

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

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Abstract: To screen and characterize the bioactive compounds in the brown seaweed *Turbinaria ornata*. 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. 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. 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.

Key words: *Turbinaria ornata* • Phytochemical screening • Bioactive compounds • GC-MS

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 phytochemical 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 [3-6]. 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 [7-10]. 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*.

MATERIALS AND METHODS

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.

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.

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 h. 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.

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.

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.

RESULTS

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.

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.

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

Table 1: Phytochemical screening of *Turbinaria ornata* in different extracts

Phytochemicals	Distilled. water	Acetone	Methanol	Ethanol	Petroleumether
Alkaloids	✓	✓	✓	✓	☒
Terpenes	☒	✓	✓	✓	☒
Phenolics	✓	☒	✓	✓	☒
Sugars	☒	✓	☒	☒	☒
Tannins	☒	✓	✓	✓	☒
Saponins	✓	✓	✓	✓	✓
Flavonoids	✓	☒	✓	✓	☒
Quinones	✓	✓	✓	✓	☒
Proteins	✓	☒	✓	✓	☒
Sterols	☒	☒	✓	✓	☒

✓ Connotes presence of phytochemicals; ☒ Connotes absence of phytochemicals

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-Tetrachlorobenzene	C ₆ H ₂ Cl ₄	215.89
2	9.50	Diethylether	C ₄ H ₁₀ O	74.12
3	15.15	1,2-Benzenedicarboxylic 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)fluoranthene	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 ₅ Cl 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 ₄ H ₇ NS	101.17
11	29.38	Di-n-octylphthalate	C ₂₄ H ₃₈ O ₄	390.55
12	34.93	Vanillylmandelic acid	C ₉ H ₁₀ O ₅	198.17
13	36.91	Tetramethrin-1	C ₁₉ H ₂₅ NO ₄	331.40
14	37.92	Acetamidrid	C ₁₀ H ₁₁ ClN ₄	222.67
15	42.09	Eicosapentaenoic acid (EPA)	C ₂₀ H ₃₀ O ₂	302.45
16	44.19	Heptanal	C ₇ H ₁₄ O	114.18
17	45.12	Humulene epoxide III	C ₁₅ H ₂₄ O	220.35

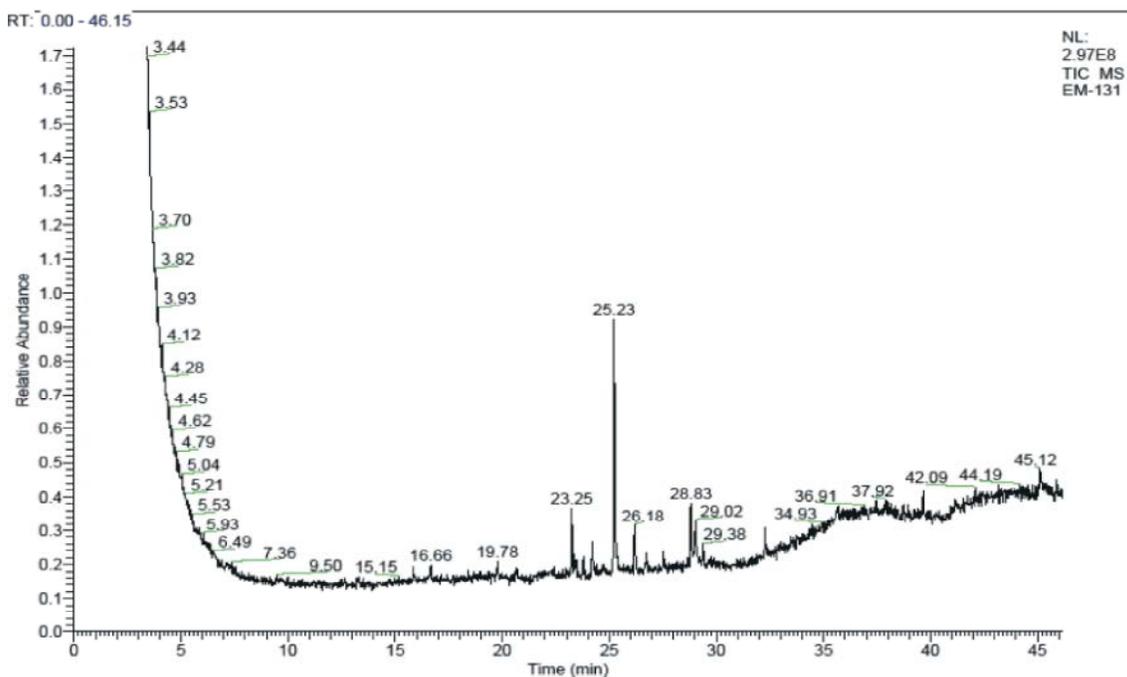


Fig. 1: GC-MS chromatogram of the crude extract of *Turbinaria ornata*

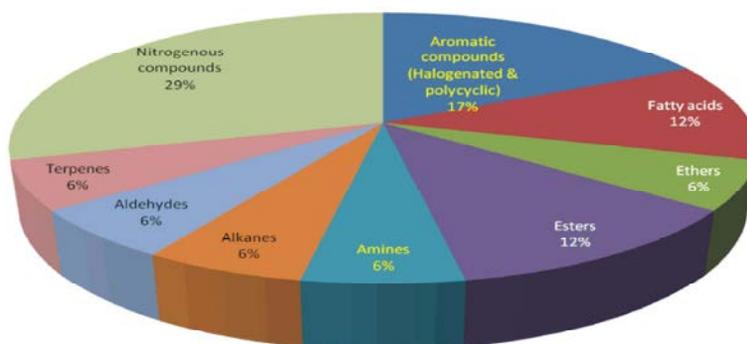


Fig. 2: Phytochemical composition of *Turbinaria ornata*

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

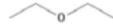
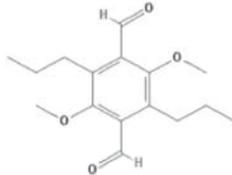
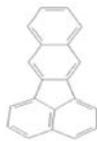
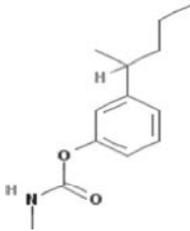
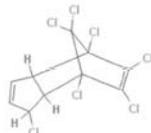
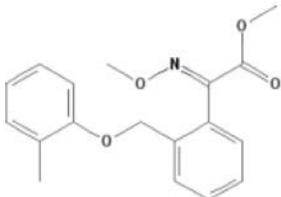
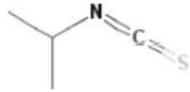
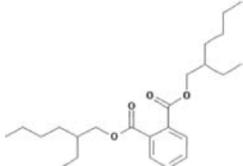
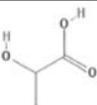
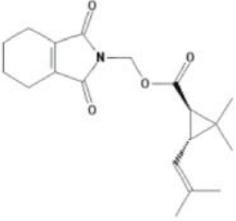
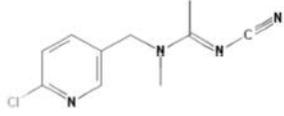
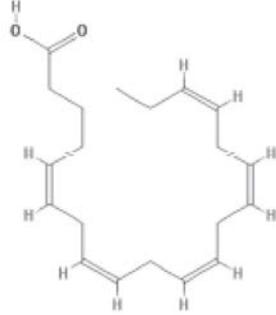
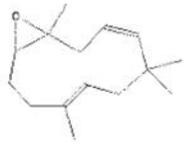
S.No.	Name of the compound	Structure of compound	Retention time (min)
1	1,2,3,4-Tetrachlorobenzene		7.36
2	Diethylether		9.50
3	1,2-Benzenedicarboxylic acid bis(2-methylpropyl) ester		15.15
4	n-Hexadecanoic acid		16.66
5	Benzo(k)fluoranthene		19.78
6	Bufencarb-2		23.25
7	Heptachlor		25.23
8	Kresoxim-Methyl		26.18
9	n-Tetradecane		28.83
10	Isopropyl isothiocyanate		29.02
11	Di-n-octylphthalate		29.38

Table 3: Continued

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

Aromatic Compounds: Halogenated aromatic compound - 1,2,3,4 – tetrachlorobenzene and heptachlor. Polycyclic aromatic compound - Benzo (K) fluoranthene and Kresoxim methyl. *Ethers:* Diethyl ether. *Fatty acids:* Saturated fatty acid - n- Hexadecanoic acid and Unsaturated fatty acid -Eicospentaenoic acid (EPA). *Esters:* 1,2- Benzene dicarboxylic acid bis (2-methyl propyl) ester and Di-n-octylphthalate. *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.

DISCUSSION

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 phytochemicals 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 fasciata* 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 decortatum*, 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.

Phytochemical 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 *Calulterpa lentillifera*, *Kappaphycus alvarezii* and *Sargassum 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.

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

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

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 [35-41]. 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].

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].

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].

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 defoliant (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.

Conflict of Interest Statement: We declare that we have no conflict of interest.

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