Indian Journal of Plant Sciences ISSN: 2319–3824(Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/jps.htm
2014 Vol.3 (1) January-March, pp.40-44/Paul
Research Article

HISTOCHEMISTRY AND FLUORESCENCE ANALYSIS OF *TURBINARIA ORNATA* (TURNER) J.AG. – AN IMPORTANT BROWN SEAWEED (PHAEOPHYCEAE)

***J. John Peter Paul**
Research Department of Botany, St. Xavier's College (Autonomous), Palayamkottai – 627 002, Tamil Nadu, India
**Author for Correspondence*

ABSTRACT
In the present study, an attempt was taken for histochemical and fluorescence analysis of *Turbinaria ornata* (Turner) J.Ag. an important beneficial brown seaweed. Histochemical analyses of the plant were carried out using light microscopy and fluorescence study was analyzed by UV lamp. Results of histochemical tests showed positive reaction to phenol compounds, polyphenol and tannin in the thallus. Fine powder and different solvent extracts of Turbinaria ornata obtained using petroleum ether, benzene, chloroform, acetone, ethanol and aqueous were examined under visible and UV light. The powdered materials were also treated with various reagents such as 50% nitric acid, 50% sulphuric acid, 1N HCl, 1N NaOH and changes in colour were recorded. From the present study it is concluded that the histochemical and the fluorescence analysis could be used for rapid identification of potential medicinal plants and bioactive compounds which is present in the particular plant.

Keywords: *Histochemical, Fluorescence, Turbinaria, Seaweed*

INTRODUCTION

Marine organisms are emerging as good candidates as an alternate source for bioactive substances. The occurrences of organic compounds from marine organisms have been reported to possess various biological activities (Kuda *et al.,* 2005) Several researchers have made attempts to identify marine organisms including seaweeds producing bioactive substances and met with success (Duan *et al.,* 2006; Mao *et al.,* 2004; Chandinia *et al.,* 2008). Seaweeds are marine macro algae and primitive type of plants, growing abundantly in the shallow waters of sea, estuaries and backwaters. Seaweeds have been used since ancient times as food, fodder, fertilizer and as source of medicinal drugs. Today seaweeds are the raw material for industrial production of agar, algin and carrageenan in various countries of the world. There are reports that seaweeds are excellent source of vitamins (A, B, B_{12}, C, D and E), riboflavin, niacin, panthothanic acid and folic acid as well as minerals such as Ca, P, Na and K (Faulkner, 2002). Recently, chemists worldwide have paid attention to the potential of marine organisms as alternative sources for the isolation of novel metabolites with interesting biological and pharmaceutical properties (Taskin *et al.,* 2002). Biological compounds extracted from some seaweed species namely Chlorophyceae, Phaeophyceae and Rhodophyceae were proven to have potential medicinal activities such as antibacterial, antiviral, antitumour, antifungal, antiprotozoal, antioxidant, antimutagenic, anticoagulant, antitumor and mosquito larva control (Thillairajasekar *et al.,* 2009; Patra *et al.,* 2008; Manivannan *et al.,* 2009).

Turbinaria, one of the marine macro algal genera belonging to the class Phaeophyceae, is widely distributed in tropical and temperate oceans. It belongs to the family Sargassaceae and order Fucales. It is a large, economically important and ecologically dominant brown algae present in much of the tropics. *Turbinaria ornata* is one of the important species belonging to the genus *Turbinaria* and a wide range of bioactive properties have been reported from this species (Vijayabaskar and Shiyamala, 2011; Stiger and Payri, 2005; Natarajan, 2011). It is widely distributed on the southern coasts of Tamil Nadu, India and it is reported to be used as animal feed, food ingredients and fertilizer. Thus, the present study was aimed to

explore the histochemical constituents and fluorescence analysis of different extracts of *Turbinaria ornata*.

MATERIALS AND METHODS

Collection of Materials

Turbinaria ornata (Turner) J.Ag. (Figure-1) was collected by handpicking at Rasthacaud coastal waters, Kanyakumari district, Tamil Nadu, India. The collected samples were cleaned well with marine water to remove all the extraneous matter such as epiphytes, sand particles, pebbles and shells and brought to the laboratory in plastic bags. It was then thoroughly washed with tap water followed by distilled water. For drying, washed seaweeds were blotted on the blotting paper and spread out at room temperature in shade. The shade dried samples were grounded to fine powder using tissue blender. The powdered samples were then stored in refrigerator for further use.

Figure 1: Natural habit of *Turbinaria ornata* (Turner) J.Ag.

Preparation of extracts

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

Histochemical studies

For the histochemical identification of thallus of *Turbinaria ornata*, a reagent was prepared consisting of a 0.05% solution of 4-nitrosophenol in conc. H_2SO_4. One drop of the reagent was applied to the adaxial surface of the thallus. The histochemical tests were carried out while using a light microscope to observe and record any colour changes. An Olympus SZH zoom stereomicroscope was used, equipped with a DF-Plan 1x objective, 2.5x camera objectives, and with the zoom setting at 50x, resulting in photographic image magnification of 125xs. The aperture was adjusted to the minimum setting. The thallus were photographed before and after application of the reagent with an Olympus C35/AD-4 camera fitted to the microscope, operated by an Olympus PM-10AD-5 automatic photomicrographic system attachment. A positive test for histochemicals was indicated by the appearance of the appropriate colour change after application of the reagent. The presence of histochemicals was confirmed by comparing the results of the histochemical tests with those of the GC-MS analyses of the in vitro spot tests (Gersbach *et al.*, 2001).

Fluorescence analysis

Fine powder and their extracts obtained using various solvents viz., petroleum ether, benzene, chloroform, acetone, ethanol and aqueous were examined under visible and UV light. The powdered materials were

also treated with various reagents such as 50% nitric acid, 50% sulphuric acid, 1N HCl, 1N NaOH and changes in colour were recorded (The Pharmacopoeia of India, 1996).

RESULTS AND DISCUSSION

Histochemical studies

The occurrence and distribution of various metabolites (phenolic compounds, polyphenols and tannins) in *Turbinaria ornata* J. Ag. are illustrated in Figure 2. Phenolic compounds (Figure 2a), polyphenols (Figure 2b) and tannin (Figure 2c) are profusely present in the outer layer of the thallus. Phenol is shown in a small quantity only in the outer layer of the cells. Phenolic compounds, tannin and polyphenols are abundantly present in the middle part of the thallus. Polyphenol and tannin showed minimal quantity and lignin was abundantly present in the central part of the thallus.

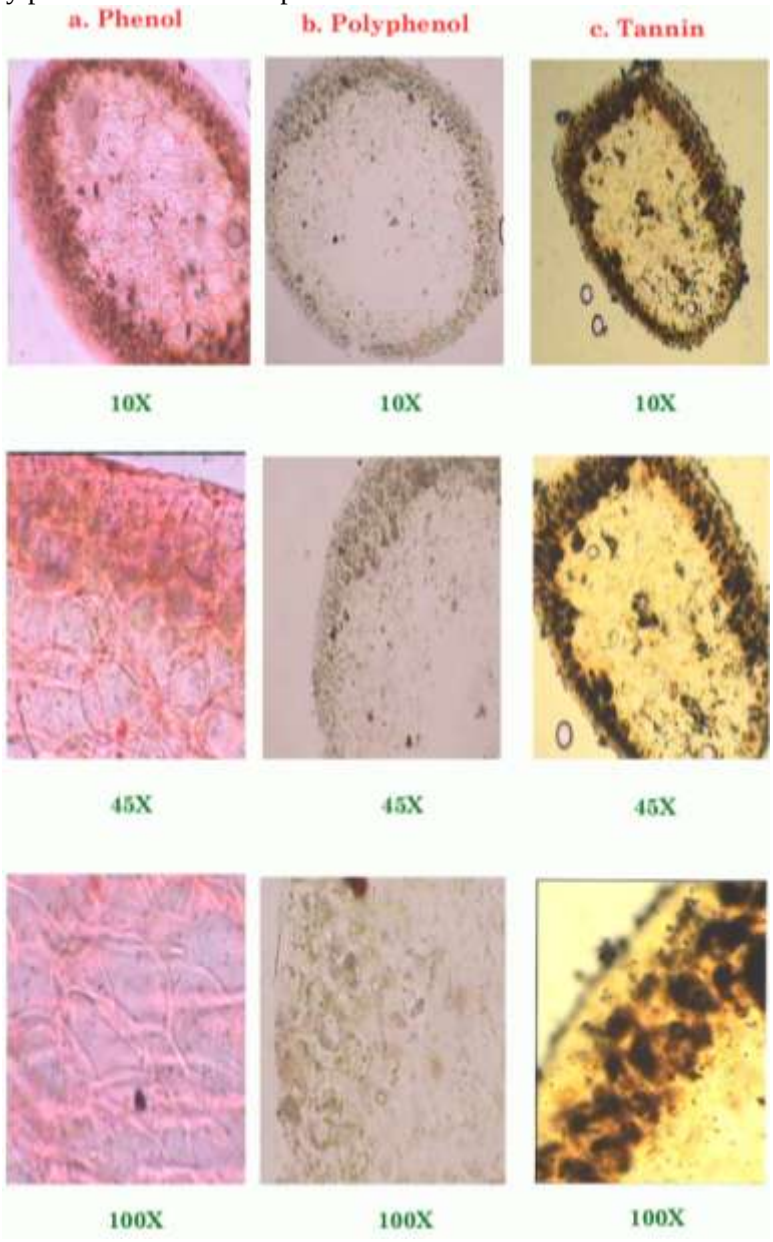

Figure 2: Histochemical studies of *Turbinaria ornata* J. Ag.

Fluorescence analysis

The characteristic fluorescent properties or colours emitted by the powdered thallus of *T. ornata* before and after treating with various reagents were recorded. The powdered thallus as such appeared very pale brown under daylight and ultraviolet radiation. After treating with various reagents such as aqueous, ethanol, acetone, benzene, chloroform and petroleum ether under daylight, it showed different shades of brown. However, under ultraviolet radiation, alkaline solutions like 1N aqueous sodium hydroxide and acids like 50% sulphuric acid, 1N HCl and 50% Nitric acid, with green, blackish brown and yellowish brown colours respectively. The characteristic fluorescent properties or colours recorded through this study could be used as a standard in the identification and authentication of the thallus of *Turbinaria ornata* in its crude form.

Table 1: Proximate analysis of *Turbinaria ornata*

	***Turbinaria ornata* (extracts)**		***Turbinaria ornata* (raw powder)**	
Solvents	**Fluorescent light**	**UV light**	**Fluorescent light**	**UV light**
Aqueous	Green	Black	Black	Turbid
Ethanol	Dark green	Light green	Light green	Green
Acetone	Dark brown	Ash colour	Dark green	Sandal
Benzene	Brown	Ash colour	Dark yellow	Sandal
Chloroform	Dark brown	Ash colour	Brown	Sandal
Petroleum ether	Dark brown	Ash colour	Light yellow	Light green
50% HNO3	Pale yellow	Light yellow	Light yellow	Light yellow
1N HCl	Dark brown	Black	Light brown	Green
1N NaOH	Greenish brown	Brown	Dark brown	Yellow
1N H2SO4	Black	Black	Sandal	Brownish green

The histochemical and fluorescence evaluations of *Turbinaria ornata* J. Ag. performed in this work revealed the presence of compounds with proven therapeutic actions such as phenolic compounds, polyphenols and tannins distributed through the tissues of those organs. The high antioxidant activity is due to the presence of phenolic compounds (Lima *et al.*, 2002). Tannins are a major group of compounds that act as primary antioxidants or free radical scavengers (Polterait, 1997). Tannins are known to possess general antimicrobial activities (Rievere *et al.*, 2009). Recent reports show that tannins may have potential value as cytotoxic and antineoplastic agents (Aguinaldo *et al.*, 2005)**.** Phenolic compounds, polyphenols and tannin are abundant throughout the species and are relatively easy to identify. They can therefore be used as chemical markers in taxonomic classifications (Zuanazzi and Montanha, 2007).

Conclusions

The present histochemical study on *Turbinaria ornata* J. Ag. produced novel histochemical 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. Fluorescence analysis can be used as effective markers in identifying authentic from its adulterants. It also suggested that *Turbinaria ornata* J. Ag. may be rich sources of polyphenols, phenols and tannin 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 bio-efficacy and bioactivity.

REFERENCES

Aguinaldo AM, El-Espeso BQ, Guovara MG and Nanoto SN (2005). Phytochemistry. In: *A Guide Book to Plant Screening Phytochemical and Biological* edited by Guevara BQ (University of Santo Tomas, Manila, Philippines).

Chandinia SK, Ganesana P and Bhaskar N (2008). *In vitro* antioxidant activities of three selected brown seaweeds of India. *Food Chemistry* **107** 707-713.

Duan XJ, Zhang WW, Li XM and Wang BG (2006). Evaluation of antioxidant property of extract and fractions obtained from a red alga, *Polysiphonia urceolata*. *Food Chemistry* **95** 37-43.
Faulkner DJ (2002). Highlights of marine natural products chemistry (1972-1999). *Natural Product Reports* **17** 1-6.
Gersbach PV, Wyllie SG and Sarafis V (2001). A new histochemical method for localization of the site of monoterpene phenol accumulation in plant secretary structures. *Annals of Botany*. **88** 521-525.
Kuda T, Tsunekawa M, Goto H and Araki Y (2005). Antioxidant properties of four edible algae harvested in the Noto Peninsula Japan. *Journal of Food Composition and Analysis* **18** 625-633.
Lima VL, Melo AG and Lima DES (2002). Fenólicose carotenóides totais em pitanga. *Scientia Agricola (Piracicaba, Brazil)* **59** 447-450.
Manivannan K, Thirumaran G and Karthikai Devi G (2008). Biochemical Composition of Seaweeds from Mandapam Coastal Regions along Southeast Coast of India. *American-Eurasian Journal of Botany* **1** (2) 32-37.
Mao WJ, Li BF, Gu QQ, Fang YC and Xing HT (2004). Preliminary studies On the Chemical characterization and antihyperlipidemic activity of polysaccharides from the brown alga *Sargassum fusiforme*. *Hydrobiologia* **512** 263-266.
Natarajan A, Moorthy R and Perumal A (2011). *In vitro* antioxidant and anticoagulant activities of sulphated polysaccharides from brown seaweed *Turbinaria ornata* (Turner) J.Agardh. *Asian Journal of Pharmaceutical and Biological Research* **1** (3) 232-239.
Patra JK, Rath SK, Jena K and Rathod VK and Thatoi H (2008). Evaluation of antioxidant and antimicrobial activity of seaweed (*Sargassum sp.*) extract: A study on inhibition of Glutathione-S-Transferase activity. *Turkish Journal of Biology* **32** 119-125.
Polterait O (1997). Antioxidants and free radical scavengers of natural origin. *Current Organic Chemistry* **1** 415-440.
Rievere CJH, Van Nguyen L, Pieters B, Dejaegher YV, Heyden CV, Minh J and Quetin J (2009). Polyphenols isolated from antiradical extracts of *Mallotus metcalfianus*. *Phytochemistry* **70** 86-94.
Stiger V and Payri CE (2005). Natural settlement dynamics of a young population of *Turbinaria ornata* and phenological comparisons with older populations. *Aquatic Botany* **81** 225–243
Taskin E, Ozturk M, Taskin E and Kurt O (2007). Antibacterial activities of some marine algae from the Aegean Sea (Turkey). *African Journal of Biotechnology* **6** 2746-2751
The Pharmacopoeia of India (1996). Controller of Publication, Government of India, India.
Thillairajasekar K, Duraipandiyan V, Perumal P and Ignacimuthu S (2009). Antimicrobial activity of *Trichodesminum erythraeum* from South East coast of Tamil Nadu, India. *International Journal of Integrative Biology* **5** 167-170.
Vijayabaskar P and Shiyamala, V (2011). Antibacterial activities of brown marine algae (*Sargassum wightii* and *Turbinaria ornata*) from the Gulf of Mannar Biosphere Reserve. *Advances in Biological Research* **5** (2) 99-102.
Zuanazzi JAS and Montanha JA (2007). Flavonóides, 6th edition. *Florianópolis* **7** 577-614.