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Research Paper

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Comparative studies on morphological and histochemical of *Padina tetrastomatica* and *Sargassum wightii*

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ABSTRACT:

Algae exhibit a very wide range of morphological diversity. The simplest forms are unicellular, microscopic and motile (or) non-motile eukaryotic cells. According to the web definitions histochemistry is the branch of science that deals with the chemical composition of the cells and the tissues of the chemical composition of the cells and tissues of the body and science the combines the techniques of biochemistry and histology in the studies of chemical constitution of cells and tissue. Fronds of *Padina tetrastomatica* shelter for aquatic organisms such as fishes and molluscs. Brown seaweeds are the most intensely studied type of seaweeds being composed of potentially bioactive polysaccharides such as fucoïdan and alginic acid. Fucoïdan from *Sargassum wightii* Greville has been reported to possess α -D-Glucosidase inhibitory activity. The Present Study revealed as the presence of Phenol crystals, Sulphated polysaccharides and Starch granules in both brown algae.

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1. Introduction

Histochemistry are to be considered as ancillary disciplines which may purely describe the chemical composition of cells and tissues through the application of a reasonably wide collection of methods and techniques. The classical study of this technique pave a way to identify the value added biomolecules for future purpose.

2. Materials and Methods

Padina Tertastromatica Huck

Classification

Class	:	Phaeophyceae
Order	:	Dictyotales
Family	:	Dictyotaceae
Genus	:	<i>Padina</i>
Species	:	<i>Padina tertastromatica</i>

Description:

Thallus flabellate, divided into several narrow overlapping blades almost to the base, in the upper part thallus split into small blades with inrolled edges, 7 – 15 cm high, 15 – 29cm broad, lightly calcified, yellowish – brown, olivegreen to dark – darkbrown, markedly zonated due to reproductive organs on both sides of narrow rows of hairs. Hairs develop in concentric bands on the lower surface of the blade. Blades in young portion composed of two cell layers, in the middle part of 3 cell layers to 90µm thick, and at the base of 4cell layers to 150µm thick. The basal part is covered by rhizoids. Attachment by stypose holdfast to low intertidal and upper subtidal rocks and dead corals, in areas with modrate wave activity.

Sargassum Wightii Greville Ex J- Agardh

Classification:

Class	:	Pheophyceae
Order	:	Fucales
Family	:	Sargassaceae
Genus	:	<i>Sargassum</i>
Species	:	<i>Sargassam wightii</i>

Description:

Plant dark brown 20-30 cm in height with a well-marked holdfast, upper portion richly branched, axis cylindrical glabrous, leaves 5-8 cm long and 2-9mm broad leaves tapering at the base and apex, midrib inconspicuous vesicles large, spherical or ellipsoidal being 5-8mm long and 3-4mm broad, stipe of the vesicle 5-7mm long seldom ending into a long tip, receptacles in clusters and repeatedly branched. It is used as raw material for the production of sodium alginate. It also contains 8-10% mannitol which can be used as substitute for sugar fertilizer and medicines.

Sample Collection:

The algae specimens are collected from Mandapam and Thonithurai coastline of Rameswaram district. The fixative is generally used 4% formaldehyde solution. The collected specimens are used to take transverse thin section of the thallus were treated with different stains used for localizing different substances in the cell wall and other cell layers. Thin free hand sections were taken and treated with different histochemical stains. The images were observed as nature

of stain binding and its remarks. The microphotographs were prepared with the help of Microscope attached with Camera at the department of Botany, St Xaviers college, Palayamkottai.

Stain Preparation of Histochemical Stains		
Name of the Stain	Preparation	Inference
Iodine Potassium iodide	Add 0.2g of potassium iodide, 0.02g iodide and of water, then shake well to make up for 100ml	Starch granules
Mercuric Bromophenol blue	1g Mercuric chloride and 10mg bromophenol blue in 10ml of water or 95% ethanol. Final volume made up to 100ml of H ₂ O	Protein oxalates
Toluidine blue	0.5% in acetate buffer 0.5 ml of Toluidine blue in 100ml of water	Starch
Acid Fuschin	1% of Fuschine dissolved in 10ml water for 1.5 minutes at 40 ⁰ c. Final volume made up to 100ml of H ₂ O	Starch
Calcium Chloride	0.125mg of iodine 0.125g potassium iodine and 4g of calcium chloride in 100ml of distilled water	Calcium oxalates
Tannic Acid Ferric Chloride	Add 2ml of 1% Aqueous acid and 6ml of 3% Aqueous ferric chloride. Final volume make up to 100ml of H ₂ O	Phenol sulphated Starch

Table:1 Binding Nature of the Stain in Samples			
S.No	Name of the Stain	Observation	Confirmity Inference
1.	Calcium chloride	Light violet colour appear	Calcium oxalates
2.	Tannic acid ferric chloride	Dark brown colouration inside the cell	Phenol, Sulphated starch
3.	Mercuric bromophenol blue	Presence of protein	Protein oxalates
4.	Toluidine blue	Dark bluish violet and brown patches observed	Starch
5.	Iodine Potassium Iodide	Bluish colour appear at the cortex region	Starch granules
6.	Acid Fuschin	Bluish violet and purplish pink colour	Starch

3. Results and Discussion

In Table (1) stain preparation of histochemical stains were Binding nature of the stains in samples were given. **Plate-I** described the plant samples were given, and identified based on standard monographs (Fritsch, 1934, &1935; Srinivasan, 1950, Boergeson,1903,1920,1923). Holm & Brodie (2004) worked on the morphology and seasonal phenology and observed some aspects of the life history in the culture of *Porphyra dioica*. Distinctive morphological features, life cycle phases and seasonal variation of *Dictyota dichotoma* from sub-tropical population was also mentioned by Ana *et.al.*, (2008). S Frizo & Faca (2013) studied the morphological growth and autoecology of two alien invasive species *Sargassum wightii* and *Undaria pinnatifida*.

In **Plate- II** *Sargassum wightii* treated with Iodine Potassium Iodide (20x) all the cells are completely changed into brown colour due to the presence of starch granules and phenol. *Sargassum wightii* with Mercuric Bromophenol Blue (40x-1) dark round shaped colonies formed due to phenol sulphated starch, phenol sugars and starch. *Sargassum wightii* with toluidine blue (20x-1) shows the cell wall layer completely changed into brown colour in the prescence of nuclear stain, sulphated fucans, proteins and inorganic salts. *Sargassum wightii* with Acid fuschin (20x-1) shows some places only have the accumulation of phenol and starch granules *Sargassum wightii* Calcium Chloride (20x-1) cell wall shows the dark black and brown colour indicates the prescence of phenol, sugars, starch, lipothilic substance, nuclear stain, sulphated fucans proteins and inorganic salts. But in **Plate –III** illustrated with experimental results of histochemical binding nature by the application of six different stains on *Padina tetrastrumatica* where, Iodine Potassium Iodide(10x-1) shows bluish colour appearance shows the starch granules. *Padina tetrastrumatica* with Mercuric bromophenol Blue (10x-1) dark blue colour appearance shows the precsence of protein oxalates. *Padina tetrastrumatica* with toluidine blue (10x-1) shows dark violet colour shows the prescence of starch. *Padina tetrastrumatica* with Acid fuschin (10x-1) shows bluish violet colour shows the presence of starch. *Padina tetrastrumatica* with calcium chloride (10x-1) shows bluish salt crystals shows the prescence of calcium oxalates. *Paina tetrastrumatica* with Tannic Acid Ferric Chloride (10x-1) dark and light brown colour shows the prescence of phenol and sulphated starch. Similar reports were in conformity with previous reports of Kaleswari and Selvi (2015), Rengasamy (1990) and Allender (1977) and Gaillard (1972).

Plate:I –Habit



Plate:II- *Sargassum wightii*

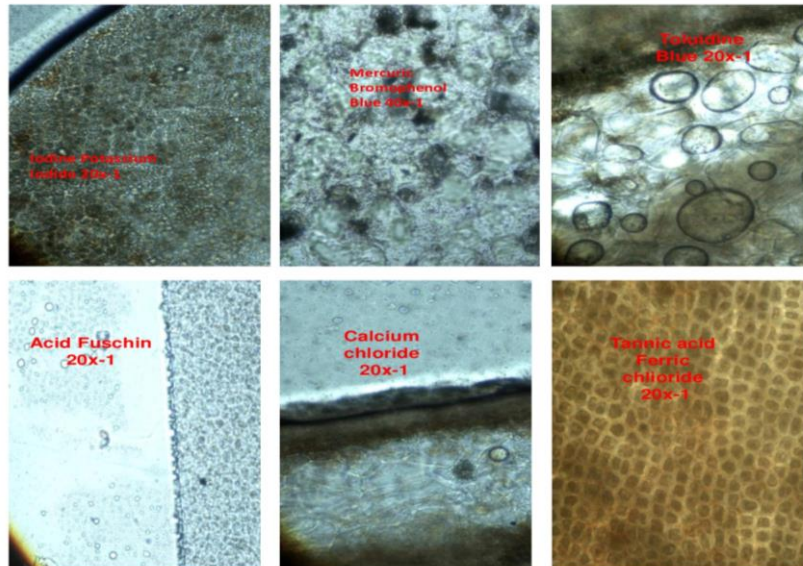


Plate:III- *Padina tetrastrum* Huck.



4. Conclusion

The present findings pertains the following results: Taxonomic identify were done based on morphological studies and with the reputed works given by marine taxonomist. The phaeophycean samples were takes samples namely *Sargassum wightii* and *Padina tetrastrumatica*. The localization of biomolecules using different stains such as Iodine Potassium Iodide, Calcium chloride, Tannic Acid Ferric Chloride, Acid Fuschin, Mercuric Bromophenol Blue and Toluidine blue treated sections where shown the transparent clear cellular details in all the algal specimens where observed and photographed under 10x, 40x, resolution. The above classical work would be lead to extract commercially important biomolecules and to develop other life support products.

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