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RECEIVED 10 November 2025
REVISED 29 January 2026
ACCEPTED 02 February 2026
PUBLISHED 24 March 2026

CITATION
Cherif W, Ktari L, Ismail A and El Bour M
(2026) Fatty acid profile, nutritional
potential and biological activity of green
macroalgae from the Northern Tunisian
coast.
Front. Mar. Sci. 13:1743547.
doi: 10.3389/fmars.2026.1743547

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Fatty acid profile, nutritional potential and biological activity of green macroalgae from the Northern Tunisian coast

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Introduction: Marine macroalgae possess very complex and diverse lipid composition. Fatty acids from marine macroalgae are a sustainable and valuable source of essential nutrients with significant health benefits, including cardiovascular protection, neuroprotection, anti-inflammatory properties, and wide applications in the nutraceutical and pharmacological industries.

Methods: To evaluate the fatty acids composition of macroalgae, from Ulvophyceae class, collected from northern Tunisian coast Cap Zebib (CZ), Rafraf (R) Ghar el Melh lagoon (G), Carthage bay (C), La Marsa (M), Tunis North Lake (L), ten species were studied (*Bryopsis muscosa* (R), *Caulerpa prolifera* (L), *Caulerpa racemosa* (R), *Cladophora laetivirens* (CZ, R), *Codium fragile* (M), *Codium tomentosum* (R), *Dasycladus vermicularis* (CZ), *Halimeda tuna* (M), *Ulva rigida* (G) and *Ulva linza* (C)). Fatty acid contents and specific profiles were determined by gas-chromatography. To assess the nutritional quality and potential health benefits of the studied macroalgae, the unsaturation index atherogenic and thrombogenic index ((U.I.), AI and TI)) were calculated. Additionally, the antimicrobial and cytotoxic activities of crude organic algal extracts against pathogenic bacteria and two microalgal species were evaluated.

Results: As results, total fatty acid contents, expressed per percentage of dry weight, ranged from 0.04% to 0.70%. Generally, saturated fatty acids (SFA) were major components percentage of total FA content 75.02%, with palmitic acid (C16:0) being the most abundant and *Codium tomentosum* containing the highest amount. *D. vermicularis* showed the better profile in terms of FA composition with a low AI (1.75) and a very low TI (0.4), making it an excellent source of healthy lipids. *Ulva linza* and *Bryopsis muscosa* exhibited the most important spectrum of antibacterial activity, inhibiting the growth of multiple strains as *P. aeruginosa* and *E. faecalis*.

Conclusion: This study provides a comprehensive overview of the lipid profiles of some green macroalgae and their potential applications in food and drug industries, with a particular emphasis on their health implications and antimicrobial properties.

KEYWORDS

atherogenic index, fatty acids, green macroalgae, Mediterranean, thrombogenic index

1 Introduction

Seaweeds are increasingly recognized as “macroalgae of the millennium” for their ecological, social and economic value, as well as their nutritional potential (Pereira et al., 2012). Owing to their minerals, fiber, polysaccharides, vitamins and diverse bioactive and despite generally low total lipid content many taxa provide meaningful amounts of essential polyunsaturated fatty acids (PUFAs) (Pereira et al., 2012; Rocha et al., 2021). Green macroalgae (Ulvophyceae) contribute significantly to primary production and nutrient cycling and include species with distinctive n-3/n-6 profiles relevant to human health (Rocha et al., 2021; Khan et al., 2024). Emerging analytical work and reviews indicate that seaweed-derived lipids (including ALA (α -linolenic acid) and LA (linoleic acid) and in some cases long-chain n-3 PUFA precursors) may help modulate inflammation and cardiometabolic risk, supporting applications in functional foods and nutraceuticals (Jaworowska and Murtaza, 2022; Matos et al., 2024; Montone et al., 2024).

Lipids in macroalgae have gained interest, particularly due to their fatty acid composition. Polyunsaturated fatty acids (PUFAs) are vital for human metabolism, yet they cannot be synthesized by the body and must be obtained through diet. The predominant PUFAs identified in macroalgae are C18 and C20 fatty acids, including linoleic, arachidonic, and eicosapentaenoic acids (Pereira et al., 2012). In Chlorophyta, PUFAs account for 17–61% of total lipids, with α -linolenic acid being the most prevalent fatty acid (Pereira et al., 2012; Schmid et al., 2018).

Fatty acids are increasingly used as chemotaxonomic markers to differentiate between algal species and groups. The specific profiles of these biomolecules offer insights into the metabolic pathways and environmental adaptability of algae. This approach has been valuable in the classification of green algae and their potential use in bioassays (Khotimchenko et al., 2002).

Green macroalgae, found abundantly along the northern Tunisian coast, contribute to the carbon and nitrogen cycles, influencing the overall health of marine habitats. Environmental conditions of the Tunisian coast, coupled with the seasonal variability, further affect the fatty acid profiles of these algae, making them ideal candidates for studies focusing on their biochemical properties and potential industrial applications in food, pharmaceuticals, and biofuels.

This study aims to explore the fatty acid composition of green seaweeds collected from the northern Tunisian coast, shedding light on their ecological significance and potential applications.

2 Materials and methods

2.1 Sampling method and identification

Macrophytes were collected in northern Tunisian coasts mainly in February through July (Figure 1). All specimens were collected by hand in shallow water (<2m). Collected samples were transported freshly in an icebox. In the laboratory, the algal material was

thoroughly cleaned from other algae, small invertebrates, and solids and was washed in freshwater 3 times before analysis and then was kept in 2% formaldehyde-seawater. Fixed materials were identified using a binocular microscope (Alphaphot-2.YS2-H; Nikon, Tokyo, Japan). The identification was based on different taxonomical keys (Hamel, 1926; Silva, 1955; Burrows, 1991; de Reviere, 2002) Table 1.

2.2 Extraction procedure

Dried macroalgae samples (20g) were subjected to successive extraction using solvents of increasing polarity, including dichloromethane (D:100), methanol (M:100), for all species. Some species were further extracted with a methanol-water mixture (MW: 50/50). Each extraction was carried out twice for 24 hours at room temperature (25 °C) under light-protected conditions. After filtration, organic solvent extracts were concentrated under reduced pressure using a rotary evaporator (Büchi).

2.3 Analysis of methyl esters of fatty acids by gas chromatography

The methylated fatty acids are analyzed by gas chromatography with a chromatograph mark HP model 19091N-133 with a polar column INNOWAX (30 m length, of 25 μ m of diameter; thickness of the film is 0.25 μ m) brand Agilent technology. The furnace temperature was developed programming 150 to 240 °C with a gradient of 2 °C/min. The injector temperature is 220 °C, that of the sensor is 275 °C, flow rate 1ml/min; Injection volume 1 μ l. The chromatogram peaks are identified by comparison with the retention time of the peaks of standard (SUPELCO), injected in the same conditions.

The unsaturation index (U.I.) was calculated by multiplying the percentage of each fatty acid by the number of double bonds followed by summing up their contributions (Poerschmann et al., 2004). Atherogenic and thrombogenic indices (AI and TI) were calculated according to De Lorenzo et al. (2001).

where:

The Atherogenic Index (AI) is an indicator used to assess the cardiovascular risk associated with the fatty acid composition of a lipid sample:

$$AI = \frac{(C12:0 + 4 \times C14:0 + C16:0)}{MUFA + PUFA}$$

C12:0 = Lauric acid

C14:0 = Myristic acid

C16:0 = Palmitic acid

MUFA = Monounsaturated fatty acids

PUFA = Polyunsaturated fatty acids

The Thrombogenic Index (TI) is an indicator of the potential risk of thrombosis, assessing the balance between pro-thrombogenic (saturated fatty acids) and anti-thrombogenic (unsaturated fatty acids) components in a lipid sample:

$$TI = \frac{C14:0 + C16:0 + C18:0}{(0.5 \times \sum MUFA) + (0.5 \times \sum PUFA_{n-6}) + (3 \times \sum PUFA_{n-3}) + (0.5 \times \sum PUFA_{total})}$$

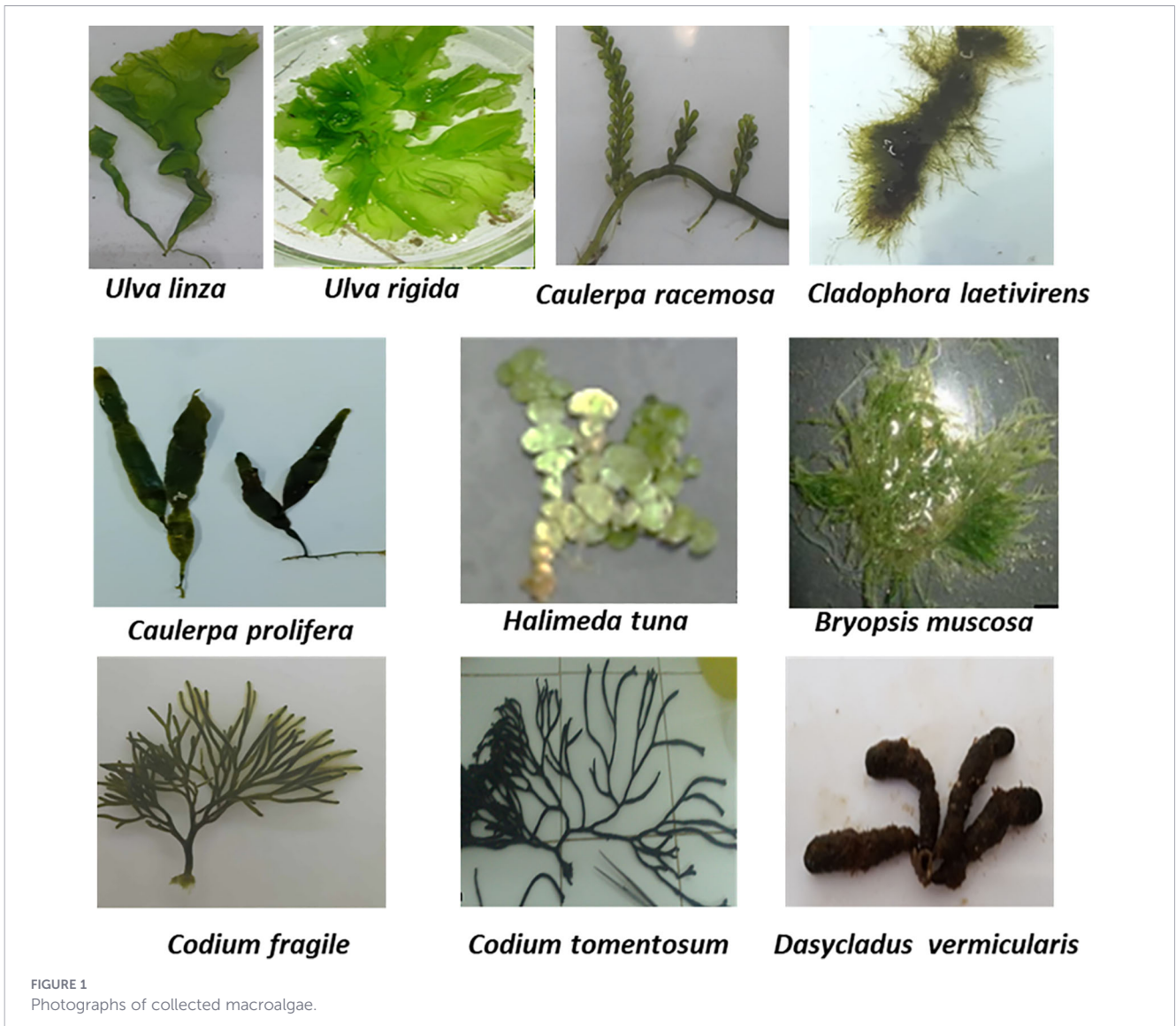


FIGURE 1
Photographs of collected macroalgae.

C14:0, C16:0, C18:0 = Saturated fatty acids involved in thrombosis.
ΣMUFA = Sum of monounsaturated fatty acids.

ΣPUFA n-6 = Sum of omega-6 polyunsaturated fatty acids.
ΣPUFA n-3 = Sum of omega-3 polyunsaturated fatty acids.
ΣPUFA total = Sum of all PUFA

TABLE 1 Macroalgae taxa and sampling sites.

Ordre	Family	Genre	Species	Geographic coordinates		Sampling site
Cladophorale	Cladophoraceae	Cladophora	laetivirens Z	37°16'3.18"N	10° 3'52.35"E	Cap zbib
			laetivirens R	37°11'50.11"N	10°12'30.47"E	Rafraf
Ulvale	Ulvaceae	Ulva	rigida	37° 9'59.75"N	10°10'9.56"E	Ghar el Melh lagoon
			linza	36°51'5.04"N	10°19'56.66"E	Carthage bay
Bryopsidale	Halimedaceae	Halimeda	tuna	36°52'56.06"N	10°20'29.24"E	La Marsa
	Bryopsidaceae	Bryopsis	muscosa	37°11'52.09"N	10°12'22.55"E	Rafraf
	Caulerpaceae	Caulerpa	racemosa	37°11'51.25"N	10°12'26.75"E	Rafraf
			prolifera	36°48'10.39"N	10°13'17.90"E	Lake nord of Tunis
	Codiaceae	Codium	fragile subsp. fragile	36°53'03.97"N	10°20'14.22"E	La Marsa
			tomentosum	37°11'46.65"N	10°12'31.80"E	Rafraf
Dasycladale	Dasycladaceae	Dasycladus	vermicularis	37°15'50.55"N	10° 4'8.97"E	Cap zbib

2.4 Antibacterial tests

The antibacterial assay of algal crude extracts was carried out against seven pathogenic bacteria: *Micrococcus* sp. (Pasteur Institute, Tunis), *Aeromonas salmonicida* LMG3780, *A. hydrophila* B3 (RVAU-Denmark), *Enterococcus faecalis* ATCC29212, *Escherichia coli* O126-B16 (ATCC 14948), *P. aeruginosa* ATCC 27853, *V. alginolyticus* ATCC 17749T. Antibacterial tests were evaluated by disc diffusion method (Casida, 1986). A total of 1 mg of algal crude extract dissolved in 10 µL of extraction solvent was loaded onto sterile filter paper discs (6 mm diameter). After solvent evaporation, the discs were placed on TSA plates, inoculated with a cultured pathogenic strain. As control, a disc loaded with solvent was simultaneously prepared. Plates were incubated overnight at 30 °C. The diameter of growth inhibition halo was measured after 24 h incubation.

2.5 Cytotoxicity tests

Cytotoxic activity was evaluated against two species of microalgae: *Tetraselmis suecica* and *Navicula* sp. The tests were carried out according to the method described by Hedio et al. (2002) with some modifications. Sterile flasks containing 5 ml of sterile sea water at a salinity of 30 psu and enriched by Conway's medium (Walne, 1966) are seeded with an inoculum of a microalgae culture concentration of 5.10^5 cells/ml. The crude extracts were added to a concentration of 200 µg/ml dissolved in 20 µl of Dimethylsulfoxide (DMSO). Flasks were incubated at 20 °C, a luminous intensity of 3000 Lux and with, a photoperiod of (12/12). In all assays cupric sulphate (CuSO_4) was also studied, they were considered as positive controls. A standard, containing no biocides and no algal extract, was also set up. Cell growth was estimated after 5 days, by direct counting of the cells in a Malassez hematocytometer. All Tests were carried out in triplicate.

The percentage of inhibition was calculated on the fifth day using the following formula:

$$\% \text{ inhibition} = \frac{N_{\text{DMSO}} - N_{\text{alg}}}{N} \times 100$$

where:

- N = number of microalgae in the control
- N_{DMSO} = number of microalgae in the DMSO control
- N_{alg} = number of microalgae treated with the extract dissolved in DMSO

2.6 Statistical analysis

Principal component analysis (PCA) was conducted to assess correlations among variables and to visualize sample clustering. PCA was applied to fatty acid composition data and nutritional indices, including saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), index of atherogenicity (IA), index of thrombogenicity (IT), index of unsaturation (IU).

Data were analyzed using one-way analysis of variance (ANOVA), followed by Duncan's multiple range *post hoc* test to identify significant differences at a 95% confidence level ($p < 0.05$).

Significant differences were indicated by different letters in the graphs.

All statistical analyses, including analysis of variance (ANOVA) and PCA were performed using XLSTAT software (version 2025.1). Graphical representations were generated to facilitate the interpretation of differences and clustering patterns.

3 Results

3.1 Fatty acids analysis

Fatty acid analysis was conducted to establish the lipid profile of the apolar algal extracts by distinguishing the relative proportions of SFA, MUFA, and PUFA. This characterization allows the identification of essential fatty acids, which are well recognized for their nutritional roles and beneficial health effects.

A high variability in fatty acid composition was observed among the studied algal species, as summarized in Table 2. SFAs predominated in *C. tomentosum* (75.03%), followed by *C. laetivirens* (50.83%) and *C. racemosa* (48.61%). In contrast, SFAs were least abundant in *D. vermicularis* (17.13%) and *C. prolifera* (18.54%). Regarding MUFAs, *C. laetivirens* exhibited the highest content (34.18%), followed by *D. vermicularis* (30.11%), whereas *C. tomentosum* showed the lowest value (7.36%). PUFA levels reached 26.65% in *C. fragile* and 27.55% in *D. vermicularis*, while they were as low as 2.04% in *C. tomentosum*. Palmitic acid (C16:0) was the most abundant fatty acid in most extracts, followed by oleic acid (C18:1 ω9) and myristic acid (C14:0). Fatty acid profiling also revealed species-specific differences. Some species clearly stood out, particularly *D. vermicularis*, which exhibited a globally favorable lipid profile, characterized by a PUFA content of 27.55%, a high linoleic acid level (16.50%), and a low thrombogenic index (IT = 0.40). Similarly, *H. tuna* showed a balanced lipid profile (PUFA = 18.12%, IT = 0.80), with notable contributions of n-3 (ALA = 3.61%) and n-6 (LA = 7.53%) fatty acids. These two species appear to be promising candidates for nutritional valorization, especially as natural sources of essential fatty acids. *U. linza* was distinguished by a high PUFA content (23.12%), dominated by linoleic acid (LA = 15.99%), and a PUFA/SFA ratio of 1, indicating a good nutritional balance. It also exhibited a very low IT value (0.59), reflecting excellent cardiovascular potential. In contrast, *U. rigida* displayed an unfavorable lipid profile, with a high SFA level (43.54%), very low PUFA content (1.21%), and an almost null PUFA/SFA ratio, indicating poor nutritional quality. Among *Caulerpa* species, *C. prolifera* showed a nutritionally interesting profile, with a PUFA content of 12.59%, appreciable levels of n-6 (LA = 2.44%) and n-3 (ALA = 5.79%), and a low IT value (0.69). Conversely, *C. racemosa* was dominated by SFAs (48.61%), with a low PUFA level (4.93%) and a high IT (3.76). *C. tomentosum* exhibited the highest SFA proportion (75.03%) and the highest IT value (7.43), indicating a highly unfavorable dietary profile.

Species belonging to the genus *Codium* displayed contrasting lipid profiles. *C. tomentosum* was characterized by a very high SFA content (75.03%), with a predominance of palmitic acid (C16:0 =

TABLE 2 Fatty acids composition of apolar extract of green algae collected from Tunisian northern coast.

Family Species	Ulva		Cladophorale				Bryopsidale					Dasycladale	
	<i>U. rigida</i>	<i>U. linza</i>	<i>C. laetivirens</i> R	<i>C. laetivirens</i> Z	<i>C. racemosa</i>	<i>C. prolifera</i> S	<i>C. prolifera</i> F	<i>B. muscosa</i>	<i>C. tomentosum</i>	<i>H. tuna</i>	<i>C. fragile</i>	<i>C. fragile</i>	<i>D. vermicularis</i>
C14:0	3.233	5.601	9.674	2.557	4.908	3.763	2.228	3.658	2.255	5.061	3.448	3.177	1.511
C15:0	1.565	0.669	0.727	0.678	0.462	0	0	–	2.19	–	0.881	1.455	0.562
C16:0	33.164	15.895	15.788	44.3	41.051	31.102	16.313	29.444	67.421	15.288	42.228	38.594	12.88
C18:0	5.575	1.855	1.133	3.295	2.187	0.933	0	3.314	3.159	1.492	4.108	5.09	2.181
SFA	43.537	24.02	27.322	50.83	48.608	35.798	18.541	36.416	75.025	21.841	50.665	48.316	17.134
C16:1 w7	5.883	8.786	6.953	8.112	4.769	12.672	2.647	13.5	–	8.934	–	–	5.39
C18:1 w9	7.786	6.707	10.867	4.729	1.763	1.257	8.097	2.78	–	2.433	1.506	–	9.069
C18:1 w7	3.591	13.097	15.295	3.557	3.504	4.529	5.012	3.033	2.135	5.591	1.282	–	13.447
C20:1 w9	0	0	1.062	0	0	0	0	0	5.229	0.434	3.49	3.729	2.207
MUFA	17.26	28.59	34.177	16.398	10.036	18.458	15.756	19.313	7.364	17.392	6.278	3.729	30.113
C16:2 w4	0	2.121	1.825	0	0	0.646	1.217	0	0	0	0	0	0.75
C18:2 w6 (LA)	1.208	15.99	2.872	0.966	1.477	9.324	2.441	2.055	0	7.529	0	0	16.496
C16:3 w4	0	0	0	0.655	1.008	3.913	3.144	0	0	6.279	0	0	2.702
C18:3 w4	0	0	0	0	0	0	0	0	0	0	1.475	0	0
C18:3 w3 (ALA)	0	0	3.368	1.35	1.902	5.293	5.788	2.346	0.401	3.607	0	0	0
C18:4 w3 (SDA)	0	0	0	0	0	0.557	0	0	1.638	0.708	1.389	0	1.647
C20:4 w6	0	3.037	0.685	1.092	0.548	0.832	0	0	0	0	1.186	0	4.034
C20:4 w3	0	0	0	0	0	0	0	0	0	0	0.367	0	0
C20:5 w3 (EPA)	0	1.971	1.496	0	0	2.118	0	0	0	0	0.441	0	0
C22:5 w3	0	0	0	0	0	0	0	1.215	0	0	0	0	1.924
C22:6 w3 (DHA)	0	0	0	0	0	0	0	0	0	0	0	0	0
PUFA	1.208	23.119	10.246	4.063	4.935	22.683	12.59	5.616	2.039	18.123	4.858	0	27.553
Total lipids	62.005	75.729	71.745	71.291	63.579	76.939	46.887	61.345	84.428	57.356	61.801	52.045	74.8

(Continued)

TABLE 2 Continued

Family	Ulvale		Cladophorale		Bryopsidale			Dasycladale				
	<i>U. rigida</i>	<i>U. linza</i>	<i>C. laetivirens</i> <i>R</i>	<i>C. laetivirens</i> <i>Z</i>	<i>C. racemosa</i>	<i>C. prolifera</i> <i>S</i>	<i>C. prolifera</i> <i>F</i>	<i>B. muscosa</i>	<i>H. tuna</i>	<i>C. fragile</i>	<i>C. fragile</i>	<i>D. vermicularis</i>
PUFA/SFA	0	1	0	0	0	1	1	0	1	0.1	0	2
n-6 PUFA	1.208	19.027	3.557	2.058	2.025	10.156	2.441	2.055	7.529	1.186	0	20.53
n-3 PUFA	0	1.971	4.864	1.35	1.902	7.968	5.788	3.561	4.315	2.197	0	3.571
n-6/n-3	0.0	9.7	0.7	1.5	1.1	1.3	0.4	0.6	1.7	0.5	0	5.7
AI	5.03	5.92	10.04	4.79	7.85	4.61	2.91	4.84	5.58	7.82	13.53	1.75
UI	63.213	110.835	91.217	79.543	72.52	117.96	68.409	72.952	86.781	75.341	52.045	122.189
TI	4.55	0.59	0.78	3.39	3.76	0.91	0.69	1.66	0.80	4.58	25.13	0.40

EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; LA, Linoleic acid; ALA, Alpha-linolenic acid; SDA, Stearidonic acid. SFAs: Saturated fatty acids, MUFAs: Monounsaturated fatty acids, PUFAs: Polyunsaturated fatty acids, U.I.: Unsaturation index, A.I: Atherogenic index, TI: Thrombogenic index.

67.42%), low PUFA content (2.04%), absence of DHA and EPA, and elevated nutritional indices (IA = 9.43; IT = 7.43), reflecting a lipid profile of limited nutritional interest. In contrast, *C. fragile* exhibited a favorable lipid profile, with moderate SFA levels (25.48%), a PUFA content of 26.65% dominated by DHA (3.61%) and omega-3 fatty acids such as ALA (9.36%). *C. fragile* also showed favorable nutritional indices (IA = 2.65; IT = 0.47). This marked contrast highlights the significant interspecific variability within the genus *Codium*.

The principal component analysis (PCA) indicates that the first axis (F1, 69.79% of the total variance) clearly separates algal species according to fatty-acid profile (Figure 2). Species positioned on the positive side of F1, such as *U. linza*, *D. vermicularis*, and *C. fragile*, are strongly associated with PUFA, the unsaturation index (UI), AGPI, and the PUFA/SFA ratio, reflecting lipid profiles rich in unsaturated fatty acids and nutritionally favorable characteristics. In contrast, *C. tomentosum*, located at the extreme negative side of F1, is closely associated with saturated fatty acids (SFA) and with the atherogenic (AI) and thrombogenic (TI) indices, indicating a more saturated and less favorable lipid composition. The close orientation of PUFA and PUFA/SFA vectors confirms their strong positive correlation, whereas SFA is negatively correlated with PUFA-related variables, highlighting the classical opposition between saturated and unsaturated fatty acids. Species located near the center or in the lower left quadrant, such as *C. racemosa*, *U. rigida*, and *B. muscosa*, exhibit intermediate lipid profiles without a strong dominance of either saturated or polyunsaturated fatty acids.

3.2 Antimicrobial activity

All results are presented in Table 3. Overall, the tested macroalgal extracts exhibited low antimicrobial activity, with limited inhibition observed against the majority of the bacterial strains. *C. fragile* showed detectable antimicrobial activity, producing moderate inhibition diameters against *P. aeruginosa*, *E. faecalis*, *V. alginolyticus*, *A. hydrophila*, and *E. coli*.

Among the species belonging to the order Ulvales, three out of the seven tested bacterial strains were inhibited. Specifically, *C. laetivirens* inhibited *E. faecalis* and *V. alginolyticus*, while no inhibitory effect was observed against *P. aeruginosa*, *A. hydrophila*, or *E. coli*. In contrast, *H. tuna* and *C. racemosa* were the least active species, as their extracts showed no antimicrobial activity against any of the tested bacteria.

B. muscosa exhibited activity against four out of the seven bacterial strains, with notable inhibition of *Micrococcus* sp., *P. aeruginosa*, *E. faecalis*, and *V. alginolyticus*, indicating a certain antimicrobial potential. The two Ulvales species, *U. rigida* and *U. linza*, showed similar inhibition profiles when extracted with dichloromethane. Overall, *Micrococcus* sp. and *A. hydrophila* were inhibited by only a few extracts, whereas *E. coli* was resistant to most of the tested extracts, except that of *C. fragile*.

3.3 Cytotoxic activity

The algal extracts were evaluated for their cytotoxic activity against a microalga (*T. suecica*) and a diatom (*Navicula* sp.). The percentages of growth inhibition of *Navicula* sp. and *T. suecica* are

TABLE 3 Antimicrobial activity of algal extracts.

Species	Mic	P.a	E.f	V.a	E.ci	A.h	A.s
<i>C. laetivirens</i> (R) D	-	-	-	+	-	-	-
<i>C. laetivirens</i> (R) M	-	-	-	-	-	-	-
<i>C. laetivirens</i> (Z) D	-	-	+	+	-	-	-
<i>U. rigida</i> D	-	+	+	+	-	-	-
<i>U. linza</i> D	-	+	+	+	-	-	-
<i>U. linza</i> M/E	-	-	-	-	-	-	-
<i>U. linza</i> E	-	-	-	-	-	-	-
<i>H. tuna</i> D	-	-	-	-	-	-	-
<i>H. tuna</i> M	-	-	-	-	-	-	-
<i>H. tuna</i> M/E	-	-	-	-	-	-	-
<i>H. tuna</i> E	-	-	-	-	-	-	-
<i>B. muscosa</i> D	+	+	+	+	-	-	-
<i>B. muscosa</i> M/E	-	-	-	-	-	-	-
<i>C. prolifera</i> D	-	-	-	-	-	-	-
<i>C. prolifera</i> M	-	-	-	-	-	-	-
<i>C. racemosa</i> D	-	-	-	-	+	-	-
<i>C. racemosa</i> M	-	-	-	+	-	-	-
<i>C. racemosa</i> M	-	-	-	-	-	-	-
<i>C. fragile</i> D	-	+	+	-	+	+	+
<i>C. fragile</i> M	-	-	-	-	-	-	-
<i>C. fragile</i> E	-	-	-	-	-	-	-
<i>C. tomentosum</i> D	-	-	-	-	-	-	-
<i>C. tomentosum</i> M	-	-	-	-	-	-	-
<i>D. vermicularis</i> D	-	-	-	-	-	-	-

(-) No inhibition; (+) Weak inhibition (6–10 mm); (+) Moderate inhibition (10.5–15 mm); (++) Strong inhibition (>15 mm); (D): dichloromethane extract; (M): methanol extract). *Ulva rigida*: *U. rigida*, *Ulva linza*: *U. linza*, *Cladophora laetivirens*: *C. laetivirens*, *Halimeda tuna*: *H. tuna*, *Caulerpa racemosa*: *C. racemosa*, *Caulerpa prolifera*: *C. prolifera*, *Bryopsis muscosa*: *B. muscosa*, *Codium tomentosum*: *C. tomentosum* *Codium fragile*: *C. fragile*, *Dasycladius vermicularis*: *D. vermicularis*; *Mic*: *Micrococcus* sp., *P.a*: *Pseudomonas aeruginosa*; *E.f*: *Enterococcus faecalis*; *V.a*: *Vibrio alginoliticus*; *E.c.*: *E. coli*; *A.h*: *A. hydrophila*; *A.s*: *Aeromonas salmonicida*.

presented in Figures 3, 4, respectively. These values ranged from 5% to 78%, with particularly high activity observed for non-polar extracts. The most pronounced inhibitory effects were mainly recorded for extracts of *C. fragile* and *C. tomentosum*, as well as for certain extracts of *C. racemosa* and *H. tuna*. Overall, *Navicula* sp. proved to be more sensitive than *T. suecica* to the majority of the extracts tested.

Two samples of *C. laetivirens* were analyzed. The non-polar (dichloromethane) extract exhibited inhibition rates of 49% and 61% against *Navicula* sp., whereas the more polar extract M/W showed low activity, with inhibition values below 20% against both microalgae tested. The dichloromethane extract of *B. muscosa* induced a 54% inhibition of *Navicula* sp. growth. In contrast, the M/W extracts of the same species exhibited lower inhibitory effects: 16% and 30% against *Navicula* sp. and 5% against *T. suecica*.

For *H. tuna*, the methanolic extract showed low activity against *Navicula* sp. 34% but a more pronounced inhibition against *T. suecica* (73%).

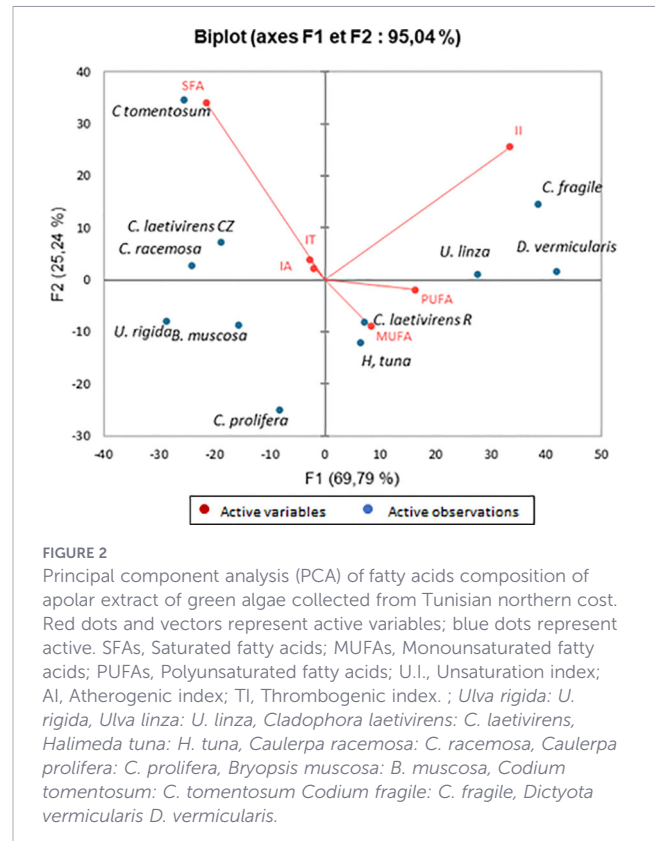


FIGURE 2 Principal component analysis (PCA) of fatty acids composition of apolar extract of green algae collected from Tunisian northern coast. Red dots and vectors represent active variables; blue dots represent active. SFAs, Saturated fatty acids; MUFAs, Monounsaturated fatty acids; PUFAs, Polyunsaturated fatty acids; U.I., Unsaturated index; AI, Atherogenic index; TI, Thrombogenic index.; *Ulva rigida*: *U. rigida*, *Ulva linza*: *U. linza*, *Cladophora laetivirens*: *C. laetivirens*, *Halimeda tuna*: *H. tuna*, *Caulerpa racemosa*: *C. racemosa*, *Caulerpa prolifera*: *C. prolifera*, *Bryopsis muscosa*: *B. muscosa*, *Codium tomentosum*: *C. tomentosum* *Codium fragile*: *C. fragile*, *Dasycladius vermicularis* *D. vermicularis*.

Species of the genus *Ulva* displayed moderate activity for dichloromethane extracts, with inhibition rates of 57% for *U. rigida* and 59% for *U. linza* against *Navicula* sp. Regarding *Caulerpa* species, extracts of *C. prolifera* exhibited variable inhibitory activity against *T. suecica*, ranging from 31% to 66%, depending on the extraction solvent used. Non-polar extracts of *C. racemosa* showed inhibition levels of approximately 44% against *T. suecica* and 74% against *Navicula* sp., whereas methanolic extracts of the same species exhibited low activity against both microalgae (<30%).

Extracts from the two *Codiales* species, *C. fragile* and *C. tomentosum*, demonstrated strong inhibitory activity against *Navicula* sp. Methanolic extracts achieved inhibition rates of up to 78%, while dichloromethane extracts resulted in inhibition levels exceeding 50%. *T. suecica* was less sensitive to *C. fragile* extracts, with inhibition values remaining below 52%.

4 Discussion

The chemical characterization of the extracts, particularly the determination of fatty acid profiles in apolar extracts, was performed. In our study, the fatty acid composition of the analyzed algae reflects their taxonomic diversity as well as their adaptation to environmental conditions. A general dominance of SFAs was observed, while PUFAs showed marked variations depending on species and collection site. These findings are consistent with previous studies (Schmid et al., 2018; Rocha et al., 2021).

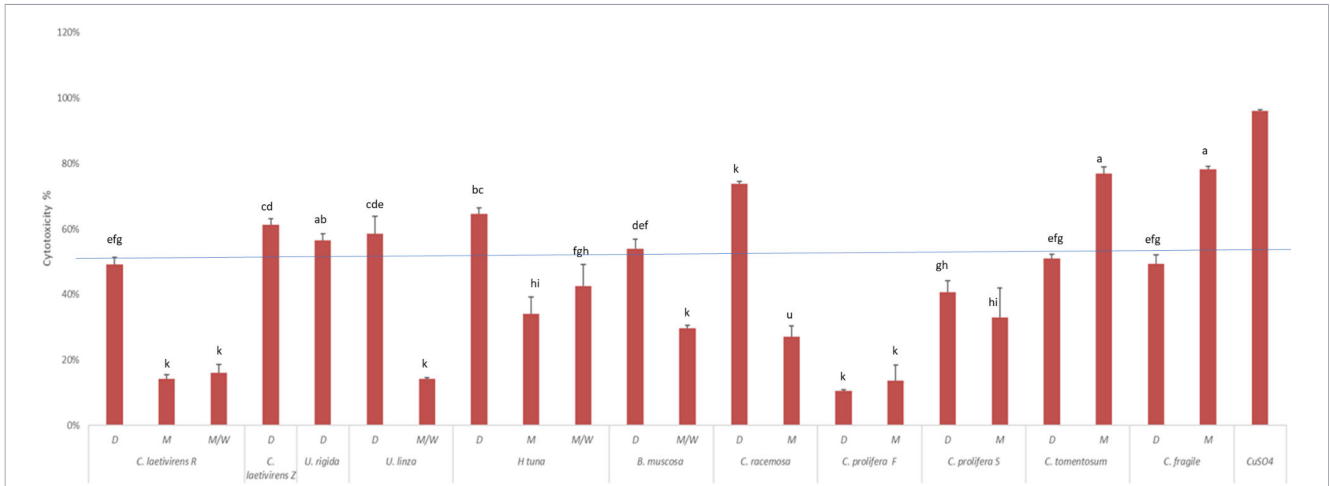


FIGURE 3 Algicidal activity against *Navicula* sp., D: dichloromethane, M: methanol, M/W: methanol/water, *Cladophora laetivirens*: *C. laetivirens*, *Halimeda tuna*: *H. tuna*, *Caulerpa racemosa*: *C. racemosa*, *Caulerpa prolifera*: *C. prolifera*, *Bryopsis muscosa*: *B. muscosa*, *Codium tomentosum*: *C. tomentosum*, *Codium fragile*: *C. fragile*, *Dictyota vermicularis* *D. vermicularis*. Different letters indicate groups of means that differ significantly according to Tukey’s HSD test ($\alpha = 0.05$). Groups sharing at least one common letter are not significantly different from each other, whereas groups with completely different letters show a statistically significant difference.

Our results indicate that *C. fragile* contains significant proportions of PUFAs (26.65%), dominated by n-3 fatty acids such as eicosapentaenoic acid (EPA, C20:5 n-3). This profile corroborates observations by Schmid et al. (2018) regarding the potential of macroalgae as sources of n-3 and highlights the nutritional value of *C. fragile*, reflected by its favorable PUFA/SFA ratio. In contrast, *C. tomentosum* is characterized by a composition heavily dominated by SFAs (75.03%), with an exceptionally high palmitic acid (C16:0; 67.42%) content. This profile results in elevated atherogenic (AI = 9.43) and

thrombogenic (TI = 7.43) indices, comparable to those of coconut oil (AI = 13.63; TI = 6.18) (Ulbricht and Southgate, 1991), limiting its nutritional value. These contrasts between *C. fragile* and *C. tomentosum* illustrate strong intra-genus variability and confirm that environmental factors influence the lipid composition of *Codium* species (Xu et al., 1998).

The presence of essential fatty acids, such as linoleic acid (C18:2 n-6) and α -linolenic acid (C18:3 n-3), underscores the nutritional relevance of these algae. Notably, *D. vermicularis* shows a high linoleic acid content (16.50%) and a very favorable n-3/n-6 ratio

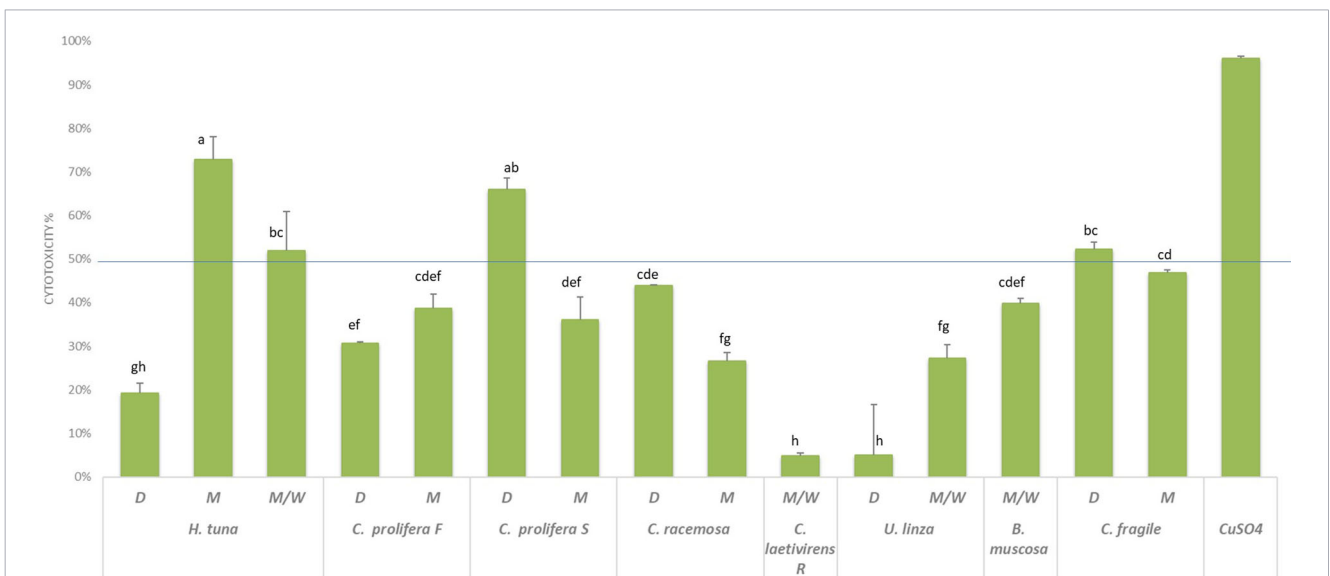


FIGURE 4 Algicidal activity against *T. suecica*, D: dichloromethane, M: methanol, M/W: methanol/water, *Ulva rigida*: *U. rigida*, *Ulva linza*: *U. linza*, *Cladophora laetivirens*: *C. laetivirens*, *Halimeda tuna*: *H. tuna*, *Caulerpa racemosa*: *C. racemosa*, *Caulerpa prolifera*: *C. prolifera*, *Bryopsis muscosa*: *B. muscosa*, *Codium tomentosum*: *C. tomentosum*, *Codium fragile*: *C. fragile*, *Dictyota vermicularis* *D. vermicularis*. Different letters indicate groups of means that differ significantly according to Tukey’s HSD test ($\alpha = 0.05$). Groups sharing at least one common letter are not significantly different from each other, whereas groups with completely different letters show a statistically significant difference.

(5.7). These findings align with Rocha et al. (2021), who emphasized the role of algae as sources of fatty acids beneficial to human health. The balance between n-6 and n-3 observed in this species is particularly important, given the detrimental effects of excessive n-6 intake in modern diets (Shaikh and Edidin, 2008).

B. muscosa exhibits a lipid profile dominated by palmitic acid (C16:0), which constitutes approximately 29% of total fatty acids, consistent with Khotimchenko et al. (2002). Its PUFA content remains modest (5.6%), limiting its nutritional value as a source of essential fatty acids.

For *Cladophora* the lipid profile is relatively balanced, with 27.3% SFAs, 34.2% monounsaturated fatty acids (MUFAs), and 10.2% PUFAs.

The fatty acid profiles of *Ulva* and *Caulerpa* species display typical characteristics of Chlorophyta, with SFA predominance, while variations in MUFA and PUFA content depend on the species. *U. linza* is notable for its high PUFA content and advantageous n-3/n-6 ratio (9.7), reinforcing its nutritional value and potential cardiovascular benefits (Garcia-Oliveira et al., 2020). In contrast, *C. prolifera* and *C. racemosa* show higher SFA concentrations, reflecting a less favorable lipid profile for human health. From a chemotaxonomic perspective, fatty acid profiles can serve as valuable markers for distinguishing macroalgal species (Pereira et al., 2012; Kumari et al., 2012).

Our study confirms that green macroalgae, particularly *Codium* and *Dasycladus*, are promising sources of bioactive lipids with nutritional and industrial potential. Optimizing cultivation conditions, as suggested by, is an essential strategy to enhance their valorization.

The antibacterial evaluation of crude algal extracts revealed heterogeneous response profiles depending on the species and bacterial strains tested. These variations suggest that antibacterial activity is closely linked to the metabolic composition of the algal species as well as to the sensitivity of the targeted microorganisms. *C. fragile* and *B. muscosa* stood out by exhibiting a broad spectrum of activity against several pathogenic bacteria, thereby confirming their pharmacological potential already highlighted in numerous studies. These species possess antimicrobial, antiviral, anti-inflammatory, and immunoregulatory properties (Kang et al., 2019), as well as anti-inflammatory and skin-protective effects demonstrated on human macrophages and keratinocytes (Jang et al., 2024). These findings reinforce previous observations regarding the therapeutic potential of *C. fragile* (Silva et al., 2020).

U. rigida, *U. linza*, and *C. laetivirens* exhibited moderate antibacterial activity, in agreement with the observations of Hussain et al. (2024), who reported the effectiveness of hexane extracts of *Cladophora* spp. against *Pseudomonas aeruginosa*. Furthermore, Ismail et al. (2018) confirmed the antibacterial activity of dichloromethane extracts of *U. rigida* collected from the Tunisian coast, attributing the observed activity to the presence of bioactive fatty acids. In the present study, extracts from *H. tuna*, *C. prolifera*, *C. tomentosum*, and *D. vermicularis* exhibited weak or negligible antibacterial activity. The literature emphasizes that algal secondary metabolites are produced at variable concentrations and often exert highly specific biological effects (Pereira et al., 2018). Overall, these results illustrate that the antibacterial efficacy of green

macroalgae depends on the species, the extraction solvent, and the specific sensitivity of the tested bacterial strains (Ibtissam et al., 2009).

The algicidal potential of the extracts was also assessed. Apolar and methanolic extracts proved to be the most active, confirming the importance of lipophilic metabolites such as terpenes, sterols, and halogenated phenols, which are widely recognized for their antifouling properties (Zhao et al., 2023). Among the tested species, the genera *Caulerpa*, *Codium*, and *Ulva* exhibited the strongest inhibitory effects, reaching up to 78% inhibition against *Navicula* sp. This result is particularly noteworthy since *Navicula* sp., a benthic fouling diatom isolated in the laboratory, is one of the earliest colonizers of submerged surfaces and plays a key role in the initial formation of marine biofilms (Mieszkin et al., 2012). According to Zerrifi et al. (2018), methanolic extracts of *Codium elongatum* showed pronounced algicidal activity against *Microcystis aeruginosa* and *Chlorella* sp. Additionally, several studies on marine macroalgae have demonstrated the ability of certain extracts to effectively inhibit diatom attachment, notably *Ulva reticulata* against *Navicula subinflata* and *Halimeda gracilis* against *Nitzschia palea* (Srikumar et al., 2014).

Compared to *Navicula* sp., *Tetraselmis suecica* exhibited lower sensitivity, with inhibition rates rarely exceeding 50%, except for the methanolic extract of *H. tuna*, which reached 73%. In contrast, the dichloromethane extract exerted strong inhibition against *Navicula* sp. This activity may be related to the presence of secondary metabolites, notably halitunal, a diterpene aldehyde isolated by Koehn et al. (1991) and recognized for its broad bioactive spectrum, including antiviral activity against murine coronavirus A59. These compounds, capable of altering membrane integrity and disrupting cell division, ultimately lead to microalgal growth inhibition. Furthermore, several species of the genus *Halimeda* are known to produce diterpenoids involved in chemical defense against herbivores (Paul and Van Alstyne, 1988), while methanolic extracts of *H. opuntia* exhibit marked antifouling activity (Gazali et al., 2025). Conversely, aqueous polar fractions, which are poorer in lipophilic metabolites, displayed reduced activity. These low inhibition rates (<20%) confirm that water-soluble polysaccharides, although bioactive under other conditions, are unlikely to play a decisive role in microalgal growth inhibition (Patil et al., 2024).

5 Conclusion

This study demonstrates that green macroalgae from the northern Tunisian coast and lagoon exhibit significant variability in fatty acid composition and bioactivity, influenced by species, environmental conditions, and collection sites. Palmitic acid (C16:0) was the predominant saturated fatty acid across all samples, while Ulvales and Dasycladales were particularly rich in polyunsaturated fatty acids (PUFAs), highlighting their nutritional and industrial potential. *C. fragile* and *B. muscosa* showed broad-spectrum antibacterial activity and favorable lipid profiles, whereas species such as *C. tomentosum* had lipid profiles dominated by

saturated fatty acids, limiting their dietary value. Non-polar and methanolic extracts displayed strong algicidal effects against early biofilm-forming diatoms, indicating the potential of *Caulerpa* and *Codium* metabolites for natural antifouling applications. Overall, these findings confirm that green macroalgae are promising sources of bioactive compounds with nutritional, pharmaceutical, and industrial relevance, and that targeted cultivation strategies could further enhance their value.

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/s.

Author contributions

WC: Formal analysis, Methodology, Writing – original draft, Writing – review & editing. LK: Supervision, Validation, Writing – review & editing, Investigation. AI: Methodology, Validation, Writing – review & editing. ME: Formal analysis, Supervision, Validation, Visualization, Writing – review & editing.

Funding

The author(s) declared that financial support was received for this work and/or its publication. The authors acknowledge the financial support provided by the Laboratory LR16INSTM05 (B3Aqua).

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Acknowledgments

The authors wish to thank Mr. Brahim Aoun for his technical assistance with gas chromatography and Prof. Saloua Sadok for providing access to laboratory equipment.

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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