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Screening and Characterization of Bioactive Compounds of Turbinaria Ornata from the Gulf of Mannar, India

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Abstract: Objective: To screen and characterize the bioactive compounds in the brown seaweed *Turbinaria ornata*. Methods: The brown seaweed, *T. ornata* was collected from Nalupanai coast of Gulf of Mannar. It was cleaned from epiphytes, washed, shade dried and powdered. Algal extraction was carried out using distilled water, ethanol, methanol, acetone and petroleum ether. The crude methanolic extract was subjected to GC-MS analysis to reveal the phyco-constituents. Results: The results obtained in the phytochemical screening supports that the seaweeds contain biologically active substance. Methanol was identified as the most appropriate solvent to extract the bioactive compounds. Different fatty acids and volatile compounds were identified in the GC-MS analysis. The volatile mixture comprised of hydrocarbons, acids, aldehydes, ketones, esters, ethers, alcohols, halogenated and aromatic compounds.

Keywords: *Turbinaria ornata*, phycochemical screening, bioactive compounds, GC-MS.

1. INTRODUCTION

Seaweeds are the extraordinary sustainable resources in the marine ecosystem which have been used as a source of food, feed and medicine. Many secondary metabolites have been isolated from them with different pharmacological activities. These pharmacological activities are due to the presence of the bioactive principles and such biopotentials of the seaweeds are revealed by its phycochemical constituents.

Owing to their possible economical use in various fields such as food, pharmaceutical and textile industries [1, 2], seaweed resources have attracted the attention of scientists all over the world. Algal dietary fiber, mainly consisting of soluble polysaccharides plays an important role in the modification of lipid metabolism in human body [3, 4, 5, 6]. They are known to have curative powers for tuberculosis, arthritis, cold and influenza and worm infestations.

They have been a source for the production of a variety of major metabolites such as polysaccharides, lipids, proteins, carotenoids, vitamins, sterols, enzymes, antibiotics and many other fine chemicals [7, 8, 9, 10]. Seaweeds are not only the source of major metabolites but are an extensive source of secondary metabolites too. More than 600 secondary metabolites have been isolated from marine algae [11, 12]. Many of the secondary metabolites of seaweeds are toxic substances which act as chemical defense systems for protecting them from grazers.

In tropical algae, production of secondary metabolites is upto 7% of dry weight and these metabolites show antibacterial, antifungal, antiviral, antitumour, antifouling, cytotoxic and other activities [13]. Although a majority of these (about 60%) are terpenes, some fatty acids are also common (20%) with nitrogenous compounds [14, 15] and many of these compounds are bioactive and have been extensively experimented in bioassays and pharmacological assays. In the present study, an attempt has been made to characterize the bioactive principles of *Turbinaria ornata*.

2. MATERIALS AND METHODS

2.1. Collection area

Turbinaria ornata was collected randomly from the intertidal regions of the Nalupanai (Lat. 09° 17.417'N; Long. 79° 08.558'E) coast (Ramanathapuram District) of the Gulf of Mannar, Southeast coast of India. The Gulf of Mannar is the first Marine Biosphere Reserve in India and in the Southeast Asia. It is one of the world's richest regions from a marine biodiversity perspective.

2.2. Collection and processing

The algae was washed with seawater and then in fresh water to remove the extraneous materials and then air-dried. The seaweeds were transported to the laboratory in sterile polythene bags at 20°C temperature. In the laboratory, algal sample was rinsed with sterile distilled water, shade dried, cut into small pieces and powdered in a mixer grinder. It was stored in air-tight polypropylene container at room temperature.

2.3. Crude extraction of phytochemicals

T. ornata (powder) was extracted successively with 500 ml of each of distilled water, acetone, methanol, ethanol, and petroleum ether by maceration and with continuous shaking on a rotary shaker at 150-180 rev/min for 72 hr. The extracts were then filtered using Whatman No. 1 filter paper and the filtrate was concentrated under reduced pressure to dryness. The residual extracts obtained were kept in desiccator for further investigation.

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2.4. Phytochemical screening of seaweed extracts

Seaweed extracts were subjected to various qualitative chemical tests to screen the phytochemical constituents [16, 17, 18]. The extract was detected for the presence of alkaloids, phenolic compounds, flavonoids, terpenoids, tannins, quinines, saponins, sterols, carbohydrates, proteins and amino acids.

2.5. GC-MS analysis for phycochemical constituents

A high resolution mass spectrum equipped with a data system in combination with Gas Chromatography was used for the chemical analysis of seaweeds. The aqueous methanolic extract of *Turbinaria ornata* was examined for its chemical composition using GC-MS THERMO GC - TRACE ULTRA Version-5.0. The standard non - polar capillary column, with dimension of 30 m X 0.25 mm X 0.25 µm was used and injection volume was one micro litre. The extract was diluted in methanol and injected in the split mode. The carrier gas was helium and the flow rate was 1.0 ml/min. The temperature was programmed to an oven temperature of 80° C raised to 260°C at 5°C /min. The run time was 43.15 min. The chemical constituents were identified after comparison with the data available in the library search results attached to the GC-MS instrument.

3. RESULTS

3.1. Phytochemical screening

Turbinaria ornata showed the presence alkaloids, terpenes, phenolics, tannins, saponins, flavonoids, quinones, proteins, sugars and sterols in various extracts are given in the table 1.

Table 1. Phytochemical screening of Turbinaria ornata in different extracts.

Phyto chemicals	Dis. water	Ace tone	Meth anol	Etha nol	Pet. ether
Alkaloids	✓	✓	✓	✓	×
Terpenes	×	✓	✓	✓	×
Phenolics	✓	×	✓	✓	×
Sugars	×	✓	×	×	×
Tannins	×	✓	✓	✓	×
Saponins	✓	✓	✓	✓	✓
Flavonois	✓	×	✓	✓	×
Quinones	✓	✓	✓	✓	×
Proteins	✓	×	✓	✓	×
Sterols	×	×	✓	✓	×

✓ Connotes presence of phytochemicals;
× Connotes absence of phytochemicals

3.2. GC-MS analysis

The GC- MS analysis of crude methanolic extract of *Turbinaria ornata* showed the presence of a mixture of volatile compounds. A total of 17 peaks were observed with different retention times as presented in Fig. 1. The molecular formula and molecular weight for the compounds identified were fetched from the library search results of the GC-MS systems and are given in Table 2.

Phycochemicals in the methanolic extract of *Turbinaria ornata* elucidated by GC-MS analysis were classified into different groups as halogenated and polycyclic aromatic compounds, ethers, esters, acids, amines, alkanes, aldehydes, nitrogenous compounds and terpenes.

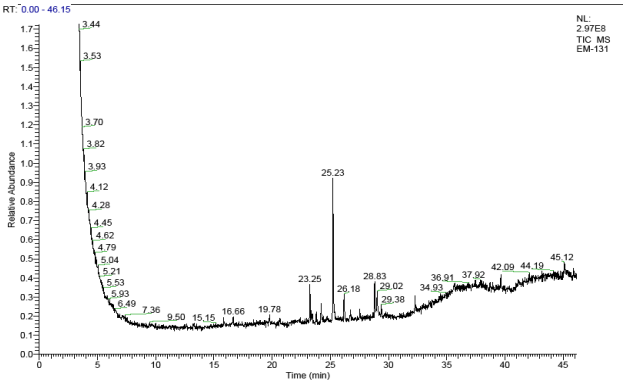

Figure 1. GC-MS chromatogram of the crude extract of Turbinaria ornata

Table 2. Phytochemical composition of methanolic extract of Turbinaria ornata by GC-MS analysis.

S. No	RT	Phytochemical compounds	Molecular formula	Molecular weight
1	7.36	1,2,3,4-Tetrachloroben zene	$C_6H_2Cl_4$	215.89
2	9.50	Diethylether	$C_4H_{10}O$	74.12
3	15.15	1,2-Benzenedicarb oxylic acid bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$	278.34
4	16.66	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42
5	19.78	Benzo(k)fluora nthene	$C_{20}H_{12}$	252.30
6	23.25	Bufencarb-2	$C_{13}H_{19}NO_2$	221.30
7	25.23	Heptachlor	$C_{10}H_5Cl_7$	373.31
8	26.18	Kresoxim-Methyl	$C_{18}H_{19}NO_4$	313.34
9	28.83	n-Tetradecane	$C_{14}H_{30}$	198.38
10	29.02	Isopropyl isothiocyanate	C_4H_7NS	101.17
11	29.38	Di-n-octylphthalate	$C_{24}H_{38}O_4$	390.55
12	34.93	Vanillylmandel ic acid	$C_9H_{10}O_5$	198.17
13	36.91	Tetramethrin-1	$C_{19}H_{25}NO_4$	331.40
14	37.92	Acetamiprid	$C_{10}H_{11}ClN_4$	222.67
15	42.09	Eicosapentaeno ic acid (EPA)	$C_{20}H_{30}O_2$	302.45
16	44.19	Heptanal	$C_7H_{14}O$	114.18
17	45.12	Humulene epoxide III	$C_{15}H_{24}O$	220.35

The presence of different bioactive compounds in each group is as follows.

Aromatic compounds: Halogentated aromatic compound - 1,2,3,4 – tetrachlorobenzene and helptachlor. Polycyclic aromatic compound - Benzo (K) fluoranthene and Kresoxim methyl. ***Ethers:*** Diethyl ether. ***Fattyacids:*** Saturated fattyacid - n- Hexadecanoic acid and

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Unsaturated fatty acid -Eicospentaenoic acid (EPA). ***Esters:***1,2- Benzene dicarboxylic acid bis (2-methyl propyl) ester and Di-n-octlyphthalate. ***Amines:*** Vanillylmandelic acid. ***Alkane:*** Tetradecane. ***Aldehydes:*** Heptanal. ***Terpenes:*** Humulene epoxide –III, a monocyclic sesquiterpene. ***Nitrogenous compounds:*** Bufencarb -2, Kresoxim methyl, Isopropyl isothiocyanate, Tetramethrin and Acetamiprid.

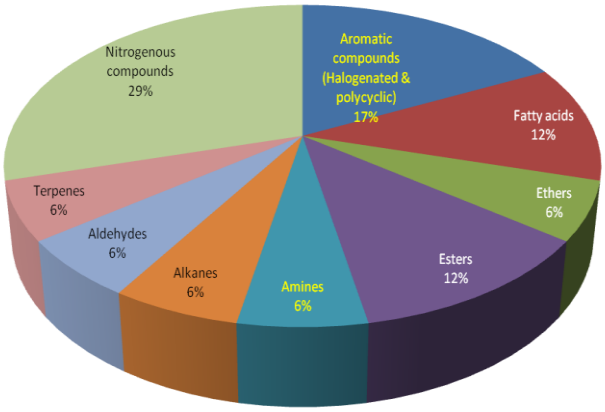
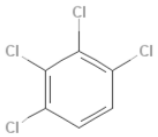
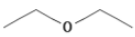
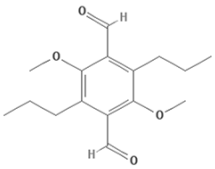
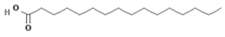
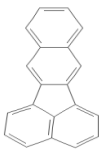
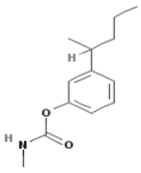
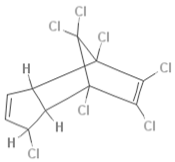
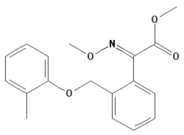
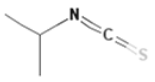
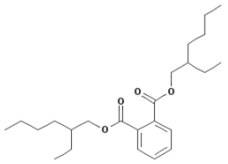
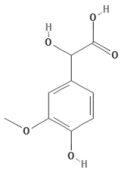
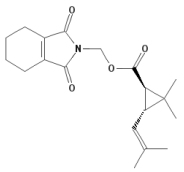
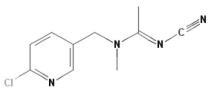
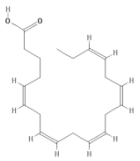
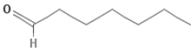
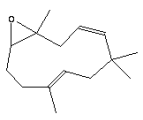


Figure 2. Phytochemical composition of Turbinaria ornata

Table 3. Structure and retention time of secondary metabolites from Turbinaria ornata by GCMS analysis.

S. No	Name of the compound	Structure of compound	Retention time (min)
1	1,2,3,4-Tetrachloro benzene		7.36
2	Diethylether		9.50
3	1,2-Benzenedicarboxylic acid bis(2-methylpropyl) ester		15.15
4	n-Hexadecanoic acid		16.66
5	Benzo(k)fluoranthene		19.78
6	Bufencarb-2		23.25
7	Heptachlor		25.23
8	Kresoxim-Methyl		26.18
9	n-Tetradecane		28.83
10	Isopropyl isothiocyanate		29.02
11	Di-n-octylphthalate		29.38
12	Vanillylmandelic acid		34.93
13	Tetramethrin-1		36.91
14	Acetamiprid		37.92
15	Eicosapentaenoic acid		42.09
16	Heptanal		44.19
17	Humulene epoxide III		45.12

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4. DISCUSSION

4.1. Screening of bioactive compounds

Active constituents synthesized by the marine genera are used in traditional and complementary medicine. Different groups of marine algae were reported to contain active ingredients that can cure diseases. Higher percentage of population prefers to use remedies of natural origin for curing illness as these claimed to produce less side effects [19].

The environment in which the seaweeds grow is harsh as they are exposed to a combination of light and high oxygen concentrations. These factors can lead to the formation of free radicals and other strong oxidizing agents but seaweeds seldom suffer any serious photodynamic damage during metabolism. This fact implies that the seaweeds contain large amount of secondary metabolites which are meant for the protective mechanisms of the plants to survive in the fragile environment [20]. In the present study also the seaweeds collected from the rocky coasts with intensive wave action revealed the presence of phycochemicals such as alkaloids, phenolics, flavonoids, terpenes, tannins, quinones, saponins, sterols, carbohydrates and proteins.

The predominant phytochemicals observed in *Turbinaria* studied were alkaloids, phenolics, flavonoids and quinones in ethanolic and methanolic extracts. Similarly, the preliminary phytochemical screening of ethanolic (70%) extract of three marine algae *Chaetomorpha antennina, Gracilaria corticata* and *Ulva faciata* from Visakhapatnam coast, Andhra Pradesh, India, showed positive results for bioactive compounds like steroids, terpenoids, alkaloids, glycoside, amino acids, carbohydrates, saponins and oils [21].

A similar result was found in *Gelidium acerosa* which contained large amount of valuable phytochemicals like saponins, flavonoids and alkaloids etc., which are known for its medicinal uses. The preparations of the seaweeds are also useful for the common ailments, including dysentery, hypertension, urinary tract infection, and some other microbial infections among people [22].

Of the five different extracts tried (distilled water, acetone, methanol, ethanol and petroleum ether), methanolic extract exhibited the presence of most of the phytochemicals. Similar result was seen in the preliminary phytochemical screening of *Codium decorticatum,* marine algae which revealed the presence of alkaloids in all the extracts [23].

The results obtained in the phytochemical screening supports that the seaweeds in general contain biologically active substances. The methanolic extract was proved to be the best solvent for the extraction of phytochemicals in seaweeds.

4.2. Phycochemical characterization

In the present study, the brown seaweed *Turbinaria ornata* exhibited the presence of alkanes, acids, aldehydes, esters, ethers, amines, terpenes, nitrogenous compounds, halogenated and polycyclic aromatic compounds. Similar compounds were identified commonly in three seaweeds *Calulerpa lentillifera, Kappaphycus alvarezii* and *Sagassum polycystum* from Borneo [24]. The bioactive compounds recorded in the present study are discussed here for their uniqueness and importance in the coastal ecosystem, highlighting their possible exploitation as leads in therapeutics.

4.2.1. Hydrocarbons:

The volatile compounds of *Turbinaria ornata* showed mainly hydrocarbons, as reported in several seaweeds [24, 25].

4.2.2. Fatty acids:

The fats and fatty acids from marine organisms can play an important role due to the wide diversity of their biological characteristics and their oxidative enzymes leading to the formation of many other bioactive secondary metabolites [26]. Seaweeds exhibit a high level of fatty acid diversity and many of which possess potential bioactivity.

The present GC-MS investigation of phytochemicals validates the presence of saturated fatty acid, n-hexadecanoic acid and unsaturated fatty acid eicosapentaenoic acid in the brown seaweed, *Turbinaria ornata*. Hai-Lan Huang, and Bin-Gui Wang (Asean Biodiversity) also identified three fatty acids, tetradecanoic acid, hexadecanoic acid and octadecanoic acid in four brown seaweeds viz. *Laminaria japonica, Plocamium telfairiae, Rhodomela confervoides* and *Symphyocladia latiuscula* at various concentrations.

Turbinaria ornata showed the presence of n-hexadecanoic acid which is also called as palmitic acid. It is a common fatty acid in brown algae and in the genus *Sargassum* represented 20 to 40% of the total fatty acids [27, 28, 29, 30]. In *S. muticum,* palmitic acid constituted 21.5% of the total fatty acids [27]. Similarly tridecatrienoic (28.1%) and palmitic (13.93%) acids in highest amounts were identified in two green seaweeds *Bryopsis pennata* and *Valoniopsis pachynema* [31].

Also, the chemical characteristic of active fraction of *Laurencia brandenii* was found to be a mixture of fatty acids. The fatty acid composition of the active fraction revealed that the major composition was octadecadienoic acid (49.75 %) followed by n-hexadecanoic acid (14.24 %) Further it was observed that in *L. brandenii,* the biological activity was due to the presence of fatty acid, octadecadienoic acid (49.75 %) in higher percentage [32]. The biological activity of *Turbinaria ornata* reported in the present study might also be attributed due to the presence of fatty acids, n-hexadecanoic acid and eicosapentanoic acid.

The eicosapentaenoic acid recorded in the present study is one of the several omega-3 fatty acids used by the biological systems. It is found in cold water fatty fish, such as salmon. It is also found in fish oil supplements, along with docosahexaenoic acid (DHA). Omega-3 fatty acids are part of a healthy diet that helps lower risk of heart disease. Getting more EPA in our diet helps to

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prevent/control coronary heart disease, high triglycerides (fats in the blood), high blood pressure, and inflammation.

4.2.3. Esters:

Turbinaria ornata showed the presence of 1,2, benzenedicarboxylic acid bis (2-methylpropyl) ester. The ethanolic extract of *Ulva pertusa* indicated the presence of 1,2-benzenedicarboxylic acid bis (2-methylpropyl) ester which exhibited strong allelopathic activity against the growth of on *Gymnodinium breve* Davis [33].

Turbinaria ornata, is also reported to contain a phthalate ester namely di-n-octylphthalate. The 1,2 benzenedicarboxylic acid bis(2-ethylhexyl) ester (or dioctyl phthalate; di-(2-ethylhexyl)-phthalate or DEHP), which is a plasticizer constitutes 16% of the active fraction of a brown algae *Sargassum muticum* [34].

Phthalate esters are the likely contaminants from plastics in the laboratory encountered during the extraction or isolation process and are commonly found during natural products isolation. However, phthalate ester may also come from the coastal environment and/or reflect a phenomenon of bioaccumulation [20].

Phthalate esters have been found in soils, plants, and aquatic organisms [35, 36, 37, 38, 39, 40, 41]. Because of their lipophilicity, they can be potentially bioaccumulated by the organisms [42]. The red alga *Bangia atropurpurea* was synthesising this compound di-(2-ethylhexyl)-phthalate *de novo* [40]. It has also been isolated from *Ceramium rubrum*, but the origin of this phthalate had not been elucidated [37].

Dioctyl phthalate has also been isolated from the brown algae *Sargassum wightii* [43], *Ishige okamurae* [41] and *S. confusum* [26] which has antibacterial potential against several species of bacteria (*Staphylococcus aureus, Proteus vulgaris, E. coli, Salmonella typhi, S. paratyphi A, S. typhiridium* and *Pseudomonas aeruginosa).*

Similarly the GC-MS profile of the algal extract of *Sargassum marginatum* revealed the presence of diethyl phthalate (tR =11.34min), (84.45%), which might be the reason for its bioactive potential. Thus *S. marginatum* might be natural source suitable for development of environmentally compatible agrochemicals [32].

4.2.4. Aldehydes:

In the present investigation, an alkyl aldehyde, heptanal was identified in *Turbinaria ornata.* It is a flavoring agent with sweet aroma. Similarly, the heptanal compound was isolated in dried Kombu (*Laminaria* spp.), a brown seaweed [44].

4.2.5. Terpenes:

Turbinaria exhibited the presence of Humulene epoxide III, a sesquiterpene, which is an essential oil present in the flower buds of hops plant (*Humulus lupulus*). Similarly, several terpenes were observed in the crude extracts of the brown seaweed *Dictyota menstrualis*, which is known to produce diterpenes as their major secondary metabolites [45].

4.2.6. Halogenated aromatic compounds:

Turbinaria ornata also contains 1,2,3,4-tetrachlorobenzene which are hazardous halogenated aromatic compounds. It is mainly used in industries as an intermediate in the production of fungicides, herbicides, and defoliants (2,4,5-T), and insecticides [46]. The source of Tetrachlorobenzenes in seaweeds might be pollution. 1,2,3,4-Tetrachlorobenzene has been found in various watercourses in Canada, primarily in the Great Lakes basin. When present above detection limits, concentrations have been reported to range from <0.000 01 to 0.126 mg×L-1. Elevated levels, some above the ranges previously noted, have been reported in industrial effluents in Ontario and Nova Scotia [47].

The bioactive compounds of *Turbinaria ornata* include essential fatty acids, essential oils, flavouring agents, plasticizer, fungicides and insecticides. These compounds are beneficial to mankind in various aspects. These compounds can be extracted from the seaweed and are utilized.

The present study is successful in identifying potential candidate seaweed from Southeast coast of India, *Turbinaria ornata* which can be exploited for the extraction of bioactive compounds. Considering its rich diversity of secondary metabolites, it is expected that this seaweed might be a promising bioactive and ecofriendly alternative.

5. CONCLUSION

The study reveals the bioactive potential of *T. ornata* and to exploit its bioactive potentials for the production of valuable therapeutics and other related compounds of economic viability and social accessibility.

Conflict of interest statement

We declare that we have no conflict of interest.

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REFERENCES

1. Ruggieri G.D., 1976. Drugs from the sea. *Science*, 194: (4264), 491-7.
2. Chen C.S., 1977. An ecological and floristic study of marine algal community along the coastal area of Ho-Pin-Tao. *Bull. Tai. Fish. Res. Inst.* 28: 113-121.
3. Guven K.C., S. B. Ulutin, E. Mutluay and O.N. Ulutin., 1973. Anticoagulant, antithrombin and fibrinolytic actions of extract of marine alga *Corallina rubens L., Haemostasis*., 2(6):260-268.
4. Ito H. and Suguira, M., 1976. Antitumour polysaccharide fraction from *Sargassum thunbergii*. *Chem. Pharm. Bull*., 24(5): 1114-1115.
5. Effinmov V.S., A.I. Usov, T.S. Olskaia, A.I. Baliunis and M. Rozkin, 1983. Comparative study of the anticoagulant activity of sulfated polyscaacharides from marine algae. *Farmakol Toksiko.*, 46(3): 61-67.
6. Okai Y., K. Higashi-Okai, Y. Yano and S. Otani, 1996. Identification of antimutagenic substances in an extract of edible red alga, *Porphyra tenera* (Asakusa-nori). *Cancer Lett.*, 100(1-2): 235-240.

7. Targett N.M. and A. Mitsui, 1979. Toxicity of subtropical marine algae using fish mortality and red blood cells hemolysis for bioassays. *J. Phycol*., 15: 181-185.
8. Fenical W., 1982. Natural product chemistry in the marine environment. *Science*, 215(4535): 923-928.
9. Colwell R.R., 1983. Biotechnology in marine science. *Sci.*, 222(1): 1924.
10. Stein J. and C. Borden, 1984. Causatine and beneficial algae: human disease condition- A review, *Phycologia*, 23: 485-501.
11. Faulkner D.J., 1984. Marine natural products, metabolites of marine algae and herbivorous marine mollusks. *Nat. Prod. Rep.,* 3: 251-281.
12. Faulkner D.J., 1986. Marine natural products. *Nat. Prod. Rep.,* 3: 2-33.
13. Oza R.M. and Zaidi, 2001. *A revised check list of Marine algae*, CSMCRI, Bhavnagar. 341.
14. Paul V.J. and W. Fenical, 1987. Natural products chemistry and chemical defense in tropical marine algae of the phylum Chlorophyta. In: *Bioorganic Marine Chemistry.* P. J. Scheuder (ed.). *Spring-Verlag, Berlin*., 1-29.
15. Van Alstyne K.L. and V.J. Paul, 1988. The role of secondary metabolites in marine ecological interactions. In: *Proc. Sixth Int. Coral Reef Conf*., Townsville, Australia.
16. Sofowara, A., 1993. *Medicinal Plants and Traditional Medicine in Africa*. Spectrum Books Ltd., Ibadan, Nigeria, 289.
17. Audu S.A., I. Mohammed and H.A. Kaita, 2007. Phytochemical screening of the leaves of Lophira lanceolata (Ochanaceae). *Life Sci. J*., 4, (4): 75-79.
18. Roy S., K. Rao, Ch. Bhuvaneswari, A. Giri and L.N. Mangamoori, 2010. Phytochemical analysis of *Andrographis paniculata* extract and its antimicrobial activity. *World J. Microbiol. Biotech*., 26: 85-91.
19. Tyagi N. and Bohra, 2002. Screening of phytochemicals of fruit plant and antibacterial potentials against *Pseudomonas aeruginosa*. *Biochem. Cell. Arch*., 2(1):21-24.
20. Peakall D. B., 1975. Phthalate esters: Occurence and biological effects. *Residue Reviews*, 54:1–41.
21. Ganga Rao Battu, E. Sambasivarao, P. Prayaga Murthy, V.S. Praneeth, and T. Mallikarjuna Rao, 2011. *In-Vitro* antibacterial activity and preliminary phytochemical screening of three algae from Visakhapatnam coast, Andhra pradesh, India. *Int. J. of Pharmcol. and Pharmaceu. Sci*., 3(4), 399-401.
22. Elsie B. Hebsibah, 2010. Evaluation of antimicrobial activity and phytochemical screening of *Gelidium acerosa, J. Pharma. Sci. and Res.*, 2(11): 704-707.
23. Anbu Jeba Sunilson, J., R. Suraj, K. Anandharajagopal, G. Rejitha, M. Vignesh and P. Promwichit, 2009. Preliminary phytochemical analysis, elemental determination and antibacterial screening of *Codium decorticatum* – A marine algae. *Acad. J. Inc*., 3(2): 84-89.
24. Vijaya Ratinam, 2009. Study of volatile compounds extraction using SPME-GCMS in local seaweeds of *Kappaphycus alvarezii, Caulerpa lentilifera* and *Sagassum polycystum*. University of Malaysia Sabah. (Unpublished)
25. Flament I. and G. Ohloff, 1984. Volatiles constituents of algae. Progress of Flavour Research Proceedings of 4th Weurman Flavour Research Symposium, Dourdan, France. 281-296.
26. Ganti V.S., K.H. Kim, H.D. Bhattarai and H.W. Shin, 2006. Isolation and characterisation of some antifouling agents from the brown alga *Sargassum confusum. J. Asian Nat. Prod. Res.,* 8(4): 309-315.
27. Vaskovsky V.E., S.V.Khotimchenko, B. Xia and L. Hefang, 1996. Polar lipids and fatty acids of some marine macrophytes from the Yellow Sea. *Phytochemistry* 42: 1347-1356.
28. Li X., X. Fan, L. Han and Q. Lou, 2002. Fatty acids of some algae from the Bohai Sea. *Phytochemistry*, 59(2): 157-61.
29. Hossain Z., H. Kurihara and K.Takahashi, 2003. Biochemical composition and lipid compositional properties of the brown alga *Sargassum horneri. Pakistan J. Biol. Sci.* 6(17): 1497-1500.
30. Kornprobst J.M., 2005. Substances of natural origin in the marine. Chimiodiversity- Pharmacodiversity-Biotechnology. *Generalites Micro-organisms,* Algues. Lavoisier, Paris. 234.
31. Aliya R., M. Shameel, K. Usmanghani, S. Sabiha, and V. U. Ahmed, 1995. Fatty Acid compositions of two siphonaceous green algae from the coast of Karachi. *Pak. J. Pharm. Sci.*, 8(2): 47-54.
32. Aseer Manilal, S. Sujith, B. Sabarathnam, G. Seghal Kiran, Joseph Selvin, Chippu Shakir and Aaron Premnath Lipton. 2010. Antifouling potentials of seaweeds collected from the Southwest coast of India, *World J. of Agri. Sci.*, 6 (3):243-248.
33. Wang Zhen-Yu, Tian Zhi-Jia, Li Feng-Min, An Zhen and Hu Hong-Ying, 2008. Allelopathic effects of large seaweeds on red tide dinoflagellate *Gymnodinium breve, Allelopathy*, 22(1):181-188.
34. Bazes A., A. Silkina, P. Douzenel, F. Faÿ, N. Kervarec, D. Morin, J.-P. Berge and N. Bourgougnon, 2009. Investigation of the antifouling constituents from the brown alga *Sargassum muticum* (Yendo) Fensholt. *J. Appl. Phycol*. 10 (1007), 1573–1576.
35. Morris R. J., 1970. Phthalic acid in deep sea jellyfish Atolla. *Nature*. 227: 1264.
36. Melancon M.J. and J.J.Lech, 1976. Distribution and biliary excretion products of di-2- ethylhexyl phthalate in rainbow trout. *Drug Metab. Dispos.* 4(2): 112-118.
37. Noguchi T., M. Ikawa, J.J. Uebel and K.K. Andersen, 1979. Lipid constituents of the red algae *Ceramium rubrum*. A search for antimicrobial and chemical defense substances. In: Hoppa HA, Levring T., Tanaka Y., editors. *Marine algae in pharmaceutical science*. New York: Walter de Gruyter & co, 711-718.
38. Wofford H.W., C.D.Wilsey and G.S.Neff, 1981. Bioaccumulation and metabolism of phthalate esters by oysters, brown shrimp and sheepshead. *Ecotox. Environ. Safe.* 5(2): 202-210.
39. Stales C.A., D.R. Peterson, T.F. Parkerton and W.J. Adams, 1997. The environmental fate of phthalate esters: a literature review. *Chemosphere* 35(4): 667-749.
40. Chen C.Y., 2004. Biosynthesis of di-(2-ethylhexyl) phthalate (DEHP) and di-n-butyl phthalate (DBP) from red alga *Bangia atropurpurea*. *Water Res.* 38(4):1014-1018.
41. Cho J.Y, J.S. Choi, S.E. Kang, J.K. Kim, H.W. Shin and Y.K. Hong, 2005. Isolation of antifouling active pyroglutamic acid, triethyl citrate and di-n-octylphthalate from the brown seaweed *Ishige okamurae*. *J. Appl. Phycol*. 17: 431-435.
42. Mackintosh C.E., J. Maldonado, J. Hongwu, N. Hoover, A. Chong, M.G. Ikonomou, and F.A. Gobas, 2004. Distribution of phthalate esters in a marine aquatic food web: comparison to polychlorinated biphenyls. *Environ. Sci. Technol.* 38(7): 2011-20.
43. Sastry V. M. V. S. and G. R. K. Rao, 1995. Dioctyl phthalate and antibacterial compound from the marine brown alga Sargassum wightii. *J. Appl Phycol*., 7:185–186.
44. Takahashi H., H.Sumitani, Y. Inada, and D. Mori, 2002. Identification of volatile compounds of Kombu (*Laminaria* spp.) and their odor description. *Nippon Kagaku Kaishi*, 49 (4): 228-237.
45. Diana Negrao Cavalcanti, Marcelo Augusto Vasconcelos Gomes; Angelo Cunha Pinto; Claudia Moraes de Rezende; Renato Crespo Pereira and Valeria Laneuville Teixeira, 2008. Effects of storage and solvent type in a lipophylic chemical profile of the seaweed *Dictyota menstrualis. Brazilian J. of Oceanography*, 56(1): 51-57.
46. USEPA (U.S. Environmental Protection Agency). 1980. Ambient water quality criteria for chlorinated benzenes. EPA 440/5-80-028. USEPA, Washington, DC.
47. Government of Canada. 1993. Tetrachlorobenzene. Canadian Environmental Protection Act Priority Substances List Assessment Report. Environment Canada and Health Canada, Ottawa.