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Research Article

Liquid Chromatography-Mass Spectrometry based Phytochemical Profiling of Marine Macroalga *Ulva compressa* Methanol Extract

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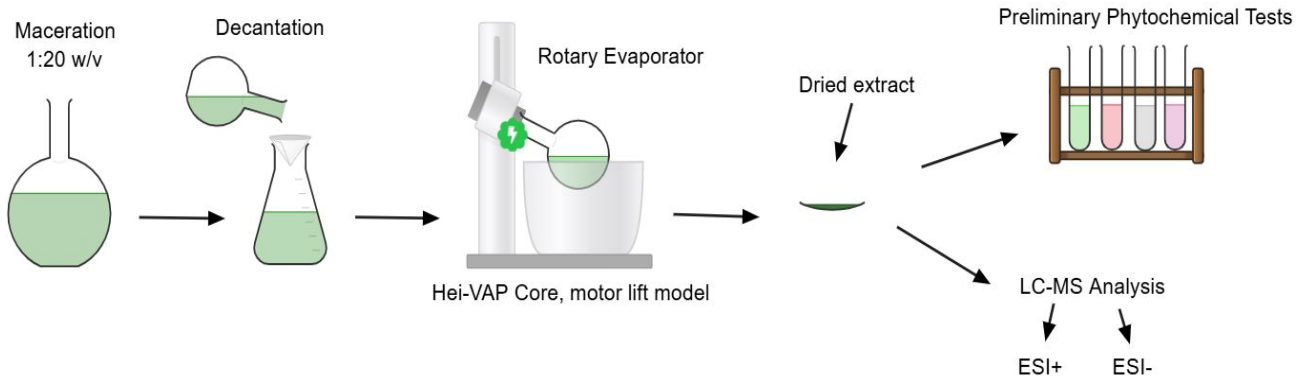
Abstract

Marine macroalgae are widely recognized as vital sources of bioactive compounds, having prospective applications in pharmaceuticals, nutraceuticals, and biotechnology. As global demands for natural therapeutics continue to grow, marine macroalgae, especially Ulva species, have emerged as a promising candidate due to their diverse profiles of phytochemicals. In such cases, qualitative phytochemical analysis is fundamental in determining these bioactive constituents, as well as their potential functional activities. In this study, *the methanolic extract of Ulva compressa was subjected to Liquid Chromatography-Mass Spectrometry analysis in both positive (ESI+) and negative (ESI-) Electrospray Ionization modes to characterize its phytochemical composition in detail*. Using ESI^-, a total of 66 phytochemicals were detected, and 29 by ESI^+, showing the phytochemical richness of the marine alga. The detected phytochemicals belonged to many biologically vital chemical classes, which include flavonoids, phenolics, peptides, fatty acids, lipids, terpenoids, and glycosides. These findings support the application of *Ulva compressa* as a marine-based resource for drug discovery and functional product development. This work also highlights the potential of LC-MS as an effective technique for marine phytochemical profiling, providing in-depth molecular insights.

Keywords: LC-MS, Phytochemical Profiling, *Ulva compressa*

Graphical Abstract



INTRODUCTION

Phytochemicals, which are non-nutritive molecules originating from plants, have been shown to alter biochemical pathways related to health and illness, making them key targets in natural product research. Although the phytochemical profiles of terrestrial plants have long been investigated, marine algae are now known for their structurally distinct and functionally powerful metabolites, especially phenolics, fatty acids, alkaloids, and terpenoids[1]. Seaweeds, commonly known as marine macroalgae, have become a vital source of biologically active compounds with immense ecological, nutritional, and pharmaceutical significance, among them, *Ulva compressa*, a green macroalga belonging to the genus Ulva and class Chlorophycota, has gathered significant interest for its potential as a source of secondary metabolites, exhibiting numerous bioactivities, such as antimicrobial, antioxidant, and anti-

inflammatory[2]. Given the chemical complexity of marine extracts, Liquid Chromatography-Mass Spectrometry (LC-MS) has emerged as the most effective method for phytochemical profiling, allowing high-resolution separation and sensitive detection of a wide range of molecules, even at low concentrations[3]. Comprehensive phytochemical investigation of the marine macroalga, *Ulva compressa,* via LC-MS is relatively unexplored, despite its potential. The precise chemical components of *Ulva compressa's* methanolic extract are unknown, as previous research has concentrated more on species like *Ulva lactuca* and *Ulva rigida*[4,5]. Thus, a thorough phytochemical characterisation of UCME using an advanced technique, LC-MS, is the aim of this investigation. Establishing a biochemical foundation for its possible therapeutic uses and clarifying the existence of bioactive compounds will support the growing field of natural products produced from marine sources.

MATERIALS AND METHODS

Collection and authentication of Ulva compressa

Fresh green alga, identified as *Ulva compressa,* was hand-harvested from the Visakhapatnam coast (Andhra Pradesh), particularly around Tenneti Park during low tide to access healthy and mature alga. It was washed immediately with seawater to eliminate epiphytes and kept in a clean, moisture-free bag for further processing. The collected alga was submitted to the Department of Marine Living Resources at Andhra University, where it was authenticated based on taxonomical characters as *Ulva compressa* (formerly known as *Enteromorpha compressa)*.

Drying of Ulva compressa

In the laboratory, *Ulva compressa* was rinsed several times with tap water, followed by distilled water to remove any residual salts, adhering sand particles, and possible contaminants. After cleaning, the alga was spread out on a filter paper in a well-ventilated area and shade dried at room temperature for 72 hours and kept in an air-tight container for further processing[6].

Extraction using methanol

Dried *Ulva compressa* was macerated in pure methanol at a ratio of 1:20 (w/v) in a long-necked round-bottom flask. The extraction was conducted at ambient temperature for 72 hours, with periodic stirring. The mixture was gently decanted to isolate the solvent extract from the residual alga. The resulting filtrate was then concentrated under reduced pressure at 400°C and 120 rpm, using a rotary evaporator (Hei-VAP Core, motor lift model, equipped with G3 vertical glassware)[7].

The dried residue was weighed, and the extraction yield was evaluated using:

$$\% \text{ Yield} = \frac{\text{Weight of extract} \times 100}{\text{Weight of dry sample}}$$

Preliminary Qualitative phytochemical analysis

A small amount of the dried *Ulva compressa's methanolic extract* (UCME) was reconstituted with methanol, and standard protocols were used to identify the presence of alkaloids, flavonoids, tannins, phenolics, fatty acids, saponins, glycosides, and lipids[8,9].

Liquid chromatography-Mass spectrometry analysis

About 1 ml of the reconstituted UCME was transferred into a sterile Eppendorf tube and sent for analysis at the MURTI Lab, GITAM School of Pharmacy. The analysis was conducted using the AB Sciex Qtrap 5500+ LC-MS system. A volume of 5µl was drawn by an autosampler syringe and subjected to ionisation via Electrospray Ionisation (ESI). The resulting ions were then detected via Channel Electron Multiplier detector. The phytochemical profile was examined in both positive (ESI^+) and negative ionisation modes (ESI^-).

RESULTS

Extraction percentage yield

The weight of the dried extract was calculated by subtracting the initial crucible weight from the final weight of the crucible plus dried extract. The percentage yield of *Ulva compressa* using methanol was found to be 3.38%.

Preliminary phytochemical screening

Preliminary tests revealed the presence of several major classes of bioactive compounds such as alkaloids, flavonoids, amino acids, phenolic compounds, and carbohydrates. The result of the qualitative analysis of UCME is presented in Table 1.

Phytochemicals identified by LC-MS

The LC-MS analysis identified many phytochemicals based on their retention times. The mass spectrum for ESI+ and ESI- is depicted in Figs. 1 and 2, respectively. The peaks obtained in the spectra were identified using the NIST database. A total of 66 phytochemical compounds were identified via ESI^- and 29 via ESI^+, depicted in Tables 2 and 3, respectively. The peaks are described as tentative due to the presence of natural products in isomeric forms, or as isobaric compounds sharing the same molecular weight but differing in elemental composition.

Table 1: Phytochemical analysis results in UCME

Phytoconstituents	Test Name	Qualitative result
Alkaloids	Dragendorff's Test	+
	Picric Acid Test	+
Flavonoids	Alkali Test	+
	Lead Acetate Test	+
Tannins	Braymer's Test	-
	10% NaOH Test	-
Amino Acids	Millon's Test	+
	Xanthopeotic Test	+
Phenolic compounds	Iodine Test	+
	Folin-Caicalteu Test	+
Carbohydrates	Molish Test	+
	Fehling's Test	+
Saponins	Froth Test	-

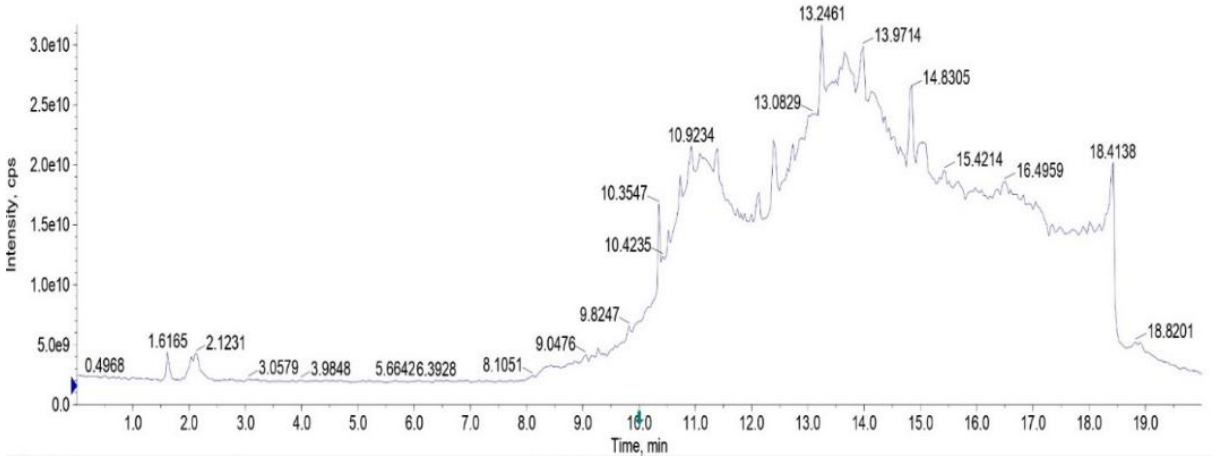


Figure 1: Mass spectrum of UCME acquired in ESI-

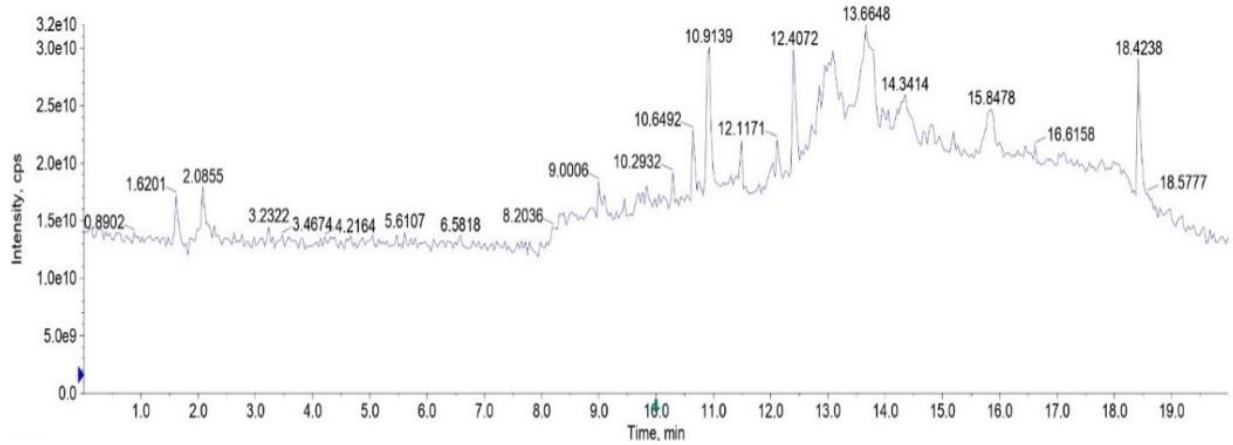


Figure 2: Mass spectrum of UCME acquired in ESI+

Table 2: List of Phytoconstituents identified using ESI- by the NIST database

Serial No.	RT (min)	MW	Adduct formed	Theoretical (m/z)	Observed (m/z)	Proposed Phytocompounds
1	1.7	254.02387	[M-H]-	253.017	253.018	Palmitelaidic acid
2	1.7	155.10785	[M-H]-	154.101	154.0932	3-Hydroxyanthranilic acid
3	1.97	230.92673	[M-H]-	229.92	229.9246	1H-Indole-3-carboxylic acid, 1-pentyl
4	2	186.88673	[M-H]-	185.88	185.9239	3-Indoleacrylic acid
5	2.26	144.15573	[M-H]-	143.149	143.1538	γ-Octalactone
6	4.3	210.95026	[M-H]-	209.944	209.9432	Glycine-Tryptophan-Arginine
7	6.43	127.96397	[M-H]-	126.957	126.962	Maltol
8	6.52	186.0415	[M-H]-	185.035	184.9956	Chamazulene
9	7.28	123.99151	[M-H]-	122.985	122.9896	4-Methylcatechol
10	8.05	117.88673	[M-H]-	116.88	116.8757	Succinamide
11	8.12	192.04673	[M-H]-	191.04	191.0362	Citric acid
12	8.16	129.04673	[M-H]-	128.04	128.0436	DL-Pyroglutamic acid
13	8.85	188.11565	[M-H]-	187.109	187.1001	Azelaic acid
14	9.18	172.12673	[M-H]-	171.12	171.1263	1-Naphthoic acid
15	9.18	253.95339	[M-H]-	252.947	253.0083	6,4'-Dihydroxyflavone
16	9.31	163.96673	[M-H]-	162.96	162.9551	p-Coumaric acid
17	9.57	152.08673	[M-H]-	151.08	151.0856	3-Hydroxyphenylacetic acid

18	9.7	114.04673	[M-H]-	113.04	113.0381	(2E,4E)-Hexa-2,4-dienoic acid
19	9.82	138.04673	[M-H]-	137.04	137.0427	Salicylic acid
20	9.82	376.36673	[M-H]-	375.36	375.33	Digitoxigenin
21	9.85	216.16673	[M-H]-	215.16	215.1623	Undecanedioic acid
22	10.24	266.08673	[M-H]-	265.08	265.0817	4-Isopropyl-4'-methylchalcone
23	10.35	290.08673	[M-H]-	289.08	289.0797	Shikonin
24	10.35	220.12673	[M-H]-	219.12	219.1218	Ethyl coumarin-3-carboxylate
25	10.42	178.36673	[M-H]-	177.36	177.3397	Glycine-Cysteine
26	10.42	208.12673	[M-H]-	207.12	207.1526	p-Methoxycinnamic acid ethyl ester
27	10.42	180.16673	[M-H]-	179.16	179.1368	Olivetol
28	10.42	182.20673	[M-H]-	181.2	181.2026	2,5-Dimethoxybenzoic acid
29	10.52	334.12673	[M-H]-	333.12	333.1204	Estrone sulfate
30	10.59	254.08673	[M-H]-	253.08	253.078	Chrysin
31	10.59	276.04673	[M-H]-	275.04	275.0383	Methysticin
32	10.73	428.44673	[M-H]-	427.44	427.4419	Oleoside 11-methyl ester
33	10.73	412.36673	[M-H]-	411.36	411.3552	L-Arginine, N2-2-(2,2-diphenylethoxy)acetyl
34	10.82	256.12673	[M-H]-	255.12	255.1192	Purpurin
35	11.16	194.20673	[M-H]-	193.2	193.1988	Alanine-Cysteine
36	11.32	264.04673	[M-H]-	263.04	263.0761	Aspartic Acid-Methionine
37	11.39	295.97785	[M-H]-	294.971	294.9881	9R-Hydroxy-10E,12Z-octadecadienoic acid
38	11.39	294.16673	[M-H]-	293.16	293.1678	13S-Hydroxy-6Z,9Z,11E-octadecatrienoic acid
39	11.53	286.01747	[M-H]-	285.011	285.0242	Hexadecanedioic acid
40	11.88	222.16673	[M-H]-	221.16	221.1707	Benzyl cinnamate
41	11.99	234.04673	[M-H]-	233.04	233.095	7-Hydroxy-4-methylcoumarin-3-acetic acid
42	12.1	248.20673	[M-H]-	247.2	247.1954	Aspartic Acid-Aspartic Acid
43	12.39	224.20673	[M-H]-	223.2	223.2089	4'-Hydroxychalcone
44	12.73	276.16673	[M-H]-	275.16	275.1662	Stearidonic acid
45	13.25	278.20673	[M-H]-	277.2	277.2139	6-Gingerol
46	13.25	474.52673	[M-H]-	473.52	473.5319	Lauroyl coenzyme A
47	13.25	466.60673	[M-H]-	465.6	465.5975	2'-Deoxycytidine 5'-triphosphate
48	13.69	304.12673	[M-H]-	303.12	303.1421	cis-5,8,11,14-Eicosatetraenoic acid
49	13.74	220.12673	[M-H]-	219.12	219.125	5-Sulfosalicylic acid
50	14.23	305.99028	[M-H]-	304.984	304.9832	cis-8,11,14-Eicosatrienoic acid
51	14.81	326.08673	[M-H]-	325.08	325.0019	Chamissonolide
52	14.85	256.12673	[M-H]-	255.12	255.1771	Apigeninidin cation
53	15.41	310.24673	[M-H]-	309.24	309.3018	Gastrodin
54	15.41	310.96673	[M-H]-	309.96	309.9646	9-Nitrooleic acid
55	15.64	414.52673	[M-H]-	413.52	413.4969	Loganin
56	15.97	298.00673	[M-H]-	297	297.0028	Ricinoleic acid

57	16.05	398.32673	[M-H]-	397.32	397.2973	Geniposidic acid
58	16.05	1332.86018	[M-3H]3-	443.28	443.3051	D-myo-Inositol-1,3,4-triphosphate
59	16.5	284.08673	[M-H]-	283.08	283.059	D-Mannose 6-phosphate
60	16.75	496.36673	[M-H]-	495.36	495.3753	1,2-Dimyristin
61	17.39	124.96673	[M-H]-	123.96	123.9923	Taurine
62	18.36	327.88673	[M-H]-	326.88	326.9007	17(R)-Hydroxydocosahexaenoic acid
63	18.43	310.48673	[M-H]-	309.48	309.4603	Neohesperidose
64	18.7	121.00673	[M-H]-	120	119.9994	O-Methyl-DL-serine
65	18.9	198.88673	[M-H]-	197.88	197.8749	N5-(1-Imino-3-butenyl)-L-ornithine
66	19.1	205.00673	[M-H]-	204	203.9743	Indole-3-butyric acid

RT: Retention time, min: minute, MW: Molecular weight, m/z: mass to charge ratio
[Peaks are described as tentative due to the presence of natural products in isomeric forms, or as isobaric compounds sharing the same molecular weight but differing in elemental composition]

Table 3: List of Phytoconstituents identified using ESI^+ by the NIST database

Serial No.	RT (min)	MW	Adduct formed	Theoretical m/z	Observed m/z	Proposed Phytocompounds
1	0.99	108.31327	[M+H]+	109.32	109.2778	m-Cresol
2	1.86	114.91327	[M+H]+	115.92	115.7905	D-Ornithine
3	1.86	85.04706	[M+CH3OH+H]+	118.08	118.0832	Betaine
4	1.86	120.31327	[M+H]+	121.32	121.2566	2-(3-Hydroxyphenyl)ethanol
5	2.05	438.07327	[M+H]+	439.08	439.0529	α,α'-Dilaurin
6	8.12	267.06241	[M+H]+	268.069	268.0689	Adenosine
7	8.86	489.31327	[M+H]+	490.32	490.3212	Tryptophyl-Glutamyl-Arginine
8	9.13	243.09217	[M+H]+	244.099	244.1003	N-Acetyl-β-D-mannosamine
9	9.21	240.13715	[M+H]+	241.144	241.137	Methyl myristoleate
10	9.27	245.23327	[M+H]+	246.24	246.241	Aspartyl-Asparagine
11	9.69	276.18423	[M+K]+	315.147	315.137	trans-4-Ketoretinoic acid
12	10.26	382.16403	[M+H]+	383.171	383.1835	Cholesta-4,6-dien-3-one
13	10.43	402.25835	[M+H]+	403.265	403.2643	Nobiletin
14	10.47	303.21842	[M+H]+	304.225	304.2238	Glutamyl-Arginine
15	10.47	217.16706	[M+CH3OH+H]+	250.2	250.1983	Lysyl-Cysteine
16	10.82	294.1807	[M+H]+	295.187	295.1676	Linoleic acid methyl ester
17	11.24	259.13057	[M+NH4]+	277.164	277.1603	9,12-Octadecadiynoic acid
18	11.38	305.36706	[M+CH3OH+H]+	338.4	338.3992	Erucamide
19	11.56	386.35327	[M+H]+	387.36	387.3636	Harpagide
20	11.66	248.18814	[M+H]+	249.195	249.1983	6,7-Diethoxy-4-methylcoumarin
21	12.08	610.15327	[M+H]+	611.16	611.2049	Cyanidin-3-O-sophoroside cation
22	12.18	382.36096	[M+H]+	383.368	383.3686	4-Dodecyloxy-2-hydroxybenzophenone
23	12.71	441.44706	[M+CH3OH+H]+	474.48	474.4616	Leucyl-Tryptophanyl-Arginine
24	12.82	312.67327	[M+H]+	313.68	313.6779	Arachidic acid

25	13.8	624.31327	[M+H]+	625.32	625.3165	Peonidin 3,5-diglucoside
26	14.02	317.83327	[M+H]+	318.84	318.8553	5-Oxo-6E,8Z,11Z,14Z-eicosatetraenoic acid
27	14.05	608.35327	[M+H]+	609.36	609.3607	1-(1,2-Dioctanoylphosphatidyl)inositol
28	14.21	638.35327	[M+H]+	639.36	639.3642	Plantamajoside
29	16.13	382.52706	[M+CH3OH+H]+	415.56	415.5585	Histidyl-Cysteinyl-Arginine

RT: Retention time, min: minute, MW: Molecular weight, m/z: mass to charge ratio
[Peaks are described as tentative due to the presence of natural products in isomeric forms, or as isobaric compounds sharing the same molecular weight but differing in elemental composition]

DISCUSSION

The diverse phytochemical profile observed in the study supports the growing evidence of the marine macroalga, *Ulva compressa*, as a rich source of bioactive compounds with pharmaceutical and nutraceutical potential.

The identification of a broad range of flavonoids, like 4'-Hydroxychalcone, 6, 4'-Dihydroxyflavone, and Nobiletin, from UCME highlights the significant phytochemical complexity and possible bioactivity of *Ulva compressa*. Previous research on Ulva species confirms a strong flavonoid content, with *Ulva compressa* having the highest values in flavonoid content among other green macroalgae, validating its antioxidant activity[10]. This aligns with earlier findings where *Ulva clathrata* and other species had high levels of flavonoids and phenolics, corresponding to significant radical scavenging activity[11]. The presence of polymethoxylated flavones, Nobiletin, has been reported to have cytoprotective and anti-inflammatory actions[12].

The detection of a broad range of phenolic and aromatic compounds, such as salicylic acid, shikonin, p-coumaric acid, and 6-gingerol, highlights the chemical intricacy of *Ulva compressa*. These compounds have been well-documented to possess antioxidant, anti-inflammatory, and antimicrobial activities, which further validates *Ulva compressa's* therapeutic potential. *Ulva compressa* has been found to have one of the highest levels of phenolic content among studies of seaweeds[10,11]. Phytochemicals like p-coumaric acid and methylcatechol exhibit a protective role against oxidative stress as well as microbial invasion[13]. In addition, gingerol and coumarin derivatives are likely to be involved in interspecies communication and UV protection within the marine ecosystem[12].

The presence of various dipeptides and amino acid derivatives like Ala-Cys, Trp-Glu-Arg, and DL-pyroglutamic acid in UCME makes it a rich source of bioactive peptides. Marine macroalgae, especially Ulva species, are gaining prominence for their ability to produce protein-derived peptides having multifunctional activities, such as antioxidant, antihypertensive, and antimicrobial[14]. The presence of peptides like Gly-Cys and His-Cys-Arg contains thiol and arginine residues responsible for radical scavenging and nitric oxide-mediated vasodilatory activity. *In silico* and biochemical studies on *Ulva lactuca* and *Ulva rigida* have also indicated the occurrence of angiotensin-converting enzyme inhibitory peptides, indicating potential cardiovascular use[15,16].

The identified fatty acids and lipid derivatives in UCME, including 5-oxo-eicosatetraenoic acid, 17(R)-hydroxydocosahexaenoic acid, and stearidonic acid, highlight its nutritional and biochemical significance. These phytochemicals include saturated, monounsaturated, and polyunsaturated fatty acids, many of which exhibit anti-inflammatory, antimicrobial, and cardioprotective properties. Many polyunsaturated fatty acids, such as stearidonic acid and arachidonic acid, produced by marine Ulva species, play an essential role in modulating immune responses and have potential nutraceutical value[13,17]. Moreover, hydroxylated fatty acids like 13s-hydroxy-octadecatrienoic acid and ricinoleic acid may contribute to the alga's defence system against oxidative stress and pathogen invasion[18]. The presence of medium and long-chain dicarboxylic acids like azelaic and hexadecanedioic acids also highlights *Ulva compressa's* multifaceted lipid profile and its potential for green bioactive lipid production[19].

CONCLUSION

Ulva compressa is a potential marine biological resource with pharmaceutical and nutraceutical applications. The LC-MS analysis identified the presence of many phytochemical compounds, such as flavonoids, phenolics, peptides, fatty acids, and lipids, which contribute to the therapeutic potential of the marine macroalga. The presence of oligopeptides in UCME distinguishes it from other Ulva species and suggests that it may be a source of novel bioactive compounds. Its potential can be further developed as an economically sustainable and viable marine macroalga through further research. Further studies could include purification techniques, evaluation, and structural characterisation of the aforementioned Phytoconstituents.

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Ethical approval: This study does not involve experiments on animals or human subjects.

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