



AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: <http://www.ajptr.com/>

Phytochemical Studies on *Turbinaria Ornata* (Turner) J.AG.

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ABSTRACT

The present study was aimed to explore the phytochemical constituents present in *Turbinaria ornata* (Turner) J.Ag. collected from the south east coast of Tamil Nadu, India. The phytochemical screening of different extracts was estimated using the standard procedure for UV-Vis spectroscopic and HPLC. The UV-Vis phytochemical profile of various extracts of *Turbinaria ornata* (Turner) J.Ag. was analyzed. The qualitative HPLC fingerprint profile of methanol extract of *Turbinaria ornata* (Turner) J.Ag. was selected at a wavelength of 254 nm due to sharpness of the peaks and proper baseline. The profile displayed one prominent peak at a retention time of 1.953 min and some moderate peaks were observed at a retention time of 3.000, 2.570 and 2.467 min respectively. The present study on *Turbinaria ornata* (Turner) J.Ag. produced novel phytochemical markers in standardization as useful analytical tools to check not only the quality of the powder but also the presence of adulterants in ayurvedic drugs.

Keywords: Phytochemistry, Seaweeds, *Turbinaria ornata*, UV-Vis, HPLC.

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Received 24 October 2012, Accepted 31 October 2012

Please cite this article in press as Paul JP *et al.*, Phytochemical Studies on *Turbinaria Ornata* (Turner) J.AG. American Journal of PharmTech Research 2012.

INTRODUCTION

For the past few decades compounds from natural sources have been gaining importance due to the vast chemical diversity. These natural sources had led to a phenomenal increase in the demand for medicines in the last few decades and the need for ensuring the quality, safety and efficacy of drugs. The medicinal value of the plants lies in some chemical substances that produce a definite physiological action on the human body. The phytochemical screening reveals the presence of primary and secondary metabolites that suggest the plant might be of medicinal or industrial importance. The screening of phytochemicals is relevant in various areas for different purposes and some of these include alkaloids, steroids, tannins, saponins, flavonoids and phenolics.



Figure 1: Turbinaria ornata

Marine macro algae are referred to as seaweeds and can be classified as red algae (Rhodophyta), brown algae (Phaeophyta) and green algae (Chlorophyta) depending on their chemical composition. Seaweeds serve as an important source of bioactive natural substances. They have some of the valuable medicinal value components such as antibiotics, antioxidant, anticoagulants, anti-ulcer products and suspending agents in radiological preparations ^{1,2}. From the literature, it is observed that the seaweeds contain a significant amount of the protein, vitamins and minerals essential for the human nutrition ³. Most of the compounds of marine algae show antibacterial activities ^{4,5}. Many metabolites isolated from marine algae have been shown to possess bioactive efforts ^{6,7}. Among the different compounds with functional properties, antioxidants are the most widely studied. Moreover the important role of antioxidants in human health has been demonstrated thus increasing the interest in such products and their demand by consumers. Marine algae serve as important resources for bioactive natural products ^{8,9}. Marine organisms are a rich source of structurally novel and biologically active metabolites. Secondary or primary metabolites produced by these organisms may be potential bioactive compounds of interest in the pharmaceutical industry. To date, many chemically unique

compounds of marine origin with various biological activities have been isolated and some of them are under investigation and are being used to develop new pharmaceuticals. Thus the present study was aimed to explore the phytochemical constituents present in *Turbinaria ornata* medicinally important brown algae.

MATERIALS AND METHODS

Collection of samples

The samples of *Turbinaria ornata* J.Ag. were collected from Rasthacaud (Lat N 08⁰08'308'' E77⁰32'80'') located in the Kanyakumari district, Tamil Nadu, India. The collections were made during the low tidal and subtidal regions (up to 1m depth) by hand picking. The collected materials were washed thoroughly with marine water in the field itself to remove the epiphytes and sediment particles. Then the samples were packed separately in polythene bags in wet conditions and brought to the laboratory, then thoroughly washed in tap water followed by distilled water to remove the salt on the surface of the thalli. They were stored in 5% formalin solution. For drying, washed specimens were placed on blotting paper and spread out at room temperature in the shade. The shade dried samples were grounded to fine powder using a tissue blender. The powdered samples were then stored in the refrigerator for further use.

Preparation of extracts

10 g of air dried powder was extracted with 60 mL of solvents viz., aqueous, methanol, acetone, chloroform and benzene,. The sample was kept in dark for 72 h with intermittent shaking. After incubation, the solution was filtered through filter paper and the filtrate was collected (crude extracts).

UV-Vis spectroscopic analysis

The different extracts of *Turbinaria ornata* (Turner) J.Ag. were centrifuged at 3000 rpm for 10 min and then filtered through Whatmann No.1 filter paper using high pressure vacuum pump. The sample is diluted to 1:10 with the same solvents. The crude extracts containing the bioactive compound was analyzed spectroscopically for further confirmation. They were scanned in the wavelength ranging from 200-1100 nm using Shimadzu spectrophotometer and characteristic peaks were detected ¹⁰.

HPLC analysis

HPLC method was performed on a Shimadzu LC-10AT VP HPLC system, equipped with a model LC-10AT pump, UV-Vis detector SPD- 10AT, Rheodyne injector fitted with a 20 μ L loop and auto injector SIL-10AT. A Hypersil BDS C-18 column (4.6 \times 250 mm, 5 μ m size) with a C-

18 guard column was used. The elution was carried out with gradient solvent systems with a flow rate of 1 mL min⁻¹ at ambient temperature (25-28°C). The mobile phase was consisted of 0.1% v/v methanol (Solvent A) and water (Solvent B). The mobile phase was prepared daily, filtered through a 0.45 µm and sonicated before use. Total running time was 15 min. The sample injection volume was 20 µL while the wavelength of the UV-Vis detector was set at 254 nm^{11,12}.

RESULTS AND DISCUSSION

UV-VIS Spectroscopic analysis

The qualitative UV-VIS fingerprint profile of the aqueous extract of *Turbinaria ornata* (Turner) J.Ag. was selected at the wavelength of 310nm to 900nm due to the sharpness of the peaks and proper baseline. The profile showed the compounds separated at 310nm, 380nm, 410nm and 520nm with the absorption 4.000, 2.181, 1.435 and 0.521 respectively (Table-1 and Figure-2A). The UV-VIS fingerprint profile of the methanol extract of *Turbinaria ornata* (Turner) J.Ag. was selected at the wavelength of 310nm to 900nm due to the sharpness of the peaks and proper baseline. The profile showed the compounds separated at the nm of 310, 380, 410 and 460 with the absorption 3.024, 3.068, 3.612 and 2.185 respectively (Table-1 and Figure-2B).

Table 1 UV-VIS peak values of different extracts of *Turbinaria ornata* (Turner) J.Ag.

| Solvents Peak No. | Aqueous | | Methanol | | Acetone | | Chloroform | | Benzene | |
|----------------------|---------|-------|----------|-------|---------|-------|------------|--------|---------|-------|
| | nm | Abs | nm | Abs | nm | Abs | nm | Abs | nm | Abs |
| 1 | 310 | 4.000 | 310 | 3.024 | 310 | 1.796 | 310 | 1.593 | 310 | 1.582 |
| 2 | 380 | 2.181 | 380 | 3.068 | 380 | 2.960 | 380 | 2.364 | 380 | 2.542 |
| 3 | 410 | 1.435 | 410 | 3.612 | 410 | 3.462 | 410 | 3.263 | 410 | 3.325 |
| 4 | 460 | 0.820 | 460 | 2.185 | 460 | 1.514 | 460 | 1.134 | 460 | 1.187 |
| 5 | 520 | 0.521 | 520 | 0.539 | 520 | 0.501 | 520 | 0.444 | 520 | 0.429 |
| 6 | 570 | 0.382 | 570 | 0.228 | 570 | 0.268 | 570 | 0.242 | 570 | 0.213 |
| 7 | 610 | 0.314 | 610 | 0.323 | 610 | 0.550 | 610 | 0.441 | 610 | 0.459 |
| 8 | 670 | 0.246 | 670 | 0.801 | 670 | 2.160 | 670 | 1.260 | 670 | 1.820 |
| 9 | 710 | 0.204 | 710 | 0.036 | 710 | 0.045 | 710 | 0.018 | 710 | 0.037 |
| 10 | 780 | 0.157 | 780 | 0.011 | 780 | 0.006 | 780 | -0.006 | 780 | 0.013 |
| 11 | 830 | 0.133 | 830 | 0.008 | 830 | 0.003 | 830 | -0.010 | 830 | 0.010 |
| 12 | 900 | 0.110 | 900 | 0.004 | 900 | 0.001 | 900 | -0.013 | 900 | 0.006 |

The UV-VIS fingerprint profile of the acetone extract of *Turbinaria ornata* (Turner) J.Ag. was selected at a wavelength of 310nm to 900nm due to the sharpness of the peaks and proper baseline. The profile showed the compounds separated at 310nm, 380nm, 410nm, 460nm and 670nm with the absorption, 1.796, 2.960, 3.462, 1.514 and 2.160 respectively (Table-1 and Figure-2C). And profile of the chloroform extract of *Turbinaria ornata* (Turner) J.Ag. was selected at a wavelength of 310nm to 900nm due to sharpness of the peaks and proper baseline. The profile showed the compounds separated at 310nm, 380nm, 410nm, 460nm and 670nm with

the absorption, 1.593, 2.364, 3.263, 1.134 and 1.260 respectively (Table-1 and Figure2D). The UV-VIS fingerprint profile of the benzene extract of *Turbinaria ornata* (Turner) J.Ag. was selected at a wavelength of 310nm to 900nm due to sharpness of the peaks and proper baseline. The profile showed the compounds separated at 310nm, 380nm, 410nm, 460nm and 670nm with the absorption 1.582, 2.542, 3.325, 1.187, and 1.820 respectively (Table-1 and Figure-2E).

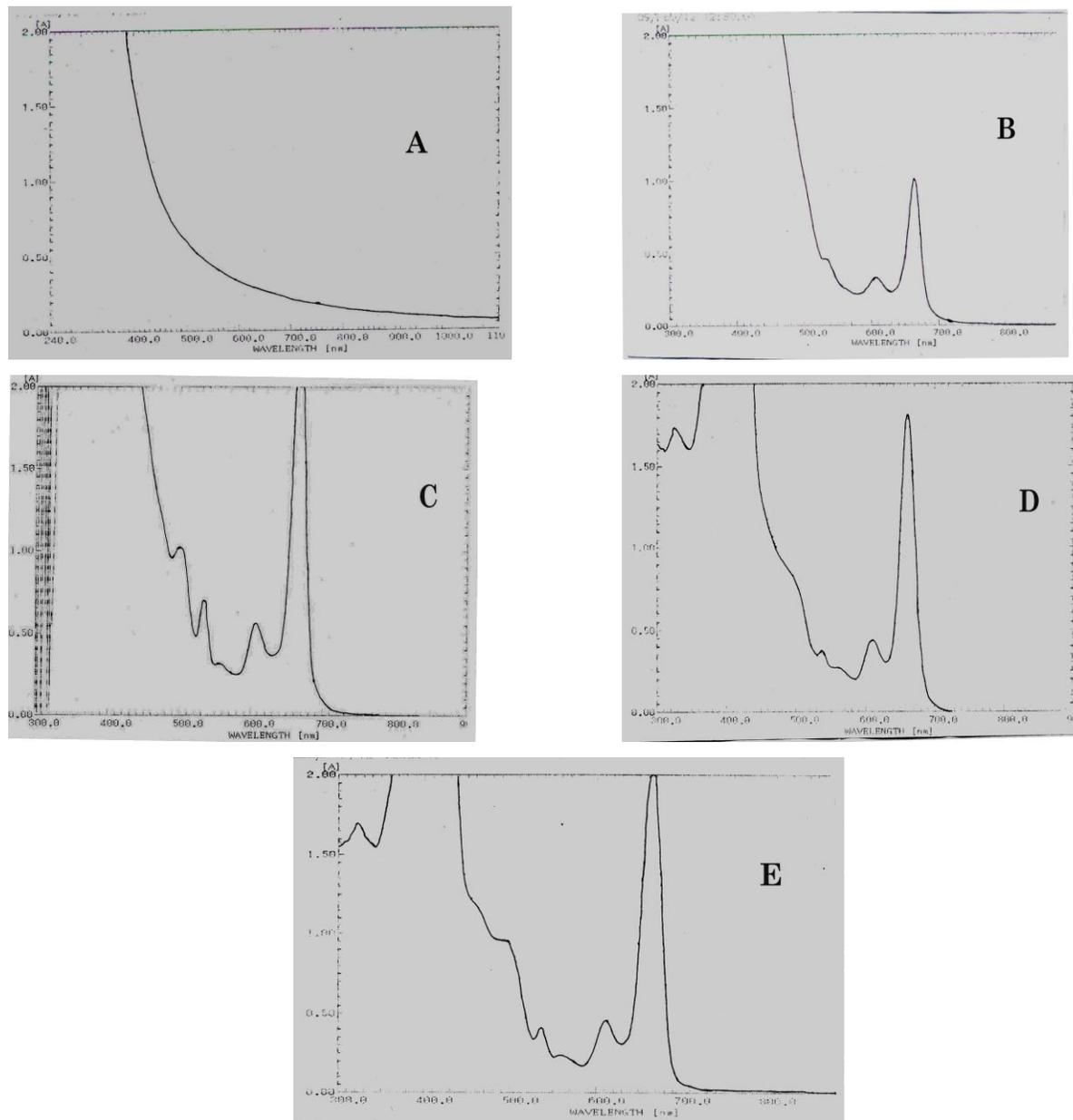


Figure 1 UV-VIS Peak Values of Different Extracts of *Turbinaria ornata* (Turner) J.Ag.

A. Aqueous Ether cold extract; B. Methanol cold extract;

C. Acetone cold extract; D. Chloroform cold extract; E. Benzene cold extract

HPLC Analysis

The qualitative HPLC fingerprint profile of the methanol extract of *Turbinaria ornata* (Turner)

J.Ag. was selected at a wavelength of 660nm due to the sharpness of the peaks and proper baseline. The methanol extract prepared by cold extraction was subjected to HPLC for the separation and identification of constituents present in the *Turbinaria ornata* (Turner) J.Ag. Six compounds were separated at different retention times of 1.957min, 2.113min, 2.573min, 3.013min, 3.377min and 3.047min respectively. The profile displayed three prominent peaks at the retention times of 1.957min, 2.113min and 2.573min and some moderate peaks were also observed at the retention times of 3.013min, 3.377min and 8.047min (Figure-3).

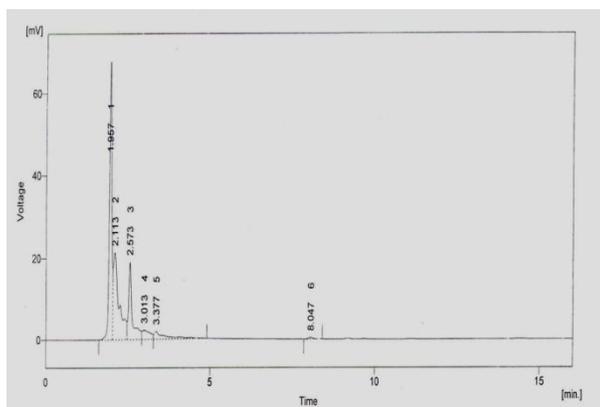


Figure 2 HPLC Profile of *Turbinaria ornata* (Turner) J.Ag. – Methanolic cold extract

Seaweeds have the great potential for the production of secondary novel bioactive compounds which are not found in terrestrial environment^{13,14}. Seaweeds are considered as the richest source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities. Compounds with cytostatic, antiviral, anthelmintic, antifungal and antibacterial activities have been detected in brown algae^{15,16}. It is a real fact that the importance of marine organisms as a source of new substances is growing. With marine species comprising approximately a half of the total global biodiversity, the sea offers an enormous resource for novel compounds, and it has been classified as the largest remaining reservoir of natural molecules to be evaluated for drug activity. A very different kind of substances have been obtained from marine organisms among other reasons because they are living in a very exigent, competitive and aggressive surrounding very different in many aspects from the terrestrial environment, a situation that demands the production of quite specific and potent active molecules¹⁷. Seaweeds known as medicinal are rich in secondary metabolites which include alkaloids, glycosides, phenols, flavonoids, tannins, saponins, steroids, related active metabolites are of great medicinal value and have been extensively used in the drug and pharmaceutical industry. Recently, a number of studies have been reported on the phytochemistry of seaweeds across the world^{18,19,20}. In the present investigation also, *Turbinaria*

ornata (Turner) J.Ag. have been selected from Tamil Nadu, India for phytochemical screening on the basis of traditional and pharmaceutical uses.

CONCLUSION

The present study *Turbinaria ornata* (Turner) J. Ag. produced novel phytochemical markers in standardization as useful analytical tools to check not only the quality of the powder but also the presence of adulterants in ayurvedic drugs. UV-VIS and HPLC analysis can be used as effective markers in identifying authentic from its adulterants. It also suggested that *Turbinaria ornata* (Turner) J.Ag. may be rich sources of phytochemicals which can be isolated and further screened for different kinds of biological activities depending on their reported therapeutic uses. Further work will emphasize the isolation and characterization of active principles responsible for bioactivity.

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