyanin. The blue component was relatively low in both, though slightly higher in ethanol (2.10 ± 0.35%) than in ChCl: LA (1.40 ± 0.07%), which could relate to pH-driven structural changes.

Using the CIE Lab color space system, the colorimetric properties were examined and shown in Table 9. The ChCl: LA extracts and ethanol extracts had lower L* values, indicating a darker color due to higher pigment concentration. The a* values were positive and higher in ChCl: LA extracts (15.21 ± 0.04), confirming stronger red coloration associated with increased anthocyanin content. The values for chroma (C*) were also significantly higher ChCl: LA (16.74 ± 0.09) than conventional extracts (1.78 ± 0.17), reflecting the greater color saturation and intensity. The hue angle of both extracts was around 24–25°, which is the red-purple region, indicating that the typical anthocyanin color was maintained. The obtained results are similar to the findings reported in other ultrasound-assisted NADES extraction studies (Velásquez et al., 2021).

3.8 FTIR

The FTIR spectra of anthocyanin extracts obtained using ChCl: LA and acidified ethanol reveal the presence of characteristic functional groups associated with anthocyanin compounds (Figure 3). The broad absorption band at 3,297 cm^{-1} for Chl: LA and 3,341 cm^{-1} for acidified ethanol corresponds to O–H stretching vibrations of hydroxyl groups in phenolic structures. The lower wavenumber in the ChCl: LA extract indicates stronger hydrogen bonding due to the highly interactive nature of the NADES, which helps to stabilize anthocyanin during extraction. Both extracts contained a peak corresponding to C=C stretching vibrations (1,633–1,634 cm^{-1}) in the aromatic rings of the anthocyanin structure, reaffirming the intact flavonoid backbone. Also of note are peaks with wavenumbers at 1,132 cm^{-1} (ChCl: LA) and 1,178 cm^{-1} (acidified ethanol), which correspond to C–O stretching typically associated with glycosidic or ester linkages found in anthocyanin glycosides. The ChCl: LA spectrum features slightly shifted and more defined peaks, indicating greater structural integrity and less

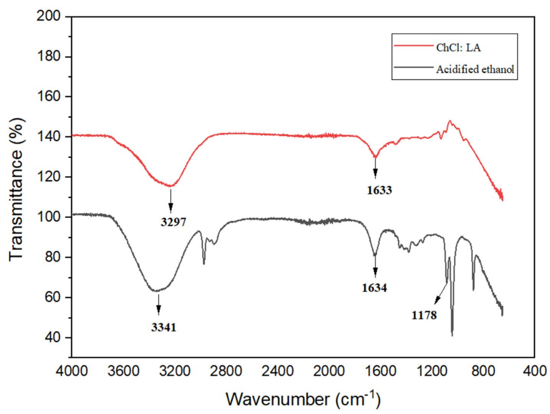


FIGURE 3
FTIR spectra of ChCl: LA and acidified ethanol.

TABLE 8 Color parameters of the extract measurement were obtained using a spectrophotometer.

Solvent	CI	Hue	% Yellow	% Red	% Blue
ChCl: LA	1.211 ± 0.23[a]	0.35 ± 0.42[a]	26.19 ± 0.13[a]	72.89 ± 0.05[a]	1.40 ± 0.07[a]
Acidified ethanol	1.093 ± 0.10[b]	1.39 ± 0.05[b]	54.99 ± 0.24[b]	40.89 ± 0.19[b]	2.10 ± 0.35[a]

Values with different small case superscript letters (a–b) in the same column for each factor indicate significant differences as estimated by Tukey's test ($p < 0.05$).

TABLE 9 Color parameters of the extract measurement were obtained using a colorimeter.

Solvent	L*	a*	b*	C*	h°
ChCl: LA	14.72 ± 0.06[a]	15.21 ± 0.04[a]	7.05 ± 0.12[a]	16.74 ± 0.09[a]	24.9 ± 0.3[a]
Acidified ethanol	0.65 ± 0.01[b]	1.63 ± 0.18[b]	0.72 ± 0.02[b]	1.78 ± 0.17[b]	23.9 ± 1.62[b]

Values with different small case superscript letters (a–b) in the same column for each factor indicate significant differences as estimated by Tukey's test ($p < 0.05$).

TABLE 10 Stability of extracted anthocyanin.

Day	Degradation %							
	Acidified ethanol				ChCl: LA			
	37 °C	4 °C	−18 °C	−40 °C	37 °C	4 °C	−18 °C	−40 °C
3	75.03 ± 0.78^{aA}	8.2 ± 0.12^{aB}	3.6 ± 0.04^{aC}	1.5 ± 0.01^{aD}	12.3 ± 0.14^{aE}	4.3 ± 0.07^{aF}	1.22 ± 0.05^{aG}	0.5 ± 0.02^{aH}
6	86.06 ± 0.98^{bA}	20.91 ± 0.26^{bB}	9.8 ± 0.11^{bC}	3.7 ± 0.02^{bD}	27.08 ± 0.23^{bE}	8.62 ± 0.17^{bF}	2.04 ± 0.02^{bG}	0.8 ± 0.01^{bH}
10	92 ± 0.85^{cA}	35.23 ± 0.44^{cB}	14.11 ± 0.27^{cC}	7.98 ± 0.1^{cD}	45 ± 0.67^{cE}	14.23 ± 0.43^{cC}	4.06 ± 0.01^{cF}	1.5 ± 0.03^{cG}
13	94.34 ± 0.92^{dA}	42.07 ± 0.52^{dB}	18.23 ± 0.31^{dC}	12 ± 0.39^{dD}	62.33 ± 0.83^{dE}	18 ± 0.23^{dC}	7.1 ± 0.06^{dF}	2.25 ± 0.06^{dG}
15	98.33 ± 1.2^{eA}	45 ± 0.56^{eB}	20.66 ± 0.18^{eC}	14.22 ± 0.65^{eD}	67 ± 0.69^{eE}	22.66 ± 0.45^{eF}	9 ± 0.13^{eG}	3 ± 0.02^{eH}
18	99.99 ± 1.56^{fA}	50.09 ± 0.62^{fB}	25.21 ± 0.38^{fC}	16.65 ± 0.43^{fD}	72.21 ± 0.85^{fE}	26.12 ± 0.28^{fF}	11.09 ± 0.17^{fG}	3.56 ± 0.07^{fH}
H30	100^{fA}	78.56 ± 0.75^{gB}	32 ± 0.41^{gC}	20.88 ± 0.51^{gD}	82.76 ± 0.92^{gE}	38.87 ± 0.32^{gF}	15.11 ± 0.12^{gG}	5.06 ± 0.05^{gH}

Values with different lowercase superscript letters (a–g) within the same column for each factor indicate significant differences according to Tukey's test ($p < 0.05$), while values in the same row with different uppercase letters (A–H) are significantly different (Tukey's test, $p < 0.05$).

degradation likely from the mild and stabilizing properties of the NADES medium. These findings are consistent with the studies of Abdelrahman et al., (2024) and Yaneva et al. (2025), where they also verified the presence of anthocyanin using FTIR with characteristic O–H, C=C, and C–O stretching vibrations. Abdelrahman et al., (2024) and Yaneva et al. (2025), also highlight that NADES, specifically choline chloride and organic acids, provide stabilizing conditions to maintain the integrity of anthocyanin and minimize degradation during the extraction procedure. In agreement with their findings, the obtained FTIR results confirm the presence of anthocyanin, with prominent spectral peaks, while also indicating that ChCl: LA can better maintain the integrity of anthocyanin compared to acidified ethanol (Abdelrahman et al., 2024; Yaneva et al., 2025). The sharper, more defined bands in the ChCl: LA spectrum, along with the slight shifts in wavenumber, suggest enhanced hydrogen bonding and reduced pigment degradation. Thus, ChCl: LA emerges as a more effective and protective solvent system for anthocyanin extraction from mango peel, affirming its potential as a green alternative to conventional solvents.

3.9 Stability of extracted anthocyanin

The storage stability of anthocyanin extracted using ChCl: LA and acidified ethanol was studied over different temperatures (4, 25, −18, and −40 °C) for 30 days (Table 10). The data demonstrate that acidified ethanol resulted in almost complete degradation of anthocyanin under all conditions after 30 days of storage. For instance, acidified ethanol extract stored at 37 °C for 30 days resulted in complete (100%) degradation at Day 30, while 20.88 ± 0.51% degradation was observed at Day 30 and −40 °C. In comparison, anthocyanin extracted by ChCl: LA had significantly better long-term storage stability, especially under freezing conditions at −40 °C (degradation of 5.06 ± 0.05% at day 30), while the degradation was 15.11 ± 0.12% after day 30 at −18 °C. Anthocyanin showed less degradation when stored in ChCl: LA. Compared to acidified ethanol, ChCl: LA provided better long-term stability due to its strong hydrogen bonding and low tendency to oxidize. ChCl: LA provides a safe and stable environment for preserving anthocyanin over time. It is a green solvent that causes less damage and is more effective than acidified ethanol. Fu et al. (2021) reported that choline chloride: oxalic acid exhibited lower degradation of anthocyanin compared to acidified ethanol.

4 Conclusion

The present study confirms the potential of hydrophilic NADES, particularly choline chloride: lactic acid (1:2), as an efficient and sustainable solvent system for anthocyanin extraction from mango peel waste. This integrated green extraction approach significantly improved the yield compared to conventional methods. Through process optimization using a Box–Behnken design, the optimal conditions were identified as a sonication duration of 30 min, microwave intensity of 45.165 W/g, and microwave duration of 20 s. Under these parameters, the maximum anthocyanin content obtained was 44.77 mg/g dry weight, indicating the high efficiency of the method. This study not only supports the potential of NADES as sustainable and non-toxic alternatives to conventional solvents but also demonstrates the synergistic effect of sonication and microwave techniques in enhancing mass transfer and reducing extraction time. Furthermore, the valorization of mango peel, a major agro-industrial by-product, into a rich source of bioactive compounds aligns with circular economy principles and adds value to food processing waste. These findings open new opportunities for the application of anthocyanin-rich extracts in the development of natural colorants, functional foods, nutraceuticals, and cosmetic formulations.

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

AK: Methodology, Software, Writing – original draft, Formal analysis, Resources, Data curation. JG: Supervision, Formal analysis, Data curation, Software, Investigation, Writing – review & editing, Resources, Methodology, Project administration, Funding acquisition, Conceptualization, Validation.

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Conflict of interest

The author(s) declared that this work 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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