Micromorphological and phytochemical studies of *Tubinaria ornata* (Turner) J. Agardh thallus (phaeophyceae)

Poonam Sethi

Department of Plant biology & biotechnology, Assistant professor, Guru Nanak college, Chennai. India.
poonam123.73@rediffmail.com

Abstract: Thallus of Turbinaria a marine alga belonging to Phaeophyceae was studied and detailed micromorphological and phytochemical evaluation was done. Morphology of the thallus has been studied to aid pharmacognostic and phytochemical evidences to aid in taxonomic species identification. Parameters presented in this paper may be proposed to establish the authenticity of this plant and can possibly help to differentiate the alga from its other species. The study revealed several interesting characters like funnel shaped terminal bodies and its cellular details. All the essential amino acids were present but isoleucine and asparagine occurred in huge amounts. Vitamin B6 formed the major part in addition to Vitamin B2 and Vitamin B1. The algae is a rich source of palmitic acid.

[Poonam Sethi. **Micromorphological and phytochemical studies of *Tubinaria ornata* (Turner) J. Agardh thallus (phaeophyceae).** *Biomedicine and Nursing* 2016;2(2): 58-64]. ISSN 2379-8211 (print); ISSN 2379-8203 (online). http://www.nbmedicine.org. 10. doi:10.7537/marsbnj02021610

Key words: alga, marine, micromorphological, phaeophyceae, phytochemistry, Turbinaria

1.Introduction

India is one of the mega diversity countries in the world and medicinal plants form the backbone of traditional systems of medicine in India, thousands of tribal communities still use folklore medicinal plants for the cure of various diseases. Indian medicinal plants have been studied for potential source of bioactive compounds. The great interest in the use and importance of medicinal plants in many countries has led to intensified efforts on the documentation of ethnomedical data of medicinal plants (Dhar et al., 1968). The recent increase in compounds isolated from land plants, has open doors to the poorly exploited marine ecosystem which appears to be a good candidate of natural resource (Baker, 1984). The aquatic ecosystem covers about 70 % of the earth's surface and India has a vast coastline of 6100 km supporting a rich flora of marine plants such as seaweeds, mangroves and sea grasses (Chapman & Chapman, 1980). Marine algae exhibit interesting nutritional properties in addition to their ecological properties. The results of the study suggest that the algae which are abundantly available in this ecosystem also have considerable potential of carbohydrates, amino acids, proteins, phenols and lipids for their use as food and pharmaceutical industry as a source in preparation of nutrient supplements, medicine and fine chemical synthesis.

2. Materials and method

i) Collection of specimens

The plant specimen for the proposed study was collected from Rameswaram, Tamil Nadu, India during the month of March During this period the experimental algae were usually in the saprophytic phase. The collection contained juveniles and few gametophytic thalli. Authenticated by Dr. R. Thevanathan, Presidency College, Chennai. Care was taken to select healthy plants and normal organs. The required samples of different organs were cut and removed from the plant and fixed in FAA. After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary –Butyl alcohol as per the schedule given by (Sass, 1940). Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60 C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

ii) Sectioning

The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the sections was 10-12 µm. Dewaxing of the sections was by customary procedure (Johansen, 1940). The sections were stained with Toluidine blue as per the method published by (O Brien, 1964). Since Toluidine blue is a polychromatic stain. The staining results were remarkably good; and some cytochemical reactions were also obtained. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies.

iii) Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon Lab Photo 2 microscopic Unit. For normal observations bright field was used. Magnifications of the figures are indicated by the scale-bars.

iv) **Phytochemical Tests**

The carbohydrates as total sugars, were estimated following the procedure of (Roe, 1955). The absorbance was read at 620 nm using HITACHI UV 2001 Spectrophotometer. (Lowry et al., 1951) procedure was followed to estimate the protein content. Freshly collected algae were used for the estimation of total soluble protein. The procedure outlined by (Bligh & Dyer., 1959) was used with modifications to determine the total lipids in the sample.

Total free amino acid content of freshly collected frozen tissues of algae was estimated by ninhydrin method (Moore & Stein, 1948). The pink colour developed was measured at 550 nm in a Spectronic 21 photocolorimeter. A composite mixture of alanine, aspartic acid, tryptophan, proline and lysine (in equal weights) was used as the standard. Vitamin content of dry, powdered sample of algae was estimated by HPLC (AOAC, 1990). HPLC system (SCHIMADZU) equipped with UV detector was used under the following analytical conditions for the estimation of nicotinic acid, vitamin B_1, vitamin B_6 and vitamin B_2. For vitamin A the algal powder was saponified with ethanolic KOH for 30 minutes and transferred to a separating funnel and repeatedly extracted with n-hexane. The final pooled extract was evaporated to dryness under reduced pressure in a rota evaporator and vitamin A level was determined by HPLC.

Fatty acids were determined and quantified by NEON II gas chromatography analysis outlined by (Niller & Bayer, 1955) Mineral analysis or elemental analysis of the shade dried sample was carried out by procedure outlined by Perkin Elmer atomic absorption spectrophotometry, 1981. Standards for the above elements were prepared according to PERKIN – ELMER'S manual.

Extraction of fucan was done following the method outlined by (Preprame et al., 2001) with some modifications. Fucoidan was extracted from the algae with water at room temperature and purified through ethanol precipitation. Ten gram of the powdered seaweed was extracted with water under mechanical stirring, for 12 hours at 25°C which was later centrifuged. The supernatant was concentrated and the residue was discarded. The supernatant was concentrated, dialyzed against water and lyophilized to yield colourless residue. This was then weighed and the yield was estimated.

3.Results

i)**Botanical description**

Plants erect and stiff, 2-20-(30) cm long when reproductive, usually isolated or in small groups, often rusty brown to dark brown; holdfast bearing one (or more) terete erect portion, basally a conical or irregular holdfast with several unbranched or dichotomously branched stolons, these often remaining when erect portion torn off, or appearing before erect portion formed. Juvenile plants with flattened blades can form new plants, become free-floating; larger plants with several orders of branching. Blades peltate, with 'petiole' and double row of stiff spines often with secondary branching from lower adaxial surface of blades; rarely irregularly triangular margin of leaves in apical view; petiole cylindrical near base, becoming traingularly compressed in distal portions; many plants with some leaves having hollow centers that function as floats. Receptacles developing into tightly branched clusters on adaxial side of leaf petiole near base, mostly cylindrical, to 1.5 cm long, with blunt apices.

ii) Macroscopical characters

The plant body consisits of branched cylindrical axis and terminal clusters of funnel shaped expanded bodies.the surface of the plant body is smooth and even. (Fig.I)

Figure: I The experimental plant

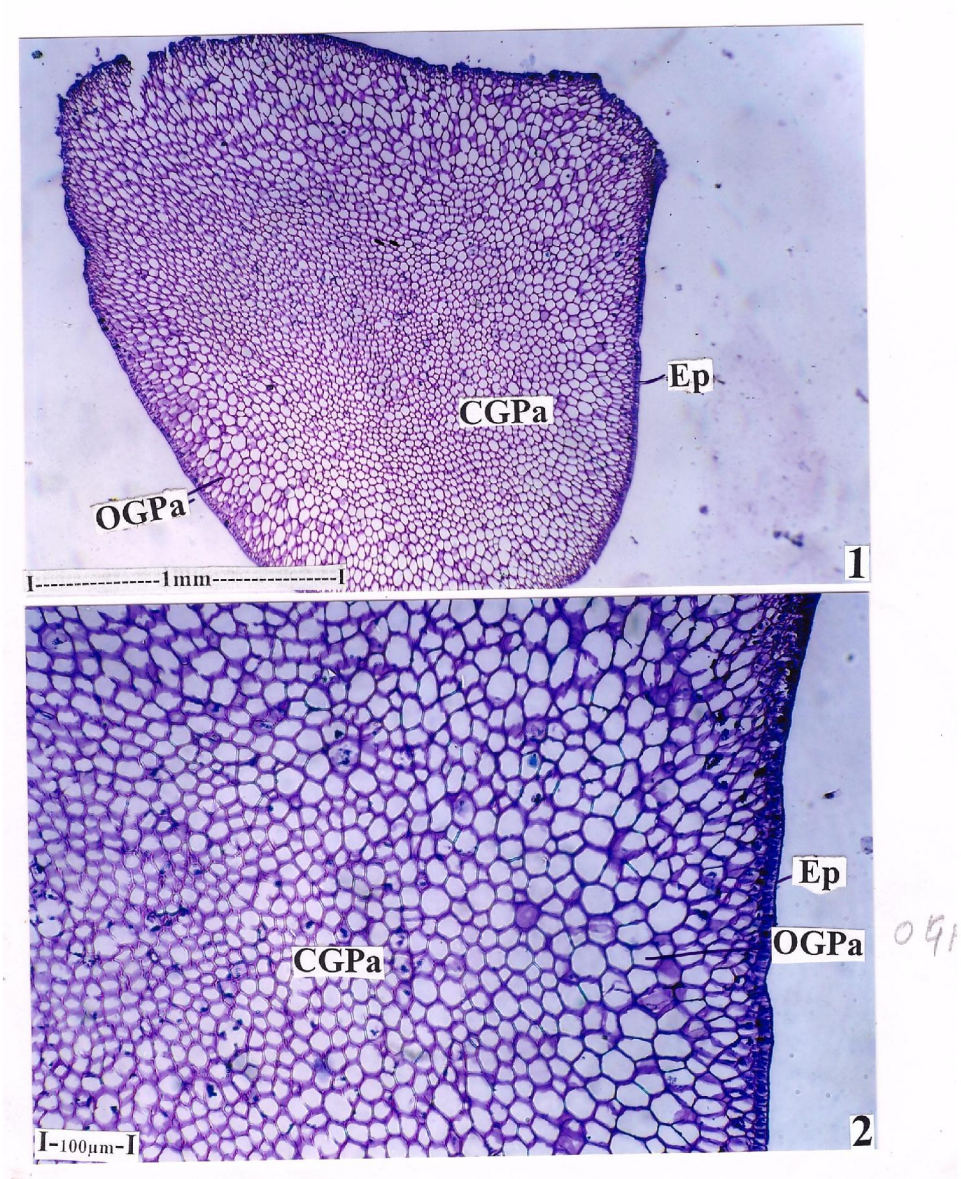
iii) **Microscopical characters**

The axis is broadly rectangular in cross sectional view (Fig.II-1.1). The axis is 1.85mm in diameter. It consists of thin layer of small thick walled darkly stained epidermis and parenchymatous ground tissue. The outer ground tissue include fairly thick walled smaller angular cells. The central ground tissue includes circular, slightly larger thin walled cells (Fig.II-1.2).

Funnel shaped terminal bodies have thick cylindrical stalk and widely expanded circular flat funnel (FigII-2.2). The stalk portion is similar to the stem. The marginal portion of the funnel appears cylindrical while the terminal part is wide and semi circular. It is 950µm thick (Fig.II-3.1). It has a small angular thick walled epidermal layer and thin walled sub epidermal layers. The ground parenchymatous cells are wide, angular thick walled and compact (Fig.II-3.2). The above parameters help in identifying the species and to establish the authenticity of this

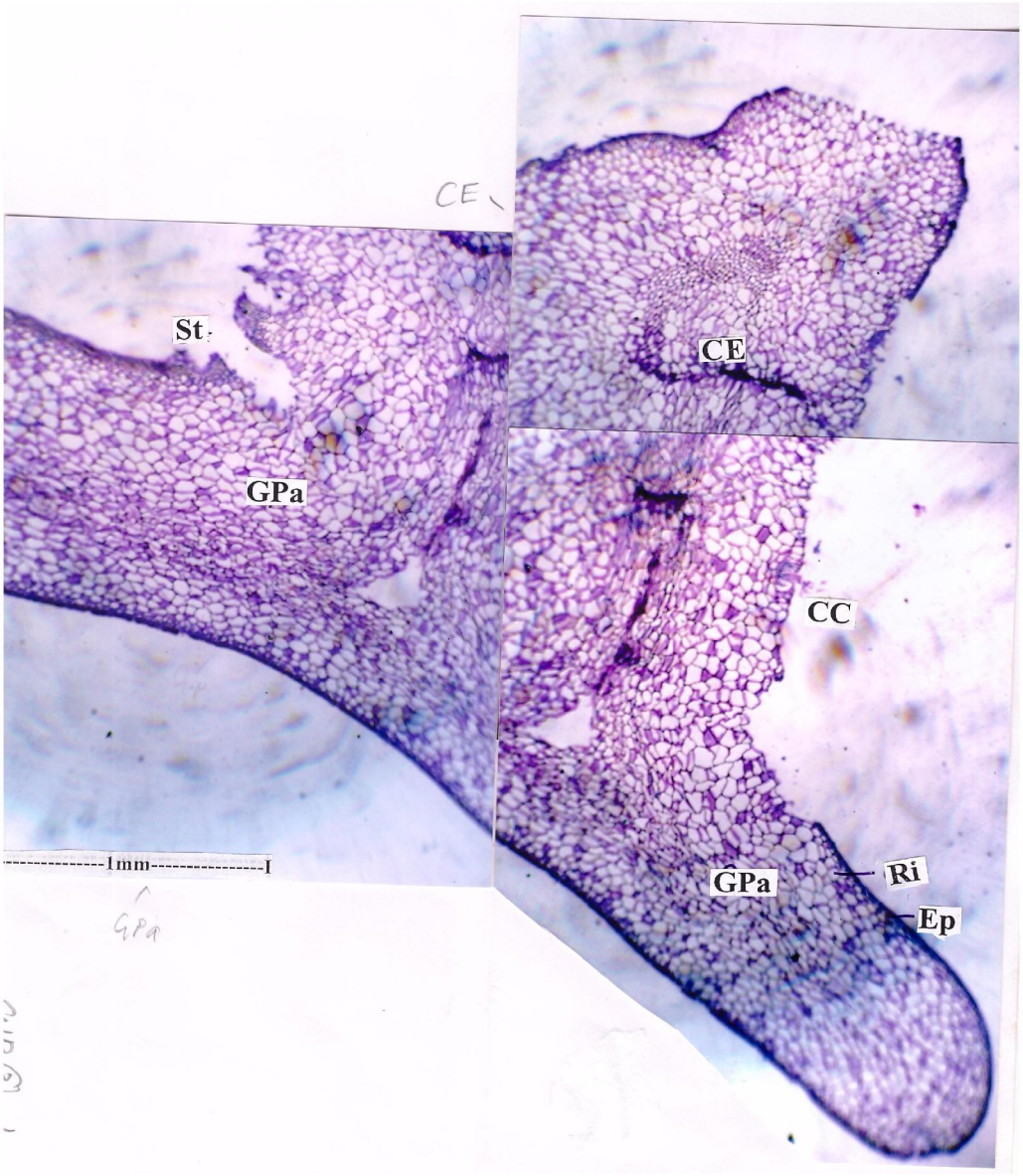
seaweed and can possibly help to differentiate it from its other adulterants.

2: T.S. of axis-a sector



[CGPa- Central Ground tissue Parenchymatous; Ep-Epidermis; OGPa- Outer Ground tissue Parenchymatous]

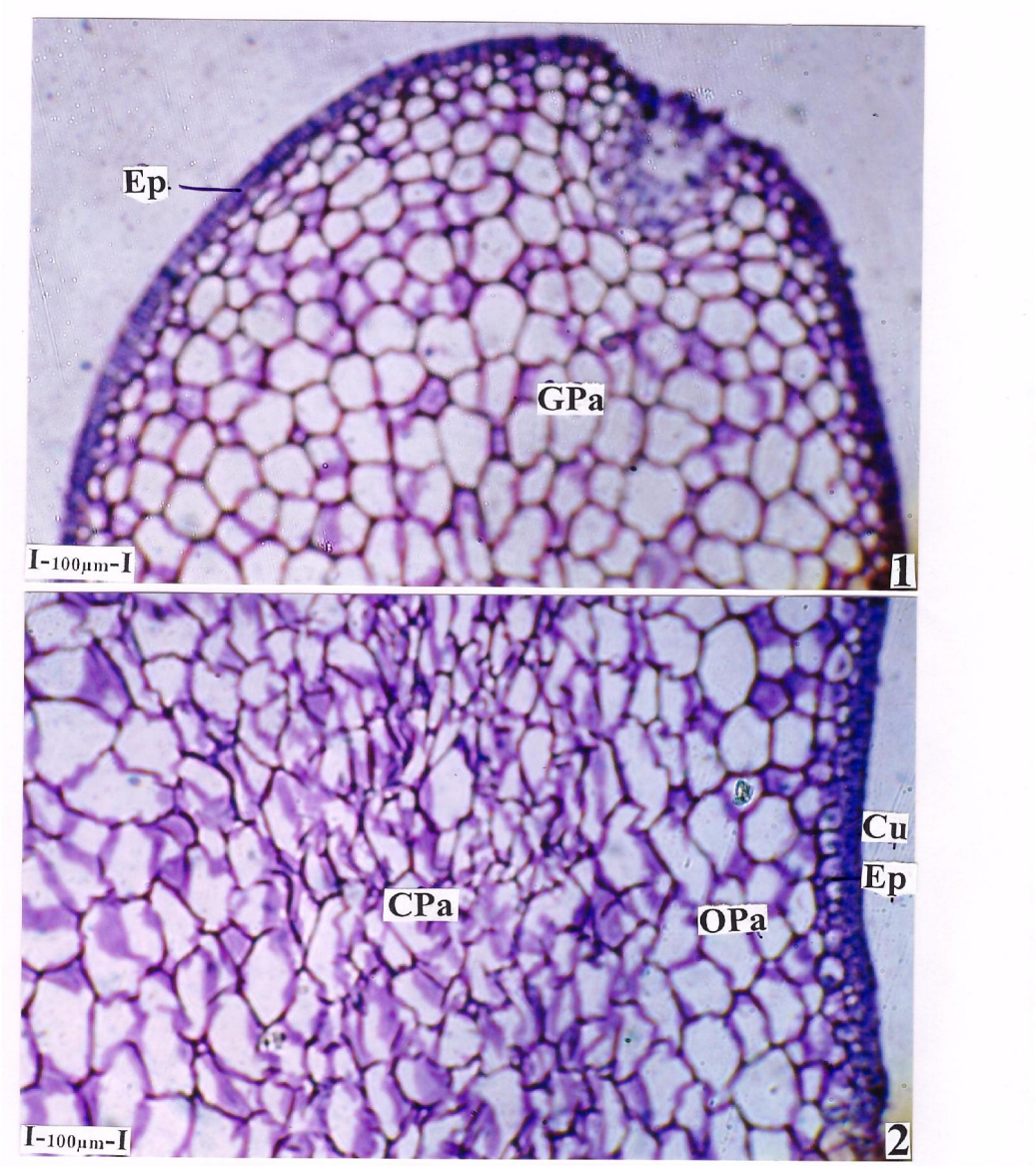
Fig. II - 1:T.S. of axis



[CC- Central Cavity; Ep-Epidermis; GPa- Ground tissue Parenchymatous; St-Stalk; CE-Conducting Elements; Ri- Rim]

Fig. III-: L.S. of funnel shaped body

2: stalk portion of the funnel



[Cu- Cuticle; Ep-Epidermis; GPa- Ground tissue Parenchymatous; CPa- Central Parenchymatous tissue; OPa- Outer Parenchyma]

Fig. IV -1: L.S. of rim of funnel shaped body

iii) Phytochemical constituents

The results are shown in the form of tables with statistical significance. Any macrobiotic diet should contain carbohydrates, lipids and protein which the algae possesses in the following proportion as given in Table 1.

Table 1: Total carbohydrates lipids and protein in the tissues of the experimental algae

Alga	Total carbohydrate mg g-1 dry wt. (mean±s.e)	Total lipid mg g-1 dry wt. (mean±s.e)	Total protein mg g-1 dry wt. (mean±s.e)
Turbinaria	46.9±0.52	182.0±1.155	12.6±0.162

The algae is rich in amino acids a total of eighteen aminoacids were reported (Table 2).

Table 2: Relative levels of amino acid in Turbinaria

Amino Acid	µg g-1 dry wt. (mean±S.E)
Aspartic acid	25.083±0.254
Glutamic acid	41.212±0.058
Asparagine	58.200±0.058
Serine	26.000±0.017
Glutamine	14.698±0.041
Glycine	33.013±0.369
Threonine	17.931±0.001
Arginine	24.562±0.030
Alanine	25.150±0.012
Cystine	26.995±0.012
Tyrosine	18.671±0.115
Histidine	44.898±0.018
Valine	45.332±0.018
Methionine	29.826±0.015
Isoleucine	74.393±0.005
Phenylalanine	50.595±0.049
Leucine	49.535±0.003
Lysine	5.022±0.013

Table 3: Vitamin content of the experimental algae

Vitamin	µg g-1 dry wt. (mean±S.E)
Vitamin B6	125±0.577
VitaminB2	76±1.732
VitaminB1	42±2.309

Table 4: Relative levels of fatty acid in Experimental alga

Fatty Acid	µg g-1 dry wt. (mean±S.E)
Caprylic acid	22.0±0.866
Lauric acid	67.0±0.577
Tridecanoic acid	27.0±0.289
Myristic acid	11.067±0.346
Pentadecanoic acid	11.6±0.176
Palmitaloic acid	35.1±0.577
Heptadecanoic acid	27.3±0.173
Stearic acid	27.4±0.866
Oleic acid	24.0±0.808
Linoleic acid	47.7±0.981
α Linoleic acid	10.0±0.173
γ Linoleic acid	8.6±0.346
Palmitic acid	5001±15.011

In addition to lysine, phenylalanine and aspartic acid, histidine too occurred as a major constituent. Table 3 gives the vitamin content of the experimental algae nicotinic acid, B1 and B2 were detected in the shade dried powdered samples of the experimental algae. B6 was present in huge amounts. Gas chromatographic analysis of the shade dried samples showed the presence of fifteen acids where palmitic acid formed the bulk of the total fatty acid content (Table 4). Among the minerals present iron formed the major one as depicted in Table 5.

Table 5: Relative levels of minerals in Experimental alga

Mineral	µg g-1 dry wt. (mean±S.E)
Copper	8.641±0.173
Zinc	19.92±0.115
Manganese	8.75±0.144
Chromium	16.6±0.115
Lead	0.4±0.006
Nickel	25.2±0.115
Cadmium	5.9±0.058
Magnesium	9.6±0.116
Sodium	39.11±0.058
Potassium	29.65±0.265
Cobalt	3.47±0.058
Calcium	12.4±5.774
Iron	858.5±0.25

The most important biochemical compound being the phycocolloids. Brown seaweeds are known to produce different polysaccharides namely alginate and fucoidans. (Table 6)

Table 6: Extractive Phycocolloids present in experimental algae

Alga	Fucoidan mg g-1 dry wt. (mean±S.E)	Alginic Acid mg g-1 dry wt. (mean±S.E)
Turbinaria	69.0±0.01	14.9±0.021

4.Discussion

The investigation revealed the richness of the algae in the carbohydrate and protein content, lipid content being far ahead. All the essential amino acids were present but isoleucine and asparagine occurred in huge amounts. Vitamin B6 formed the major part in addition to Vitamin B2 and Vitamin B1.The algae is a rich source of palmitic acid and iron formed the major bulks in the mineral content. The bulk of the algae contained a phycocolloid fucoidan which is a sulphated polysaccharide and trace amounts of alginate a carboxylated polysaccharide. These phycocolloids are promising in the field of pharmaceuticals. Thus the nutraceutical value of the alga shows its diversification in the field of medicine, nutrition and industry.

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6/24/2016