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Compounds identification through Gas Chromatography Mass Spectroscopic analysis, phytochemical screening and antioxidant properties of Ulva fasciata

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ABSTRACT

Marine algae have been considered promising resources for new bioactive compounds in addition to their nutritional significance, multiple pharmacological, industrial, food industry, etc., activities. The *Ulva fasciata* is a common green alga consumed worldwide, current study focused the *Ulva* collected from coastal region of Ramnad, collected samples are dried made into powder, powders are dissolved in methanol and ethanol solvents then made extracts, its focused qualitative phytochemical analysis, different types of compounds identification by GC-MS analysis and free radical scavenging antioxidant activity by DPPH method. In results the crude methanol extract showed yield 0.12 g and ethanol extract showed 0.12 g yield. Qualitative analysis of methanol and ethanol extracts showed presence of tannins, saponin, cardiac glycosides, etc., GC-MS analysis of methanol extract showed 10 compounds among that the main component are glycerol tricaprylate, it showed 57.72% peak intensity and the retention time (RT) noticed 47.26, followed by 10-Nonadecanone shows 22.13% with RT 47.42, 9-Octadecenoic acid, 1,2,3-propanetriyl ester showed 2.81% peak intensity and the RT noticed 44.36. The ethanol extract showed hexadecane peak intensity 33.65% was obtained in RT 19.59, followed by hexanedioic acid, bis(2-ethylhexyl) ester showed 19.59% with the RT 42.99, azulene showed 11.88% peak intensity with RT 6.86. Methanol extract showed potential antioxidant activity 91.03% with IC_{50} value 284 mg/ml methanolic extract and 84.65% with IC_{50} value 298 mg/ml ethanolic extract, the antibacterial activity potential showed against *E. coli*, we concluded that methanolic extract showed potential antioxidant activity, it's useful for pharmacological industries for human societies.

INTRODUCTION

The marine environment contains bioactive natural materials with unique structural and chemical characteristics that differ from those found on land (Al-Saif et al., 2014). Marine organisms create bioactive metabolites in response to environmental stressors. Marine macroalgae are well-known for producing diverse products. Polyunsaturated fatty acids are among the biogenic substances (El-Din and El-Ahwany, 2015). Various compounds, including flavonoids, terpenoids, alkaloids, quinones, sterols, polyketides, chlorotannins, polysaccharides, glycerols, peptides, and lipids, have antimicrobial, antifouling, anti-inflammatory, and antiviral properties (Bouhlal et al., 2015).

Marine algal cell walls typically contain sulphated polysaccharides such as carrageenan from red algae, ulvan from green algae, and fucoidan derived from brown algae (Thanh et al., 2016). Phenolic chemicals derived from macroalgae have been shown to have antiallergic, antiaging, and whitening benefits in food, medicine, pharma, and cosmetic items. Furthermore, consuming a suitable amount of polyphenols, such as myricetin, morin, and quercetin contained in *U. lactuca* (Caf et al., 2015), may help to avoid diseases including obesity, metabolic syndrome, Alzheimer's, and cancer. Carotenoids in *U. lactuca*, including astaxanthin, ß-carotene, fucoxanthin, and lutein, have been shown to have anti-inflammatory, anti-aging, antioxidant, and other properties (Ambiga et al., 2022). They are commonly utilized in vitamin supplements and cosmetics, as well as natural food colors and fish and poultry feed additives (Morais et al., 2021).

Carotenoids have anti-inflammatory characteristics because they can neutralize free radicals, providing chemical protection against the development of cancer cells (Jimenez et al., 2021).

Common locations for *Ulva fasciata* (Worldwide: Eastern Atlantic, Caribbean, Indian and Pacific Oceans) include reef flats, tidepools, and intertidal rocks, frequently prevalent in nutrient-rich freshwater runoff locations, such as those adjacent to stream mouths and runoff pipelines (Madhusudan and Baskaran, 2023; Yohannan et al., 2024). *U. fasciata*, also known as *Limu palahalala*, is a favorite seaweed to eat in Hawaii. Chopped in salads with other varieties of limu, cooked in soups, or served as a condiment are some of the preparation techniques. The nutritional composition of *U. fasciata*, commonly known as sea lettuce, can vary depending on factors such as environmental conditions, harvesting season, and geographical location (Pereira, 2015; Daoud et al., 2021). However, seaweeds like *Ulva fasciata* are generally considered to be highly nutritious and may contain a variety of vitamins, minerals, proteins, carbohydrates, and other bioactive compounds (Karthik et al., 2021; Pereira, 2023).

The present study focused *Ulva fasciata* ethanol and methanol extracts to identify the bioactive compounds by GC-MS, phytochemical quantifications and free radical scavenging DPPH antioxidant activity.

Materials and Methods

Algae sample collection and extract preparations

The fresh *Ulva fasciata* commonly known as green seaweed collected from Mandapam, Ramanathapuram district, Tamil Nadu, India. The sample was then rinsed in distilled water to remove impurities and particles. And shade dried (without direct sun exposure) and preserved for further processing.

The dried sample is grounded using mortar and pestle and soaked in (volatile solvents) methanol and ethanol. The 5 grams of each sample were weighed and soaked in 50 ml of methanol and ethanol separately. The extract was allowed to stand overnight and filtered using sterile filter paper. The filtrate was collected and evaporated at 10 to 20ºC used for heating the mantle. After evaporation the dried extracts were weighed and found by calculating the yield.

Qualitative phytochemical analysis

Secondary metabolites analysis of *U. fasciata* methanol and ethanol leaf extract focused confirmatory analysis, either presence or absence of bioactive secondary metabolites, its focused tannins, saponins, flavonoids, alkaloids, and polyphenols, the standard biochemical protocols were followed in accordance with established previously procedures (Vinothini et al., 2017).

Gas Chromatography and Mass Spectrometry

The bioactive compounds in *U. fasciata* leaf methanol extract were identified through GC-MS analysis; GC/Q-TOF was utilized for proper collection transmission with the aid of GC/MS analysis, allowing for expanded compound identification using MS/MS. The instrument was equipped with low-energy electron ionization and free engineered procedures, with a hidden region temperature of 30°C and heating to 300°C at 10°C per 4 min (Saranya et al., 2024). The ionization energy was set at 70 Ev, with a MS check substitute of 45–450 MHz. The total length of an occurrence was 55 min. The GC-MS made the counter-length parts restrictive. The mass spectra were differentiated and saved in the mass spectrometer measurement, with each part selected from the standard zenith point of each segment of recording to compare the assortment of identification parts of molecular weight, formula, and structure for the identification of study materials in the NIST (National Institute of Standards and Technology) library (Arjun et al., 2017).

DPPH assay

The extracted *Ulva*'s antioxidant activity was assessed using two conventional methods: 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging, the antioxidant activity of extracted briefly, 2 mL extracts were diluted with solvents to create various concentrations (100, 200, 300, 400, 500 mg/mL), subsequently, 1 mL of a 0.1 mM DPPH solution was added to the diluted solution. After thorough mixing, the mixture was stored in a dark room at room temperature, and the radical scavenging activity was determined by measuring the absorbance at 517 nm for each sample after a 30-min incubation period, with ascorbic acid serving as a positive control. The extract concentrations that inhibited DPPH radicals by 50% (IC_{50}) were calculated.

Inhibition (%) = [(A0 x A)/A0] × 100

In this context, A0 represents the DPPH absorbance without the sample, whereas A represents the DPPH absorbance in the presence of the sample (Sumathy et al., 2024).

Results and discussion

Collection and extraction of Samples

The fresh *Ulva fasciata* (green seaweed) collected from Mandapam, Ramanathapuram district, Tamil Nadu, India, filtered extracts, after complete evaporation of solvents, then extracted, yield of the sample methanol extract 0.12 g and ethanol extract 0.12 g (Figure 1).

Figure 1. Fresh sample of *Ulva fasciata* collected and dried, made into powder, extracted with methanol and ethanol

Phytochemical Analysis

Primary analyses of secondary metabolites were observed in methanol and ethanol extracts, tannins, saponins, flavonoids, alkaloids, proteins, steroids, quinones, terpenoids, phenols, cardiac glycoside identified and confirmed (Table 1, Figure 2).

Table 1. phytochemical analysis of methanol and ethanol extracts of *Ulva fasciata*

S. No.	Contents	Methanol	Ethanol
1	Tannins	+	-
2	Saponin	-	+

3	Flavonoids	-	-
4	Alkaloids	-	-
5	Proteins	+	+
6	Steroid	-	+/-
7	quinones	-	-
8	terpenoid	-	-
9	Cardiac glycosides	+/-	-
10	Phenol	-	-

Note: (+) Positive and (-) Negative

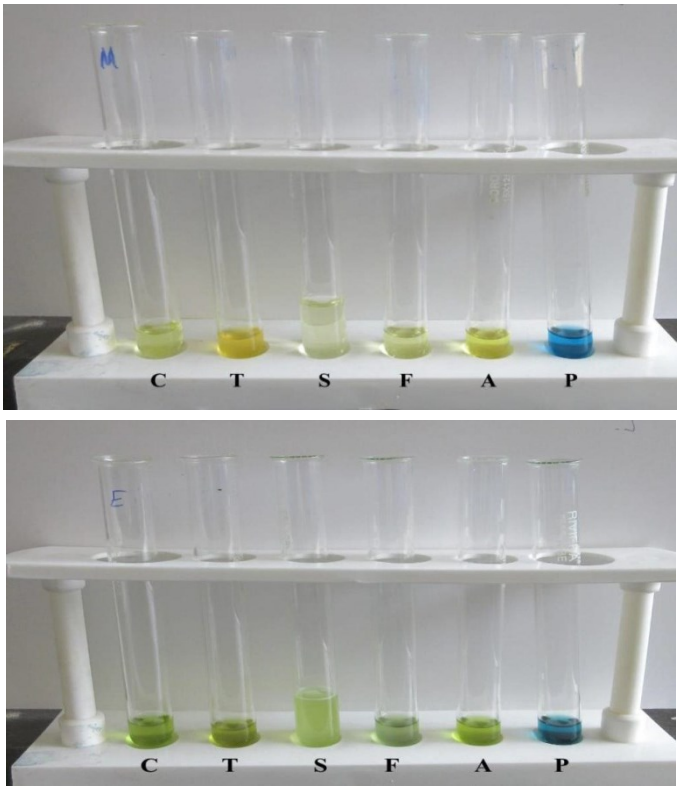

Figure 2. Phytochemical analysis of methanol and ethanol extract

Tannins, as secondary metabolites, are sequestered in vacuoles within the plant cell, protecting the other components. They are found naturally in the roots, wood, bark, leaves, and fruit of many plants, especially in the bark of oak (*Quercus*) species, sumac (*Rhus*), and myrobalan (*Terminalia chebula*). They also exist in galls, which are diseased growths caused by insect infestations (Okuda, 2011; Azhagu Madhavan and Arjun, 2024).

Tannins are a varied category of polyphenols, secondary metabolites in plants generated in response to biotic and abiotic stress inducers. The phenolic rings and hydroxyl groups contained in their chemical structures offer them antioxidant and protein-binding characteristics, as they have a large range of molecular weight (500–20,000 Da) that it also demonstrated in their extensive structure variety (Vuolo et al., 2018; Sumathy et al., 2023), their nature and concentration of hydroxyl radicals, tannins are very hydrophilic molecules, soluble in aqueous solutions as well as having a high potential to securely link with proteins and carbohydrates. This property is common to all tannins, although it seems that their association with polysaccharides diminishes the probability of attaching and interacting with proteins (Molino et al., 2020).

Antioxidant Assay

The antioxidant activity of methanol and ethanolic extract tested Figure 3 summarize the results of DPPH free radical scavenging activity based on dose dependent manner, the DPPH-free radical scavenging activity of methanol extracts of *Ulva lactuca* increased with increasing concentration (100 to 500 mg/ml) it showed highest scavenging activity 91.03% followed by ethanol extract showed 84.65%. The IC_{50} values are calculated for methanol extracts were 284 mg/mL and ethanol extract 298 mg/mL, respectively. The IC_{50} values show substantial differences (Figure 3).

Previous research demonstrated that the antioxidant properties of sulphated polysaccharides from several groups of green algae were determined by a variety of structural characteristics. Qi et al. (2015), discovered that ulvan with a high sulphate concentration has greater antioxidant properties than natural ulvan isolated from *Ulva pertusa*. Qi et al. (2005) discovered that the antioxidant activity of acetylated ulvans did not increase with increasing substitution degree. Qi et al. (2005) showed that ulvan from *Ulva pertusa* with a low molecular weight has higher antioxidant activity. Li et al. (2013) found that sulphated polysaccharides with high Mws from *Enteromorpha prolifera* exhibited higher superoxide radical scavenging capabilities.

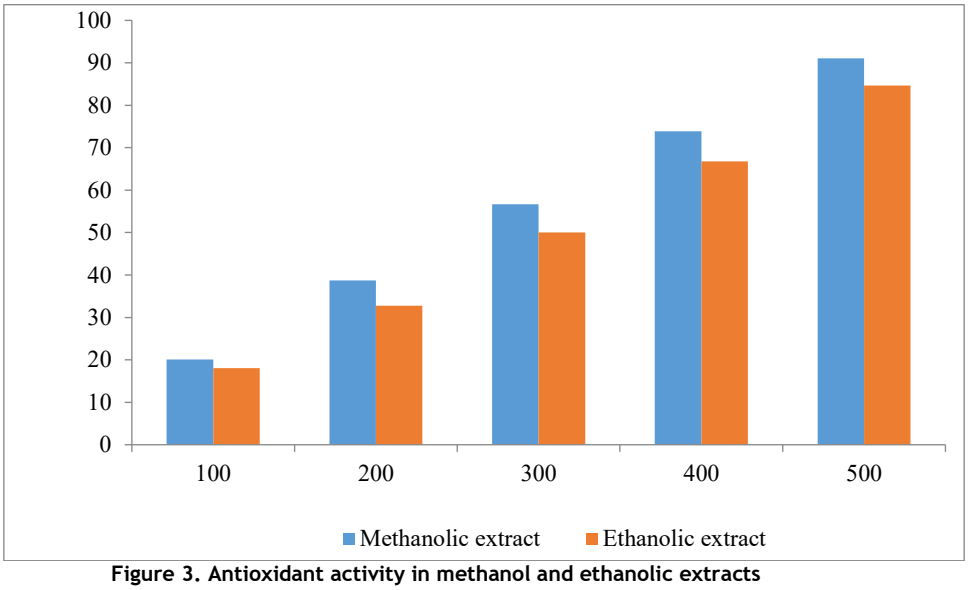

Figure 3. Antioxidant activity in methanol and ethanolic extracts

Gas Chromatography and Mass Spectrometry

The chromatogram of *Ulva* methanol extract obtained from the GC-MS analysis of the fraction obtained after elution of *U. fasciata* extract showed 10 chromatographic peaks from 7 to 50 min, in this duration 10 components were identified most of the compounds. The main component glycerol tricaprylate showed 57.72% in the retention time (RT) of 47.26, its triglycerides of saturated fatty acids; excipients often employed in lipophilic formulations were evaluated for their ability to improve skin penetration. The 10-Nonadecanone contain 22.13% with RT 47.42, this long-chain aliphatic ketone, commonly known as caprinone, appears as a white or nearly white crystalline powder. Herbal medications are said to have fewer side effects than allopathic drugs. Followed by glycerol tricaprylate showed 8.08% in the retention time (RT) of 47.04, the lowest area peak percentage was noticed diethyl heptyloxy octadecyloxy- 0.43%, RT 44. 18 (Table 2, figure 4).

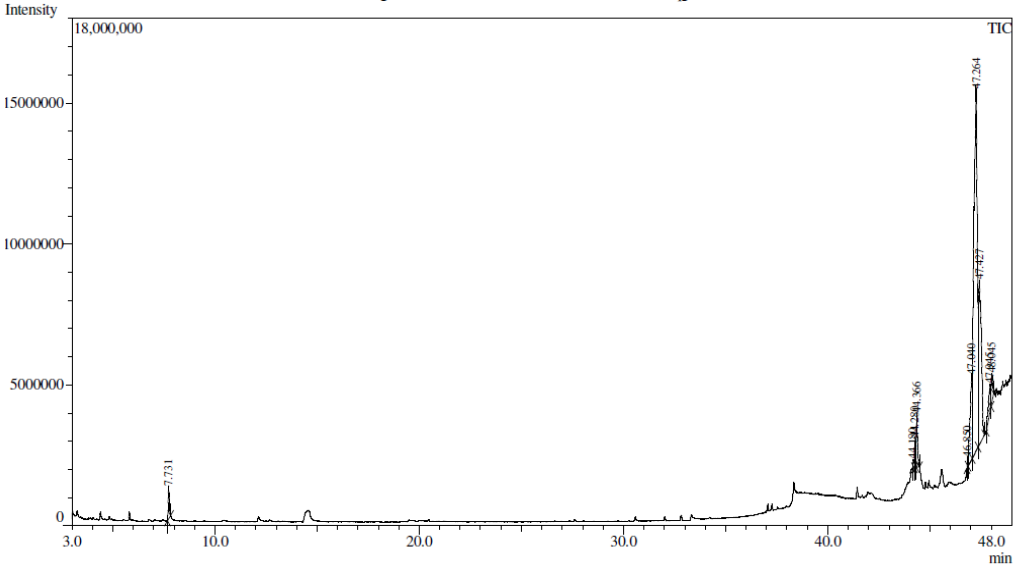

Figure 4. GC-MS analysis of methanol extract

The gas chromatography mass spectroscopy of ethanol extract of *Ulva* showed 10 compounds, maximum Hexadecane amount 33.65% was obtained in RT 19.94, it's also called cetane is an alkane hydrocarbon, Liquefied Petroleum Gas (LPG) is a commercial fuel made from hydrocarbons like propane and butane. Hexanedioic acid, bis(2-ethylhexyl) ester showed 19.59% with the RT 42.99; is primarily utilised as a plasticizer in the flexible vinyl plastic sector, and is commonly employed in flexible polyvinyl chloride (PVC) items such as food cling wrap packaging. Then followed by azulene showed 11.88% with RT 6.86; is an aromatic hydrocarbon that lacks six-membered rings. It is an isomer of naphthalene, with a similar odour. The lowest amount of 2-Amino-4-dimethylamino methylene pentanedinitrile was obtained 2.16 in RT 3.02; as a clear, red liquid with an ammonia-like odour, less dense than water, and insoluble in water. Scientists agree that developing new antimicrobial agents is crucial for humanity's future, these results increase the knowledge on the phytochemical composition of *U. fasciata* extract and it should add to the understanding of the product pharmacological properties (Table 3, figure 5).

Table 3. Methanol extract of *Ulva* GC-MS analysis

Peak #	Retention time	Area %	Compound names
/nb1	7.73	1.77	2-Furancarboxaldehyde
2	44.18	0.43	Silane, diethylheptyloxyoctadecyloxy-
3	44.28	1.60	4-Methyl-8-hexadecyn-1-ol
4	44.36	2.81	9-Octadecenoic acid, 1,2,3-propanetriyl ester
5	46.85	0.35	Didecyl 1,2-Cyclohexanedicarboxilate

6	47.04	8.08	Glycerol tricaprylate
7	47.26	57.72	Glycerol tricaprylate
8	47.42	22.13	10-Nonadecanone
9	47.94	3.16	Tetradecanoic acid, 2-hydroxy-1,3-propanediyl ester
10	48.04	1.94	Dodecanoic acid, 1,2,3-propanetriyl ester

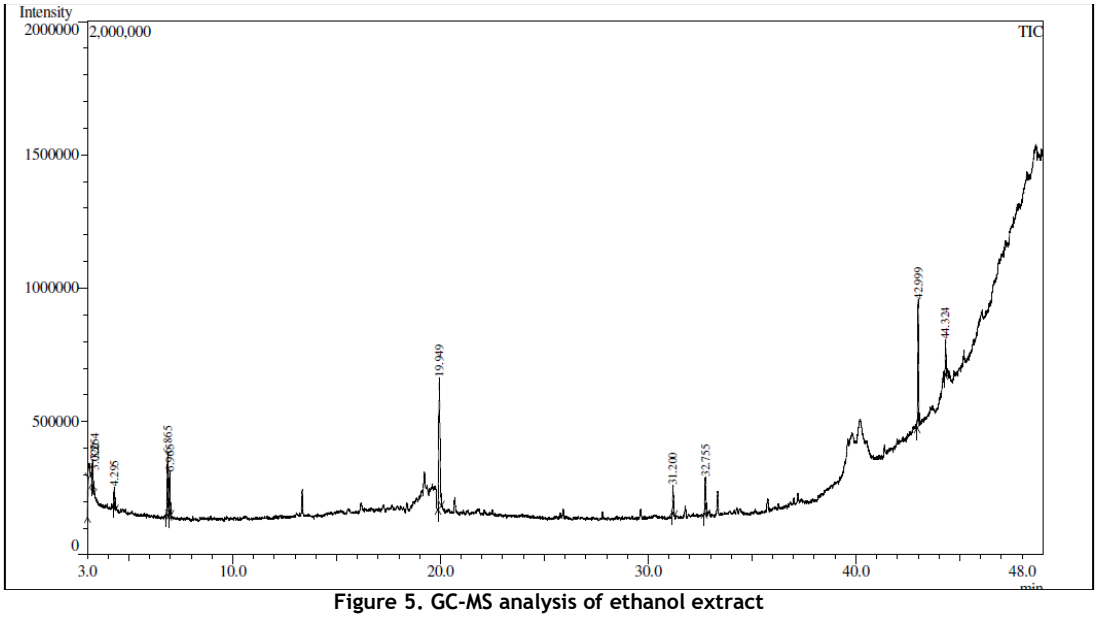

Figure 5. GC-MS analysis of ethanol extract

Table 3. *Ulva* GC-MS analysis of ethanol extract

Peak #	Retention time	Area %	Compound names
1	3.02	2.16	2-Amino-4-dimethyl amino methylene pentanedinitrile
2	3.25	3.80	Decane
3	4.29	2.78	Propanoic acid, 2-methyl-, 1-methylbutyl ester
4	6.86	11.88	Azulene
5	6.96	6.95	Dodecane
6	19.94	33.65	Hexadecane
7	31.20	5.15	Dibutyl phthalate
8	32.75	8.19	Dibutyl phthalate
9	42.99	19.59	Hexanedioic acid, bis(2-ethylhexyl) ester
10	44.32	5.85	9-Octadecenoic acid, 1,2,3-propanetriyl ester

CONCLUSION

The *Ulva fasciata* methanol and ethanol crude extracts showed tannins, saponin, cardiac glycosides, etc., compare to ethanol, methanol extract showed potential antioxidant activity, in GC-MS analysis of methanol extract contains major constituent of glycerol tricaprylate, 10-Nonadecanone contain, ethanol main component of hexadecane, hexanedioic acid. The isolated *Ulva*

has potential for use in pharmaceutical, medical and food applications.

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