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# Extraction, isolation and identification of bioactive compounds of *Turbinaria ornata* (Turner)

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**Key words:** FTIR, TLC and UV- VIS spectroscopic, *Turbinaria ornata*

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## Abstract

In the present study of investigation suggest the *Turbinaria ornata* is tropical brown algae of the order fucales native to coral reef ecosystem of the south pacific. *Turbinaria ornata* is more commonly referred to as crowed sea bells in the US and crowned sea bells worldwide. In the present study, the active substances in the methanolic extract of Turbinaria ornata were analyzed using FTIR, TLC, and UV-VIS spectroscopy. The FTIR analysis revealed the presence of 18 compounds of recognized such as N-H Stretching vibrations primary, free two bands, Amides, O–H stretch, H–bonded, Alcohols, Phenols, O–H stretch, Carboxylic acids, C-H stretching, Alkane, C-H Stretching vibrations and bonds, Aldehydes, C-H Stretching, Alkane, S-H Stretching vibrations, sulfur compounds , sulfoxides, –C≡C– stretch, Alkynes, C≡N Stretching vibrations, isocyanide, Unsaturated Nitrogen Compounds, C≡N Stretching vibrations, isocyonides, C–C Multiple bond stretching, Alkene, disubstituted, *gem,* C–C stretch (in–ring), Aromatics, C–H bend, Alkanes, C–N stretch, Aliphatic amines, Sulfur compounds, S=O Stretching vibrations, sulfoxides, Amines, C–N Vibration, Aliphatic, –C ≡ C–H: C–H bend, Alkynes each showing significant peaks. The phytochemical compounds of flavonoid and phenol were identified may be by TLC from the methanolic extract of *Turbinaria ornata*. The bioactive compounds of flavonoid and phenol which was indicated for maximum amount of secondary metabolites in the *Turbinaria ornata* were estimated by UV-VIS spectroscopic analysis were confirmed.

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## Introduction

*Turbinaria ornata* is a very common brown algae found intertidally on Hawaiian reefs and throughout the pacific and Indian ocean. It is normally found in small clusters attached to the crevices of basalt rocks in high wave action areas as well as in the crevices of coral heads at 20- 30 meters deep. The morphological characteristics of this alga enable it to survive extreme environmental conditions. The algae touch thallus is able to withstand the high energy hydrodynamics of the intertidal environment as well as resist herbivore. The strong holdfast provides a stable grasp on the substrate and is capable of recolonization if the thalli are removed.

The species has also exhibited seasonal changes. The thalli of *Turbinaria ornata* are often scoured from the holdfast in the winter season, and the remaining viable holdfast propagates new blades. *Turbinaria ornata* successfully reproduces from either sexual reproduction in fragmentation. Fragments of the stolon and blade can attach to the substrate and initiate new plants.

The southwest coast of India has a diverse marine habitat of seaweeds, with brown algae being the most prevalent (Viswanathan *et al.*, 2013). The brown algae are differentiated by their colour which differs from olive green via light golden shades of brown. This is due to the occurrence of a golden-brown xanthophyll pigment fucoxanthin in their chromatophores. The brown algae are brownish in colour because of the huge quantities of the carotenoid and fucoxanthin covering the residual pigment chlorophyll a and c, carotene, and other xanthophylls. The cell walls are composed of alginic acid, which was extracted as alginate or agent for industrial use. Brown algae range from smaller cords to the largest seaweed, and the majority are found in the intertidal belt. Brown seaweeds are mostly utilized to cure hypothyroidism, fatigue, cellulite, cough, asthma, stomach ailments, and headache. Brown seaweeds are also utilized to encourage weight loss besides assistance in skincare. The prospective antioxidant compounds in brown seaweeds were recognized as polyphenols and pigments mostly (Yoshie-Stark *et al.*, 2013).
These compounds are dispersed in plants or algae and are widely known for displaying antioxidant activities by reactive oxygen species (ROS) recovery activity and lipid peroxidation inhibition (Heo *et al.*, 2005). The isolated compound's functional groups and structural properties were identified using Fourier Transform-Infrared (FTIR) spectroscopy. The FTIR spectrum of laminarin which was isolated from *Turbinaria ornata* exhibited the typical absorption peak.

## Materials and methods

*Collection of seaweeds*

*Turbinaria ornata* were collected from Gulf of Mannar, Tamil Nadu, India. The collected samples were cleaned well with sea water to remove all the extraneous matter such as epiphytes, sand particles, pebbles and shells and brought to the laboratory in sterile bags. The sample was submitted to Botanical survey of India, Coimbatore, Tamil Nadu and authenticated (BSI/SRC/5/23/2021/Tech/91) as *Turbinaria ornata* (SARGASSACEEAE).

Then the samples were washed with tap water and distilled water and spread in the dark room for drying, after which the dried samples were powdered and subsequently stored at 4°C.

*Chemicals*

All chemicals and reagents used in the study were obtained commercially and were of analytical grade from Ranchem and Merck.

*Preparation of extract*

About 50g of *Turbunaria ornata* (Brown Seaweed) powdered samples was kept in Soxhlet apparatus with 200ml of 100% ethanol for 12 hours. The ethanolic extract was filtered and concentrated at 40-50°C using a rotary evaporator. The extract was stored in air and moisture tight container.

*FTIR analysis (Iqbal Hussain, 2010)*

FTIR analysis was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks in ranging from 400-4000 cm-1 and their functional groups. The peak values of the FTIR were recorded. Each and every analysis was repeated twice for the spectrum confirmation.

*TLC analysis (Katoch, 2011)*

Flavonoid and phenolic compounds of brown seaweed (*Turbinaria ornata*) methanolic extract was subjected to thin layer chromatography (TLC) as per conventional one-dimensional ascending method using silica gel G were cut with ordinary household scissors. Plate markings were made with soft pencil. Glass capillaries were used to spot the sample for TLC applied sample volume 1-micro litre by using capillary at distance of 1 cm. In chamber with solvent system (n-Butanol: Acetic acid: Water (4:1:5) was used as mobile phase. After presaturation with mobile phase for 20 min for development were used. After the run plates are dried and sprayed freshly prepared ammonia reagents were used to detect the bands on the TLC plates. The movement of the active compound was expressed by its R value was calculated for sample.

*UV–Visible spectrophotometric analysis (Nanzeen Bobby, 2012)*

The ethanolic extract was examined under UV Visible spectral analysis. The extracts were centrifuged at 3000 rpm for 10 min and filtered through Whatmann No. 1 filter paper For UV and FTIR spectrophotometer analysis by using high pressure vacuum pump. The sample was diluted to 1:10 with the same solvent. The extracts were scanned in the wavelength ranging from 260-900 nm using Perkin Elmer Spectrophotometer and the characteristic peaks were detected.

**Results and discussion**

The current investigation to determine the functional groups present in the active components, based on peak values within the infrared radiation range. The findings, along with the functional groups and bonds, were illustrated in (Fig. 1, Table 1). Upon passing the *Turbinaria ornata* extract through the FTIR, the functional groups were distinguished by their peak ratios. The FTIR analysis results confirmed the presence of various functional groups and bonds such as N-H Stretching vibrations primary, free: two bands, Amides, O–H stretch, H– bonded, Alcohols, Phenols, O–H stretch, Carboxylic acids, C-H stretching, Alkane, C-H Stretching vibrations and bonds, Aldehydes, C-H Stretching, Alkane, S-H Stretching vibrations, sulfur compounds , sulfoxides, S-H Stretching vibrations, sulfur compounds, sulfoxides, –C ≡C– stretch, Alkynes, C≡N Stretching vibrations, isocyanide, Unsaturated Nitrogen Compounds, C≡N Stretching vibrations, isocyonides, C–C Multiple bond stretching, Alkene, disubstituted, *gem,* C–C stretch (in–ring), Aromatics, C–H bend, Alkanes, C–N stretch, Aliphatic amines, Sulfur compounds, S=O Stretching vibrations, sulfoxides, Amines, C–N Vibration, Allphatic and –C ≡ C–H: C–H bend, Alkynes with major peaks at 3969.50, 3413.86, 2949.82, 2968.59, 2867.95, 2842.72, 2596.38, 2524.82, 2120.18, 2075.83, 2051.60, 1648.50, 1411.88, 1455.03,1111.20, 1054.48 1016.21 and 658.36, respectively**.**

**Table 1.** Identification of functional groups using FTIR techniques

| Peak value | Functional group |
|---|---|
| 3969.50 | N-H Stretching vibrations primary, free: two bands, Amides |
| 3413.86 | O–H stretch, H–bonded, Alcohols, Phenols |
| 2949.82 | O–H stretch, Carboxylic acids |
| 2968.59 | C-H stretching, Alkane |
| 2867.95 | C-H Stretching vibrations and bonds, Aldehydes |
| 2842.72 | C-H Stretching, Alkane |
| 2596.38 | S-H Stretching vibrations, sulfur compounds , sulfoxides |
| 2524.82 | S-H Stretching vibrations, sulfur compounds , sulfoxides |
| 2120.18 | –C ≡C– stretch, Alkynes |
| 2075.83 | C≡N Stretching vibrations, isocyanide |
| 2051.60 | Unsaturated Nitrogen Compounds, C≡N Stretching vibrations, isocyonides |
| 1648.50 | C–C Multiple bond stretching, Alkene, disubstituted, gem |
| 1411.88 | C–C stretch (in–ring), Aromatics |
| 1455.03 | C–H bend, Alkanes |
| 1111.20 | C–N stretch, Aliphatic amines |
| 1054.48 | Sulfur compounds, S=O Stretching vibrations, sulfoxides |
| 1016.21 | Amines, C–N Vibration, Aliphatic |
| 658.36 | –C ≡ C–H: C–H bend, Alkynes |

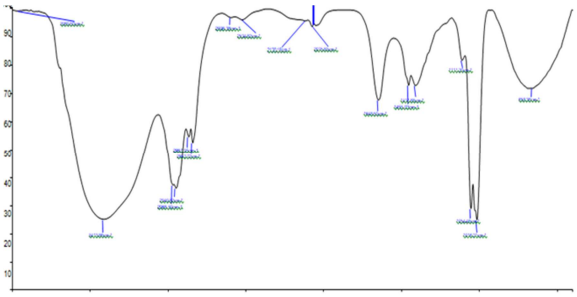
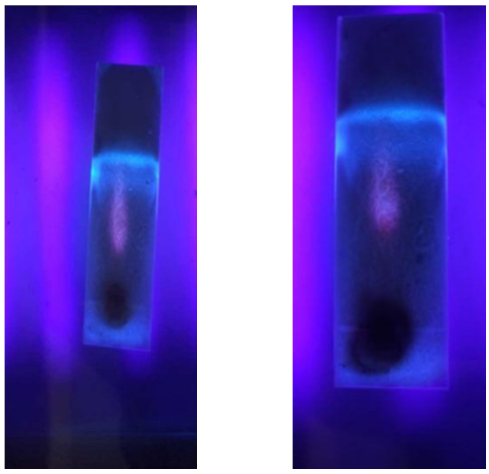


**Fig. 1.** Identification of functional groups using FTIR techniques

The FTIR spectra of *Laminaria ochroleuca* Na-alginate and synthesized Ag NPs were performed in the range of 4000 to 500 cm−1, as (Soukaina Kaidi *et al.*, 2022). Broadband appeared at 3204.68 cm−1 could be assigned to the stretching vibration of –OH group (Voo *et al.*, 2015). The peak noted at 2919.75 cm-1 is related to carboxylate O=C–O asymmetric stretching vibrations. According to Fenoradosoa *et al.* (2010) and Leal *et al.* (2007), the characteristic peak of alginate found at 1599.21 cm−1 can be attributed to asymmetric stretching vibrations of carboxylate salt ion. The strong peak at 1404.91 cm−1 may be assigned to C–OH deformation vibration with the contribution of O–C–O symmetric stretching vibration of the carboxylate group, while the weak band at 1023 cm-1 may be assigned to C–O, and C– C stretching vibrations of pyranose ring (Gomez-Ordonez and Ruerez, 2011). The following bands are important for the alginate characterization as they correspond to the anomeric region (950 to 750 cm-1), in which two peaks were reported. The first one was observed at 877.95 cm-1 which corresponds to the C1–H deformation vibration of β-D-mannuronic acid residues. The second absorption brand band at 810,93 cm−1 was often reported for alginates, due to mannuronic acid residues (Lawrie *et al.*, 2007).

Fig. 2 displayed a thin layer chromatogram of the methanolic extract of *Turbinaria ornata*, while Table 2 provided the corresponding Rf value. The TLC analysis of the methanolic extract showed the presence of a spot with Rf values of 0.69 for flavonoid and 0.71 for phenol using an n-Butanol: Acetic acid: Water (4:1:5) solvent system. This spot was identified as a flavonoid and phenol compound in the methanolic extract of *Turbinaria ornata*.



Flavonoid Phenol

**Fig. 2.** Identification of flavonoid and phenolic compound by using TLC

The Thin Layer Chromatography (TLC) approach for identifying antibiotic chemicals in putative endophytic bacterial extracts revealed 14 stains on the TLC silica gel plate. Visualization with visible light does not reveal stains; however visualization with 365 nm-long UV light does reveal the spots that are produced. Endophytic bacterial extract To.09.pp produced 5 spots (0.35, 0.62, 0.7, 0.82and 0.9), To.10.pp produced 5 spots (0.35, 0.6, 0.75, 0.82and 0.88), and Sc.06.pp produce4 spots (0.62, 0.75, 0.82 ando.88).

**Table 2.** Analysis of flavonoid and phenol in methanolic extract of *Turbinaria ornata* by thin layer chromatography

| Phytoconstituent | Rf value | Results |
|---|---|---|
| Flavonoid | 3.4/4.9 | 0.69 |
| Phenol | 3.5/4.9 | 0.71 |

Based on fucoxanthin analysis using thin layer chromatography (TLC), Retention factor (Rf) value of fucoxanthin from ethanol, methanol and ethyl acetate was 0.25 with an orange- brown color. According to Zaelani and Hartati Rf value of fucoxanthin pigment is 0.25 (orange) and Rf value with range from 0.26 to 0.28 (orange). Rf value used for estimate the type of extract contained in the extract (Arifah *et al.*, 2019).

The UV-Vis spectrum profile of the methanolic extract of *Turbinaria ornata* was carefully chosen from 210.7 nm to 977.0 nm due to the sharpness of

peaks and a proper baseline. The UV-Vis spectrum profile of the methanolic extract of *Turbinaria ornata* was presented in Fig. 3, with absorption values detailed in Table 3.

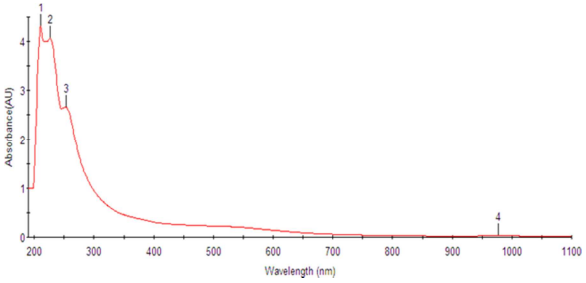



**Fig. 3.** UV-Vis spectrum of methanolic extract of *Turbinaria ornata*

**Table 3.** UV-Vis spectrum of methanolic extract of *Turbinaria ornata*

| Wave length (nm) | Absorption peak | Compounds |
|---|---|---|
| 210.7 | 4.3160 | Phenol and flavonoid |
| 226.7 | 4.0733 | |
| 252.6 | 2.6607 | |
| 977.0 | 0.0405 | |

The profile exhibited absorption peaks at wavelengths 210.7 nm, 226.7 nm, 252.6 nm, and 977.0 nm, with corresponding values of 4.3160, 4.0733, 2.6607, and 0.0405, respectively. The UV-Vis spectroscopic analysis results confirm the presence of the Phenol and flavonoid compounds in the seaweed extract (Table 3). UV-Vis spectrum of methanolic extract of *Turbinaria ornata*. UV–visible spectral analysis UV-VIS spectrum profile of methanolic extract of *Sargassum wightii* was selected from 200 –1100 nm due to the sharpness of the peaks. The profile showed the compounds separated at the nm of 242,356, 607 and 664. These absorption spectra are distinctive for flavonoids and its derivatives. The flavonoids spectral bands characteristically comprise of two absorption spectra maximum in the ranges 230-290 nm and 300-360 nm (Rajeswari and Jeyaprakash, 2019). The exact position and virtual intensities of these maxima give enormous valuable information on the nature of the flavonoids 20. Then peak occurrence of at 234-676 nm exposes the presence of phenolic and alkaloids compounds in the *Turbinaria ornata* (Sargasaaceae*)*. On comparison of the spectra of seeds and flowers, shows that the extract has some similar alkaloid, flavonoids and glycoside compounds reported (Neha Sahu and Jyoti Saxena, 2013).

## Conclusion

The study revealed that *Turbinaria ornata* possesses a significant amount of secondary metabolites. These results suggest that *Turbinaria ornata* has the potential to be a valuable natural antioxidant, which could be used as a therapeutic agent to prevent degenerative diseases caused by oxidative stress. Our future studies will focus on purifying, identifying, and characterizing the active compounds found in *Turbinaria ornata*.

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