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PHYTOCHEMICAL ANALYSIS OF HALIMEDA *MACROLOBA* FROM GULF OF MANNAR-BIOSPHERE RESERVE, SOUTHERN INDIA.

Amala jeyakumar J[1], Karuppusamy S[1],and Pannerselvam N[2]

[1] Department of Botany, [2] Department of Microbiology, The Madura College, Madurai, India.

Abstract:

Halimeda macroloba (Chlorophyceae) a green alga was subjected for phytochemical analysis by GC-MS and FTIR spectroscopy methods. GC-MS has recorded the presence of eight peaks among them 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (phytol), hexadecanoic acid, N- hexadecanoic acid, 1-Hexyl-2-nitrocyclohexane and β-sitosterol are major compounds. FTIR analysis has down and the presence of various phytochemicals such as alkane, aldehyde, aromatic, methyl and aliphatic compounds . The present study highlighted the presence of phytocompound from the marine alga H.macroloba can be used for biological and pharmacological studies.

Keywords: *Seaweed, Halimeda macroloba , bioactive compound, GC-MS, FTIR.*

Corresponding author:

Amala jeyakumar J,
Department of Botany,
The Madura College, Madurai, India.
amala.jeyakumar@yahoo.com
Mobile No.: 9842196878

QR code

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INTRODUCTION:

Seaweeds which are multicellular algal species occupied on rock and hard surface in coastal region; diverse of these species are evaluated for the exploration of bioactive compounds with a wide range of biological activities, such as antibiotics, antifungal, antibacterial [1] antiviral [2], antioxidant and anti-inflammatory [3,4]. In *Halimeda* species has polyphenol content of catechin, epicatechin, epigallocatechin gallate, and gallic acid recorded [5] and many studies showed polyphenol potential to induce of apoptosis and cytotoxic activity in vitro studies [6]. Seaweeds are known for a highly nutritive food containing vitamin, protein, mineral, fiber contents, and essential fatty acids. Alginate, carrragenean and agar as phycocolliods have been used for prolong period in medicine and pharmacy and highly renewable living resources. Considering about secondary metabolites of nature products especially from seaweeds has differed because it depends upon parts of specimen, geographic area, season and temperature of water and specific of solvent extraction [7]. Some reports indicate that seaweed is still employed in folk medicine in many parts of the world as treatments of a variety of diseases [8]. Increasing demand of seaweeds in food and chemical industries alternative identification of chemical sources and seaweed cultivation are also parallel increased worldwide. Indian oceanic recorded 1019 species of marine algae [9] and contribution volume nearly 1millon metric tones of total seaweed production [10]. The worldwide aquatic seaweed production rose from 11 million metric tones to 23 millon metric tones by 2003 to 2012 [11]. Consuming of dry algae by Asian people groups approxiamatly1.6 kg per year [12]. To date approximately 16,000 marine natural products have been isolated from marine organisms and reported in approximately 7,000 publications [13].

Gas chromatography mass spectrometry (GCMS) compiled technology and Fourier transform infrared (FTIR) has been emerged as key technological platform to find secondary metabolite isolated from natural source. Fundamentally, FTIR spectra illustrate absorption bands with characteristic frequency attributed to different functional groups whilst GCMS reveals the compounds eluted at different retention times with mass spectra corresponding to compounds present. The signal processing tool exclusively designed for FTIR is not applicable to GCMS chromatograms due to differences in data nature and characteristics [14].

Halimeda macroloba is a green alga, 1- 1.5 cm rounded calcareous thallus (80% $CaCO_3$), calcified segments joints and which occurs lagoon floor along the side of the deep channel above 50 m.

Diterpenoid metabolites halimedatrial and halimedatetraacetate isolated from the *Halimeda* sp. [15,16] and clionasterol, a triterpenoid has been reported [17]. It has been reported antimicrobial [18,19], antioxidant [4] antidiabetic [20] and antitumor properties[21].

MATERIALS AND METHODS:

Sample collection and extract preparation

Fresh specimens of *Halimeda macroloba* was collected by handpicked method in intertidal region of the Mandapam coastal area of Gulf of Mannar Biosphere Reserve and immediately brought to the laboratory in plastic bags containing water to prevent dehydration. Then the plants were washed thoroughly with tap water to remove extraneous materials and epiphytic. Seaweed material as a whole was shade dried to prevent photolysis and powdered with a mixer grinder. The algal sample stirred with ethanol ambient temperature for 20-30 days. The extract was then concentrated and dried under reduced pressure and the semi solid used for experiments.

GC-MS analysis:

The Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250µm df) and the components were separated using helium as carrier gas at a constant flow of 1 ml/min. The injector temperature was set at 260°C during the chromatographic run. The 1µL of extract sample injected into the instrument and the oven temperature was as follows: 60 °C (2 min); followed by 300 °C at the rate of 10 °C min−1; and 300 °C, where it was held for 6 min. The mass detector conditions were: transfer line temperature 240 °C; ion source temperature 240 °C; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS NIST (2008) library.

FTIR Analysis

FT-IR analysis performed with ethanolic extract by attenuated total reflection Shimadzu model. The molecular functional vibrations of chemical groups present in the sample recorded with Shimadzu IR Affinity-1 spectrophotometer operated at a resolution of 2 cm^{-1} ranging from 4000 to 400 cm^{-1}.

RESULTS:

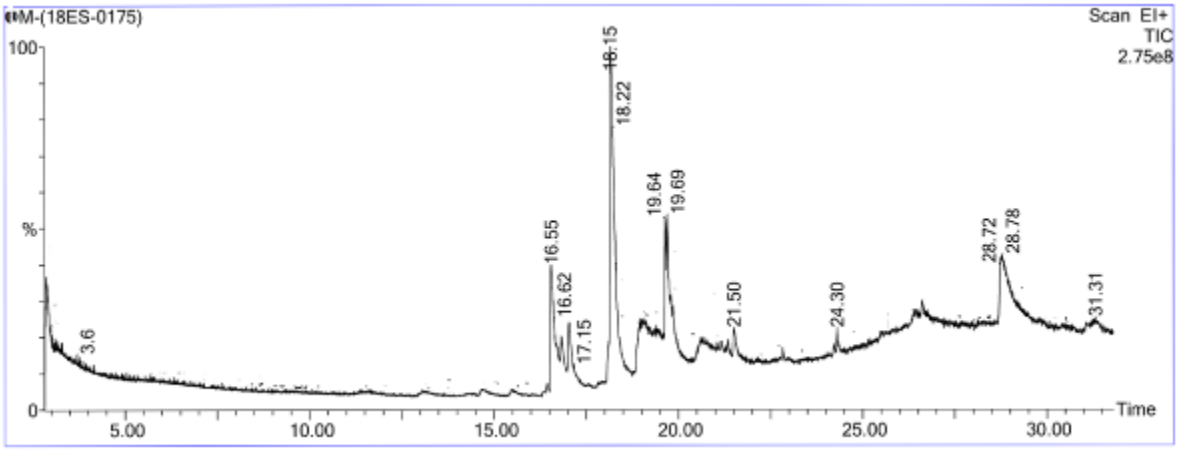

Fig1.GCMS analysis of *Halimeda macroloba* ethanolic extract

GC-MS analysis of *Halimeda macroloba* ethanol extract of led to the identification of eight compounds (Table 1). These compounds were identified through mass spectrometry attached with GC. The identified compounds with their retention time, molecular formula, molecular weight and concentration (peak area %) are presented in Table 1. The Major compounds are observed as 3,7,11,15-Tetramethyl-2-hexadecen-1-ol,hexadecanoic acid, N-hexadecanoic acid,9,12-octadecadienoic acid, 1-hexyl-2-nitrocyclohexane, β-sitosterol.

Table 1: GC-MS analysis of *Helimeda macroloba* ethanolic extract

Peak	RT	Compound	Mol. Wt.	Structure	Area %
1	16.549	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	296	$C_{20}H_{40}O$	13.851
2	16.819	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	296	$C_{20}H_{40}O$	3.049
3	17.034	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	296	$C_{20}H_{40}O$	3.413
4	18.170	Hexadecanoic acid, ethyl ester	284	$C_{18}H_{36}O_2$	38.926
5	19.050	N-Hexadecanoic acid	256	$C_{16}H_{32}O_2$	9.520
6	19.635	9,12-Octadecadienoic acid (z,z)-	280	$C_{18}H_{32}O2$	3.852
7	19.690	1-Hexyl-2-nitrocyclohexane	213	$C_{12}H_{23}O_2N$	6.019
8	28.789	Beta.-sitosterol	414	$C_{29}H_{50}O$	21.368

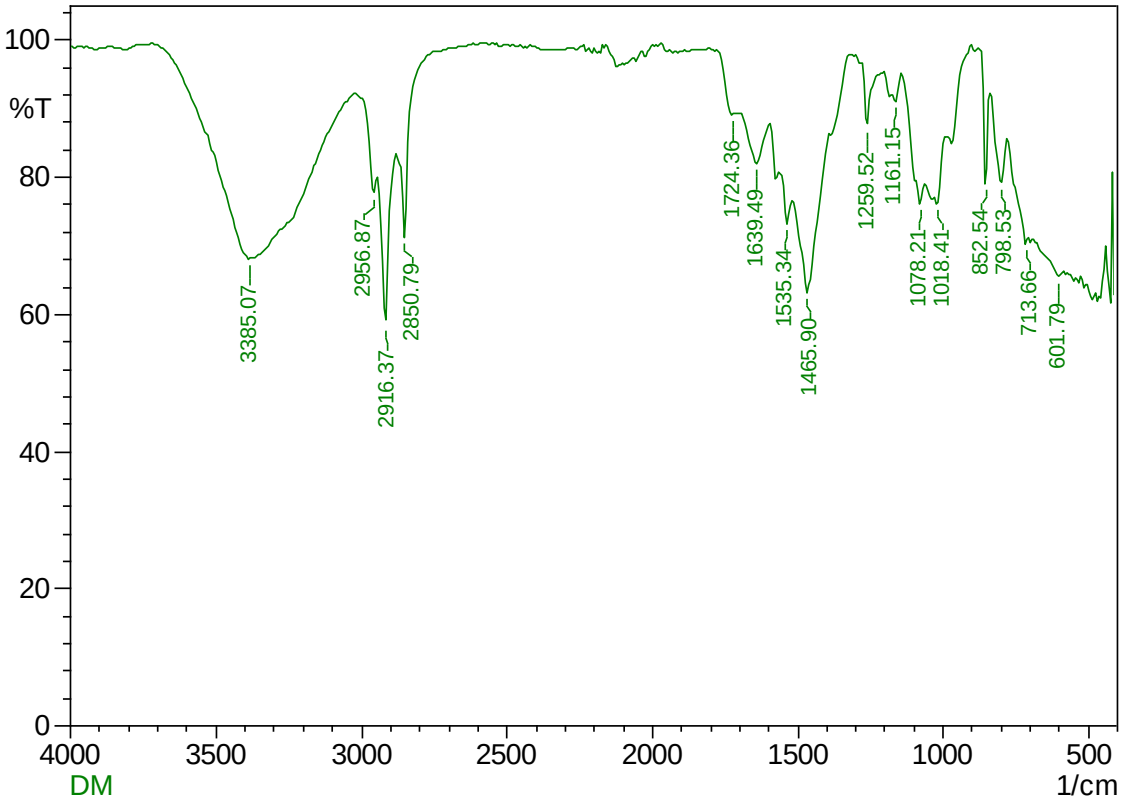

Fig. 2: FTIR analysis of *Helimeda macroloba* ethanolic extract

The more intense bands occurring stretch at 3385.07 alkane (C-H), 2956.87, 2916.37 stretching aldehyde (C-H); 2850.79 alkane (C-H), 1724.36 aldehyde (C=O), 1639.49 aromatic (C=C),1535.34 nitro compounds asymmetric (N–O), 1465.9 methylene(C-H), 1259.52, 1161.15, 1078.21 are stretching for secondary alcohol (C-O), 852.54 aliphatic chloro compound(C-X),798.5, 713.66 aliphatic chloro compound (C-Cl) and 601.79 stretching disulphides (C-S) found with the help of FT-IR spectra radiation.

DISCUSSION:

Analysis of GC-MS and Fourier transform infrared showed matching about the bond of carbon, hydrogen and nitrogen to stretch on particular wavelength helps to find active compound of ethanolic extract of *H.macroloba* and its secondary derivatives have create ionic exchange has eight peaks observed and in this studies high amount of 3,7,11,15-Tetramethyl-2-hexadecen-1-ol(phytol) present and which act as anticancer activity[22] hexadecanoic, which mono unsaturated fatty acid , 9,12-Octadecadienoic acid , β-sitasterol. β-Sitosterol is nontoxic, effective nutritional supplement and has amazing potential health benefits in many diverse applications including antibacterial activity[23].

Aqueous extract of *H.gracilis* and *H. macroloba* showed 29 and 41 compounds respectively, the major compounds are hexadecanoic acid, dibutyl phthalate, n-hexadecanoic acid, octadecenoic acid and benzenedicarboxylic acid [24].

FTIR has sixteen stretching is implicated alkane, aldehyde, amine, potential to create. 3385.07 stretch alkane (C-H), 2956.87, 2916.37 stretching aldehyde (C-H); 2850.79 alkane (C-H), 1724.36 aldehyde (C=O), 1639.49 aromatic (C=C),1535.34 nitro compounds asymmetric (N–O), 1465.9 methylene(C-H), 1259.52, 1161.15, 1078.21 are stretching for secondary alcohol (C-O), 852.54 aliphatic chloro compound(C-X),798.5, 713.66 aliphatic chloro compound (C-Cl) and 601.79 stretching disulphides (C-S) . FT-IR analysis of *Halimeda micronesia* acetone extract showed peak CH_2 and CH_3 aliphatic compounds formation occurred between 2850, 1458 and 1376cm^{-1}. The regions from 1734 to 1638 cm^{-1} represented C=O in anhydrides and C=O secondary amides, C-N band of aromatic amines appeared between 1237 cm^{-1}. The SO_3H in solfonic acids, C-NH_2in primary aliphatic amines, Ar-OH in phenol are those bands of esters that appeared approximately at the range of 1610, 1110 and 669 cm^{-1} [26]. Chloride is important for digestion of food and to absorb many trace elements

that what we need to survive. The FTIR studies on methanol extract of *Acanthophora specifera* shows major peaks at 3371.74, 2835.07,f 2216.74, 2044.31, 1599.53, 1113.83, 1025.47 and 657.53 shows the presence of phenol, alkane, alkene, carboxylic acid, aromatic compound, nitro compound, alcohol, benzene and bromo alkane compounds[26]. Some studies shows [18] acetone and methanol extract of *H.macroloba* inhibit the *Pseudomonas aeruginosa, Proteus mirabilis, Staphylococcus aureus, Escherichia coli, Streptococcus pneumoniae, Candida* sp. and also[27] studies exhibit the various solvent of *H.opuntia* inhibit the *E.coli, Salmonella typhi* and *Klbesiella pneumonia.* Another studies show the 70% of ethanol extract of *H.opuntia* inhibition of *E.coli* and *S. aureus* [28].

Based on different solvent has capable to isolate various compound and FTIR data shows on stretching peak area noted. The GC-MS analysis based on molecule weight and retention time cross checking NIST library in molecule level the bonding between C,H, O and N showed in result [29]. FTIR stretched peak area based on wavelength and transmittance classify aldehyde, carboxylic acid (organic hydrocarbons), amines, alkane, carbonyl compounds, aliphatic compound, halogens and disulphides bond formation based [30].

The present data gives idea about free of non-toxic elements such as Pb, Cd and Al and also above-mentioned compound potential as pharmaceutical aspect. The collective data help to find the compound and purification also needed to find novel compound.
Conclusion

The study on phytochemical leads for novel compound properties is a continuing process of researcher due to arising new era diseases now a day. Trends on exploited much for screening and identification of promising compound from marine sources of algae and another marine biota. *Halimeda macroloba* shows as a good source for potential exploration of promising compound which would be used for pharmaceutical.

REFERENCES:

1. Indira K, Balakrishnan S, Srinivasan, M, Bragadeeswaran S, Balasubramanian T; Evaluation of in vitro antimicrobial property of seaweed (*Halimeda tuna*) from Tuticorin coast, Tamil Nadu, Southeast coast of India. 2013; 12(3): 284-289.
2. Mu J, Hirayama M, Sato Y, Morimoto K, Hori K. A Novel High mannose specific lectin from the green alga *Halimeda renschii* Exhibits a potent antiinfluenza virus activity through high affinity binding to the viral hemagglutinin. Marine Drugs. 2017; 15(8): 255.
3. Boonchum W, Peerapornpisal Y, Kanjanapothi D, Pekkoh J, Amornlerdpison D, Pumas C, Sangpaiboon P, Vacharapiyasophon P. Antimicrobial and antiinflammatory properties of various seaweeds from the gulf of Thailand. International Journal of Agriculture and Biology. 2011;13: 100–104.
4. Santoso J, Stark Y, Suzuki T. Antioxidant activity of methanol extracts from Indonesian seaweeds in an oil emulsion model. Fisheries Science. 2004; 70: 183–188.
5. Yoshie Y, Wang W, Hsieh YP, Suzuki T. Compositional difference of phenolic compounds between two seaweeds, *Halimeda* spp. Journal of Tokyo University Fisheries. 2002; 88:21-24.
6. Braicu C, Pilecki V, Balacescu O, Irimie A, Neagoe IB. The relationships between biological activities and structure of flavan-3-ols. International Journal of Molecular Sciences. 2011; 12(12): 9342-9353.
7. Rajasulochana P, Dhamotharan R, Krishnamoorthy P, Murugesan S. Antibacterial activity of the extracts of marine red and brown algae. Journal America Science. 2009; 5(3):20–25.
8. Liu L, Heinrich M, Myers S, Dworjanyn SA. Towards a better understanding of medicinal uses of the brown seaweed *Sargassum* in traditional Chinese medicine: A phytochemical and pharmacological review. Journal of Ethnopharmacology. 2012; 142: 591–619.
9. Rao PSN and Gupta RK. Algae of India: A checklist of Indian marine algae (excluding Diatoms & Dinoflagellates), Botanical Survey of India.Kolkata, 2015; p93.
10. Dhargalkar VK, Pereria N. Seaweed: promising plant of the millennium. Science and Culture. 2005; 71(3-4): 60-66.
11. FAO.Food and Agricultural Organisation of the United Nations. Year book of fishery statistics. 2012.
12. Fujiwara AT, Mino N, Kuroda M. The protein value in human nutrition of edible marine algae in Japan. Hydrobiologia. 1984;116: 513-516.
13. Blunt JW, Copp BR, Munro MH, Northcote PT, Prinsep MR. Marine natural products. Natural Product Reports. 2005; 22: 15–61.
14. Guillen MD, Cabo N, Ibargoitia ML, Ruiz A.Study of both sunflower oil and its headspace throughout the oxidation process. Occurrence in the headspace of toxic oxygenated aldehydes. Journal of Agricultural and Food Chemistry. 2005;53(4):1093-1101.

15. Paul VJ and Fenical W. Isolation of halimedatrial: chemical defense adaptation in the calcareous reef-building alga *Halimeda*. Science. 1983; 221(4612):747-749.
16. Paul VJ and Fenical W. Bioactive diterpenoids from tropical marine algae of the genus *Halimeda*. Tetrahedron. 1984b; 40: 3053-3062.
17. Dzeha T, Jaspars M, Tabudravu J. Clionasterol, a triterpenoid from the Kenyan marine green macroalga *Halimeda macroloba*. Western Indian Ocean Journal of Marine Science. 2003; 2(2): 157-161.
18. Govindasamy C, Narayani S, Arulpriya M, Ruban P, Anantharaj K, Srinivasan R. In vitro antimicrobial activities of seaweed extracts against human pathogens. Journal of Pharmacy Research. 2011;4(7):2076-2077.
19. Nor Afifah S, Darah I, Shaida Fariza S, Mohd Jain Nordin MK, Nurul Aili Z. Antimicrobial activity of various extracts of a tropical Chlorophyta macroalgae, *Halimeda discoidea*. Journal of Applied Sciences. 2010; 10: 3007-3013.
20. Chin YX, Lim PE, Maggs CA, Phang SM, Sharifuddin Y, Green BD. Antidiabetic potential of selected Malaysian seaweeds. Journal of Applied Phycology. 2015;27(5): 2137–2148.
21. Sanja M, Tatjana K, Vesna M, Jelena AS, Nada G, Ivana D, Ivan S, Tatjana S. Anti α-glucosidase, antitumour, antioxidative, antimicrobial activity, nutritive and health protective potential of some seaweeds from the Adriatic coast of Montenegro. Farmacia. 2017; 65(5)**:**731-740.
22. Pejin B, Kojic V, Bogdanovic G. An insight into the cytotoxic activity of phytol at in vitro conditions. Natural Product Research. 2014; 28(2):2053-2056.
23. Sen A, Dhavan P, Shukla KK, Singh S, Tejovathi G. Analysis of IR, NMR and antimicrobial activity of β-sitosterol isolated from *Momordica charantia*. Science Secure Journal of Biotechnology. 2012; 1(1):9–13.
24. Mangaiyarkarasi R, SabariAnandh JV, Thamiz Selvan E, Kumar Pillai A. Metabolomic chemical signature of the seaweeds (marine macro algae) *Halimeda gracilis* and *Halimeda macroloba* extracts by GC-MS analysis. In International Summit on Fisheries and aquaculture, 2018, Netherlands.
25. Ganeshamurthy R, Ajithkumar TT, Dhayanithi NB. Effect of secondary metabolites of the seaweed (*Halimeda micronesia*) at Lakshadweep islands against aquatic pathogens. International Journal of Pharma and BioSciences. 2012; 3(2):213-220.
26. Rajeshkumar R and Jeyaprakash K. Screening of UV-VIS, TLC and FTIR spectroscopic studies on selected red seaweed (*Acanthophora specifera*) collected from Gulf of Mannar, Tamilnadu, India. World Journal of Pharmaceutical Sciences. 2016; 4: 28-33.
27. Al -Judaibi A. Antibacterial effects of extracts of two types of red sea algae. Journal of Biosciences and Medicines. 2014; 2:74-82.
28. Mishra JK, Srinivas T, Madhusudan T, Sneha S. Antibacterial activity of seaweed *Halimeda opuntia* from the coasts of South Andaman. Global Journal of Bio-Science and Biotechnology. 2016; 5(3): 345-348.
29. Adams R.P. Identification of essential oil compounds by gas chromatography and mass spectrometry; 4th edn. 2009; Allured publishing Corporation, Coral Stream, IL.
30. Thangarajan S, Paramasivam R, Chinthamony A, Palanisamy C, Perumal V, Kanniappan G. Element and functional group analysis of *Ichnocarpus frutescens* r. br. (Apocynaceae). Int J Pharma Pharmace Sci. 2012;4(5): 343-345.