

Phytochemical Profiling of the Red Seaweed, *Halymenia dilatata* by GC-MS Analysis

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ABSTRACT: Though numerous studies are being carried on marine algae for their structurally diverse bioactive compounds, there is a lot more to be explored. The present study was designed to bring out the phytochemical composition of the red algae, *Halymenia dilatata*, on which there is not much information. *Halymenia dilatata* was collected from the coastal area of Mandapam, Tamil Nadu, India and was subjected to methanol extraction. GC-MS analysis of the methanol extract was performed to analyze the phytochemical constituents of the algae and the analysis revealed the presence of seventeen phytocomponents. Majority of the identified compounds were found to be from the fatty acid group and others were from alkane, acetate, amide, alkenyl, alcohol and steroid group. The major constituents of the extract includes Hexadecanoic acid, methyl ester, n-Hexadecanoic acid, 6,10,14-Trimethylpentadecan-2-one, 9-Octadecenoic acid (Z)-methyl ester and 2-Dodecen-1-yl(-)succinic anhydride. Most of the identified compounds were reported to possess antimicrobial, antioxidant, anti-inflammatory, antitumor and antifouling properties. From the results, it is evident that *Halymenia dilatata* contains various bioactive compounds and can be recommended as seaweed of phyto-pharmaceutical importance.

Keywords: *Halymenia dilatata*, marine algae, GC-MS analysis, phytochemical.

INTRODUCTION

Marine algae, commonly known as seaweeds are the renewable living source with medicinal, industrial and environmental uses. The isolation of biogenic compounds and determination of their structure could provide leads for future development [1]. In recent years, there have been many reports of marine algae derived compounds that have a broad range of biological activities, such as antibiotic, antiviral, antioxidant, antifouling, anti-inflammatory, cytotoxic and antimutagenic activities [2]. They have been screened extensively to isolate life-saving drugs and biologically active substances all over the world [3].

Seaweeds are receiving much attention mainly because of their contents of functional ingredients such as polyunsaturated acids, carotene and their pigment carotenoids, sulphated polysaccharide and sterol [4, 5]. Though diverse phytoconstituents from various seaweed species has been reported and several compounds have been derived from them for prospective development of novel products, there is still an enormous number of species to be explored. One such algal species is the marine red algae, *Halymenia dilatata*.

Halymenia dilatata belongs to the family Halymeniaceae and is found distributed in the coastal lines of Asia (India, China, Taiwan, South-west and South-east Asia), Pacific Islands, Africa, Australia and New Zealand. The algae look like clumps of several sheets, with 10-20cm long thallus which are translucent, flexible, slippery and slimy. Edges of the thallus are smooth often in regular ruffles and the surface may be plain or might have large paler blotches or mottles. Color of the thallus may be brownish-orange, dark pink to reddish depending on the geographical location of occurrence [6].



Figure 1: Image of *Halymenia dilatata*

In spite of abundant distribution, there are no detailed studies on the active components reported to date on this seaweed. Hence, the present study was performed to analyze the phytochemicals of the not much explored seaweed, *Halymenia dilatata*, by using Gas Chromatography-Mass Spectrometry (GC-MS) analysis.

MATERIALS AND METHODS

Seaweed collection and identification

Fresh algae (*Halymenia dilatata*) were collected from the Mandapam coastal line in the Gulf of Mannar (Lat. 09° 17.417'N; Long. 079° 08.558'E), Ramanad district, Tamil Nadu, India by hand picking during low tide. The collected material was transferred to dark polythene bags and stored in isothermal boxes to prevent photo- and thermal degradation during transport to the laboratory. A small portion of the seaweed was taken separately for preservation in 4% formalin and submitted for taxonomic identification at the Botanical Survey of India (BSI), Coimbatore. The BSI examined the sample and authenticated the identification.

Extract preparation

In the laboratory, wet algae were cleaned thoroughly by washing in running tap water (3-4 times) to remove the attached sediments, epiphytes and other marine debris. Finally it was washed with distilled water and allowed to dry under shade for 5 – 7 days, until the moisture was completely removed. The dried material was ground to a coarse powder using an electrical mixer and stored in air tight container at room temperature. Finely ground algal material was extracted with methanol by soaking 10g of the powdered sample in 100ml of solvent (1:10 – w/v) in a conical flask and kept on a shaker (120 rpm) for 24 hrs. The mixture was then filtered using Whatman. No.1 filter paper and re-extraction was done until the solvent becomes colorless. After extraction, the solvent was filtered and vacuum-evaporated to yield the crude organic extract [7].

Gas Chromatography–Mass Spectrometry (GC–MS)

GC-MS analysis was performed using the Agilent technologies 6890 N JEOL GC-MS Mate II model instrument (IIT, Chennai). The sample was injected into a HP-5 column (30 m X 0.25 mm i.d with 0.25 μ m film thickness). Helium was used as a carrier gas at a flow rate of 1.0 ml/min. The injection port was maintained at 200°C and column oven temperature was programmed as 50-250°C at a rate of 10°C/min injection mode. Mass spectra were obtained at an ionization voltage of 70 eV with ion source temperature of 250°C and the interface temperature was maintained at 250°C. The scanning range was 50-600 mass units.

Identification of compounds

Interpretation of mass spectrum (GC-MS) was done using the database of National Institute of Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown compounds was compared with the spectrum of the known compounds stored in the NIST Library. The name, molecular weight and peak area of the compounds of the test material was ascertained.

RESULTS AND DISCUSSION

GC-MS analysis of *Halymenia dilatata* revealed the presence of seventeen compounds (Fig.1) in the methanol extract. The compounds with their retention time (RT), molecular formula, molecular weight (MW), percentage composition and structure are presented in Table 1. Most of the identified compounds were found to be from the fatty acid group and others from alkane, acetate, amide, alkenyl, alcohol and steroid group (Table 2).

It was found that many of the identified compounds were reported to possess antimicrobial, antioxidant, anti-inflammatory, antitumor, cancer preventive and antifouling properties (Table 2). In a study, it was reported that fatty acids exhibit antibacterial, anti-inflammatory and antifungal activity [8]. The antifouling property of palmitic acid was also identified and reported [9]. Phthalic acid derivatives were found to possess antitumor, anti-inflammatory and antimicrobial properties [10, 11]. Many authors have reported that natural aromatic

compounds possess important biological activities, such as antioxidant, anti-inflammatory and antibacterial activities [12, 13, 14].

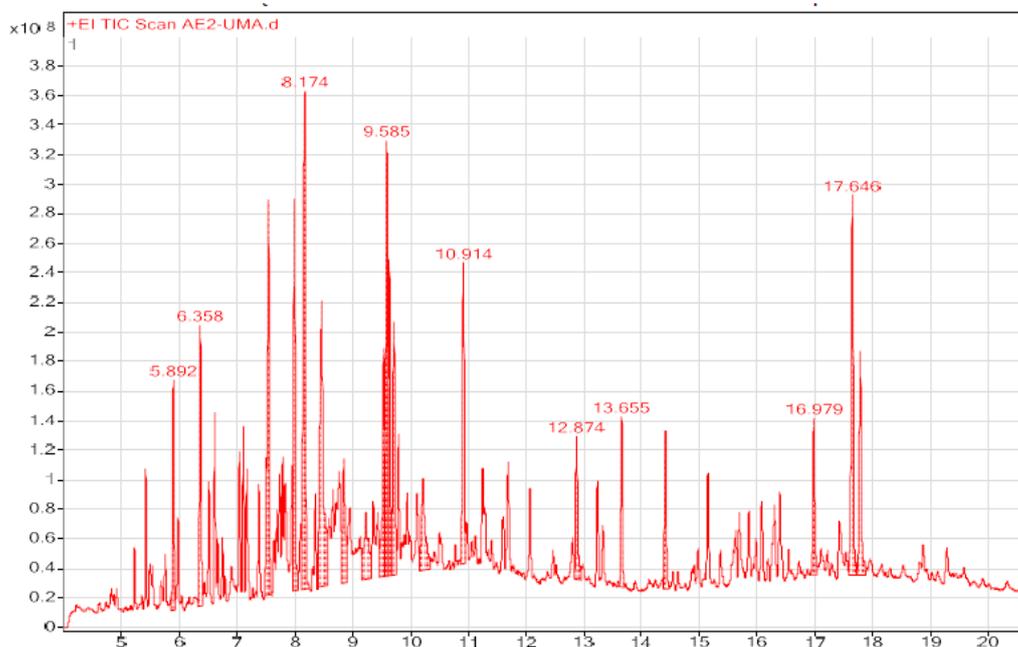
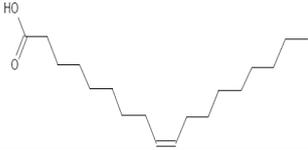
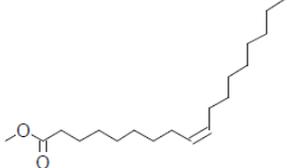
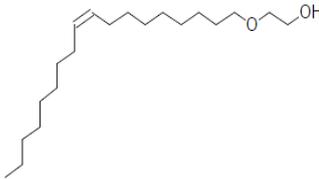
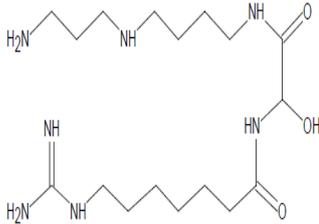
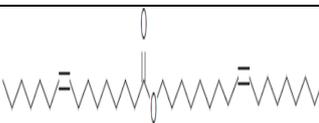
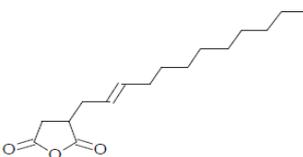


Fig 2: Chromatogram of the methanol extract of *Halymenia dilatata*

Table 1: Active compounds identified in the methanol extract of *Halymenia dilatata*

S.No	Name of the compound	RT	Peak Area (%)	Mol.wt g/mol	Mol. Formula	Structure
1	Diethyl Phthalate	5.89	3.05	222.24	$C_{12}H_{14}O_4$	
2	1-Hexadecanol	6.36	4.02	242.44	$C_{16}H_{34}O$	
3	6,10,14-Trimethylpentadecan-2-one	7.53	7.56	268.49	$C_{18}H_{36}O$	
4	9-Hexadecenoic acid, methyl ester, (Z)-	7.99	6.06	268.44	$C_{17}H_{32}O_2$	
5	Hexadecanoic acid, methyl ester	8.17	11.87	270.46	$C_{19}H_{34}O_2$	
6	n-Hexadecanoic acid	8.46	9.11	256.43	$C_{16}H_{32}O_2$	

7	Oleic Acid	8.84	2.97	326.57	$C_{18}H_{34}O_2$	
8	8,11-Octadecadienoic acid, methyl ester	9.53	4.57	294.47	$C_{18}H_{34}O_2$	
9	9-Octadecenoic acid (Z)-, methyl ester	9.59	7.2	296.50	$C_{19}H_{36}O_2$	
10	Ethanol, 2-(9-octadecenyloxy)-, (Z)-	9.71	5.05	312.54	$C_{20}H_{40}O_2$	
11	Deoxyspergualin	10.214	3.29	423.99	$C_{17}H_{37}O_3$	
12	11,13-Dimethyl-12-tetradecen-1-ol acetate	10.914	5.4	254.41	$C_{18}H_{34}O_2$	
13	Heptacosane	12.874	2.61	380.75	$C_{27}H_{56}$	
14	Hexacosane	13.655	2.6	366.72	$C_{26}H_{54}$	
15	Z)-9-Hexadecenoic acid (Z)-9-octadecenyl ester	16.979	2.62	504.87	$C_{34}H_{64}O_2$	
16	2-Dodecen-1-yl(-)succinic anhydride	17.646	7.3	266.38	$C_{16}H_{26}O_3$	

17 Lathosterol 17.783 4.25 386.66 $C_{27}H_{46}O$

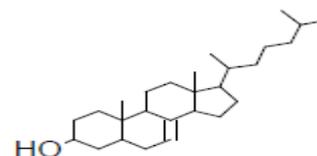


Table 2: Chemical nature and biological activity of phyto-components identified in the methanol extract of *Halymenia dilatata*

S.No	Name of the compound	Chemical nature	Biological Activity
1	Diethyl Phthalate	Phthalic acid ester	Antimicrobial
2	1-Hexadecanol	Palmityl alcohol	Antimicrobial
3	6,10,14-Trimethylpentadecan-2-one	Alcoholic compound	Flavoring Agent, antimicrobial
4	9-Hexadecenoic acid, methyl ester, (Z)-	Palmitic acid ester	Antioxidant, nematocide, pesticide, antiandrogenic, flavor
5	Hexadecanoic acid, methyl ester	Palmitic acid ester	Antioxidant, nematocide, pesticide, antiandrogenic, flavor
6	n-Hexadecanoic acid	Palmitic acid	Antioxidant, nematocide, pesticide, antiandrogenic, antifouling
7	Oleic Acid	Oleic Acid	Anti-inflammatory
8	8,11-Octadecadienoic acid, methyl ester	Linoleic acid ester	Anti-inflammatory, Cancer preventive.
9	9-Octadecenoic acid (Z)-, methyl ester	Oleic acid ester	Anti-inflammatory, Antiandrogenic, Cancer preventive
10	Ethanol, 2-(9-octadecenyloxy)-, (Z)-	Alcoholic compound	Antimicrobial
11	Deoxyspergualin	Amide	Antitumor, cytoprotection, immunomodulation
12	11,13-Dimethyl-12-tetradecen-1-ol acetate	Acetate compound	Not reported
13	Heptacosane	Alkanes	Antioxidant
14	Hexacosane	Alkanes	Antioxidant
15	Z)-9-Hexadecenoic acid (Z)-9-octadecenyl ester	Fatty acid	Not reported
16	2-Dodecen-1-yl(-)succinic anhydride	Alkenyl	Antineoplastic agents, Antioxidants, Antimicrobial
17	Lathosterol	Steroid	Steroid activity

CONCLUSION

The present study has shown that the methanol extract of *Halymenia dilatata* is rich in chemically active compounds with phyto-pharmaceutical importance. Further studies on the isolation of compounds and investigation of biological activity might present more significant results. The findings of the study provide the scientific basis on the importance of seaweeds and the need to explore the naturally available abundant resource of the ocean which can lead to the discovery of novel compounds useful to mankind.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding this manuscript.

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