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Studies on Morphological, Economic Value and Histochemistry of Padina and Sargassum Species

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Abstract

A typical life history of seaweeds is involving a seasonal growth cycle caused by climatic changes occurring throughout the year. Almost all the algae are show to exhibit seasonal growth, development and morphological changes. Brown seaweeds are the most intensely studied type of seaweeds being composed of potentially bioactive polysaccharides such as fucoidan. Fucoidan from *Sargassum wightii* Greville (*S. wightii*) has been reported to possess α-D-Glucosidase inhibitory activity. 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.

***Key words*: *Sargassum wightii*, Sargassum species, Histochemistry, Morphology, Economic value**

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. This is achieved through the application of a diverse array of methods and techniques. By utilizing these approaches, histochemistry provides detailed insights into the biochemical and molecular makeup of biological specimens, thereby supporting and enhancing the understanding gained from other biological and medical research fields. The study of the phytochemical characteristics of marine natural resources, such as seaweeds, is crucial due to their role as an alternative source of new bioactive molecules. Seaweeds have been used since ancient times as food and medicinal sources. The diverse life conditions of seaweeds have driven them to develop unique bioactive molecules [1], which exhibit antioxidant activity [2] and can be applicable for the treatment of oxidative-damage-related diseases or diabetes. Similarly, marine natural products with antibacterial capabilities [3] offer rich pharmacological potential. Many studies have demonstrated the usefulness of seaweeds in these contexts [4]. Padina's life cycle is considered perennial, but the thallus detaches every winter and regrows in spring [5]. During winter, the alga stays in the form of rhizoids, filamentous thalli or sporelings, until the conditions are suitable for full regrowth [6]. Like most brown algae, it has a haploid-diploid reproduction cycle [7]. Paul *et al*. [8] studied the morphology of the brown algal genus *Padina* from the southern Philippines, identifying three new species of this genus in the region. *Padina* species are widely distributed throughout tropical regions and are easily recognizable in the field due to their distinctive features. However, detailed morphological verification at the microscopic level is necessary for accurate infrageneric segregation. The study by Paul and colleagues highlights the importance of morphology in distinguishing species within the genus *Padina*, emphasizing the need for careful examination of physical characteristics to accurately classify and understand these algae. Holm and Brodie [9] 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*. [10]. *Sargassum wightii* is known for its rapid growth and ability to adapt to various environmental conditions. This species can form dense mats on the water surface, which can outcompete native vegetation for light and nutrients. The study found that *Sargassum wightii* thrives in a wide range of temperatures and salinities, contributing to its invasive potential [11]. *Undaria pinnatifida*, commonly known as wakame, is another highly invasive species. It has a fast growth rate and can attach to a variety of substrates, including rocks, shells, and artificial structures. The study highlighted that *Undaria pinnatifida* can significantly alter the local marine environment by displacing native species and changing the habitat structure [12-14]. Both species pose significant threats to biodiversity and ecosystem function. They can alter habitat structures, displace native species, and change nutrient cycling processes. Managing these invasive species requires a thorough understanding of their growth habits and ecological impacts to develop effective control strategies. Sfriso and Facca [15] studied the growth and

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auto ecology of two alien invasive species *Sargassum wightii* and *Undaria pinnatifida*. Species of the marine brown alga genus *Padina* are widely distributed throughout the tropics and are very easy to recognize in the field. However, infrageneric segregation requires the morphological verification at the microscopic level.

MATERIALS AND METHODS

Padina tertastromatica
Classification

Class	:	Phaeophyceae
Order	:	Dictyotales
Family	:	Dictyotaceae
Genus	:	*Padina*
Species	:	*Padina tertastromatica*

Description

Thallus flabellate, divided into several narrow overlapping blades almost to the base, in the upper part thallus split into small blades with enrolled edges, 7 – 15 cm high, 15 – 29cm broad, lightly calcified, yellowish – brown, olive-green to dark – dark-brown, 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 interdidal and upper subtidal rocks and dead corals, in areas with moderate wave activity.

Sargassum wightii Greville ex J- Agardh

Class	:	Eukaryota
Order	:	Fucales
Family	:	Surgassaceae
Genus	:	Sargassum
Species	:	Sargassam wightii

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.

Histochemistry

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.

Fixation: The fixative is generally used 10% formalin or the equivalent 4% formaldehyde solution.

Dehydration

Fixatives, such as formaldehyde, have the potential to further react with any staining procedure which may be used later in the process. Consequently, any remaining fixatives is washed out by placing the blocks in running water overnight or by successive changes of water and or a buffer. There are myriad means of washing the tissues, but usually simple washing in tap water is sufficient. If the tissues are to be embedded in paraffin or plastics, all traces of water must be removed: Water and paraffin are immiscible. The removable of wear is dehydration. The dehydration process is accomplished by passing the tissue through a series of increasing alcohol concentrations. The blocks of tissue are transferred sequentially to 30%, 50%, 70%, 80%, 90%, 95%, and 100% alcohols about two hours each. The blocks are then placed in a second 100% ethanol solution to ensure that all water is removed.

Embedding

After dehydration, the tissues can be embedded in xylol are the most commonly used clearing agents, through some histologist prefer mixtures of various oils.

The microphotographs were prepared with the help of Microscope attached with Camera at the department of Botany, St Xaviers College, Palayamkottai. The results were given in the table.

Economic important of Sargassum and Padina

Different industries and specialists have highlighted some of the benefits that *Sargassum* has. For example, it contains alginic acid, which is used as chickened and emulsifier in the food industry it has cellulose and hemicellulose, which are raw materials of paper it generates ethanol one of the main compounds for brio fuel, and it was discovered that its properties also make it an excellent fertilizer. Some of the products that are being made with *Sargassum* are note books and paper that can also be used for packaging and crafts. It's a friendly material as it avoids the use of trees and gives a new life to this seaweed. Hair strengthening treatments, as well as makeup moisturizing creams and sunscreens are some of the products that are being produced. The food industry is not getting behind in the use of *Sargassum*. The first cocktail drink based on this seaweed has been created. It is the "Pineapple" gift, whose name is a tribute to the Mayan culture that in ancient time. For some years now around Tulum area farmers families have using *Sargassum* to fertilize their crops. One of the reasons for the declining in the rate of antibiotic drug discovery is the high court of in vivo testing of antibacterial activity using mammalian model systems. At present there is a vast number of original articles reporting the antibacterial activity of crude extracts prepared from a diverse of natural products including Indonesian medicinal plants. Although most of those extracts and compounds fielded promising results in the in vitro stage many are not further characterized or even tested in the whole reason for such research to be done in the first place. A class of drugs that can inhibit the growth of bacteria known as antibiotics has been widely introduced as one of the potent arsenals in the treatment of infection in humans. Bacterial loud has been suggested to play an important role in the increasing death rate of the infected host.

Economic importance of Padina

Macroalgae belonging to the genus *Padina* produces antibacterial compounds that are important to inhibit the growth of various pathogens. Seaweeds are well known for their medicinal important and reported by several workers from all over the world. They are potential source of biologically active metabolites that are not found in other organisms and are used in pharma and drug discovery. The marine algae or seaweeds are one of the most important natural resources of marine ecosystems. Applications of *Padina* extracts showed significant nymphal mortality as compared to many other plant species. It can also be used as in organic fertilizer. Aqueous extract of *Padina* is rich in macronutrients like nitrogen (N), phosphorous (P), and potassium (K), that are essential for plant growth and development. The seaweeds can be exploited as bioorganic eco-friendly fertilizer to stimulate the growth of various economic important crops instead of using synthetic chemical fertilizer that are harmful for the environment as they can undergo biological magnification. Algae are important role in environmental monitoring and management. They are the primary producers in all aquatic ecosystems. They provide the energy for the functioning of aquatic ecosystems. They respond rapidly even to a minor change in their environmental.

RESULTS AND DISCUSSION

The data described in (Table 1) stain preparation of histochemical stains was binding nature of the stains in samples were given. In (Plate – I) the plant samples were given.

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 prescience 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 presence of phenol, sugars, starch, lipothilic substance, nuclear stain, sulphated fucans proteins and inorganic salts.

Table 1 Stain preparation of histochemical stains

S. No.	Name of the stain	Preparation	Inference
1.	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
2.	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_2O	Protein oxalates
3.	Toluidine blue	0.5% in acetate buffer 0.5 ml of Toluidine blue in 100ml of water	Starch
4.	Acid Fuschin	1% of Fuschine dissolved in 10ml water for 1.5 minutes at 40^0c. Final volume made up to 100ml of H_2O	Starch
5.	Calcium chloride	0.125mg of iodine 0.125g potassium iodine and 4g of calcium chloride in 100ml of distilled water	Calcium oxalates
6.	Tannic acid ferric chloride	Add 2ml of 1% aqueous acid and 6ml of 3% aqueous ferric chloride. Final volume makes up to 100ml of H_2O	Phenol sulphated starch

Table 2 Binding nature of the stain in samples (*Sargassum wightii* and *Hypnea valentiae*)

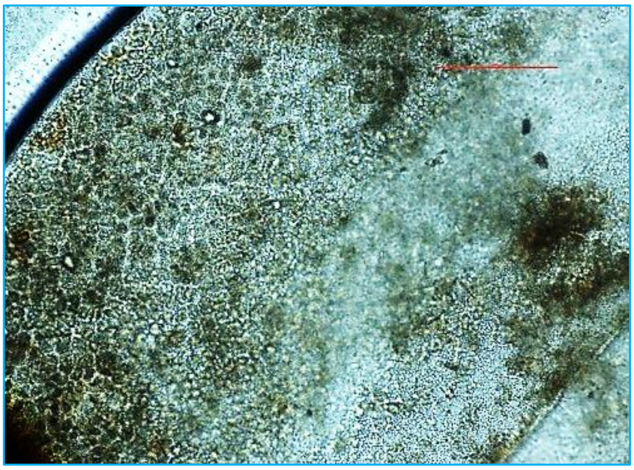
S. No.	Name of the stain	Observation	Inference
1.	Calcium chloride	Light violet colour appear	Calcium oxalates
2.	Tannic acid ferric chloride	Dark brown colouration inside the cell	Phenol, Sulphated starch
3.	Mercuric bromophenol blue	Presence of protein	Protein oxalates
4.	Toluidine blue	Dark bluish 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

Sargassam wightii Greville

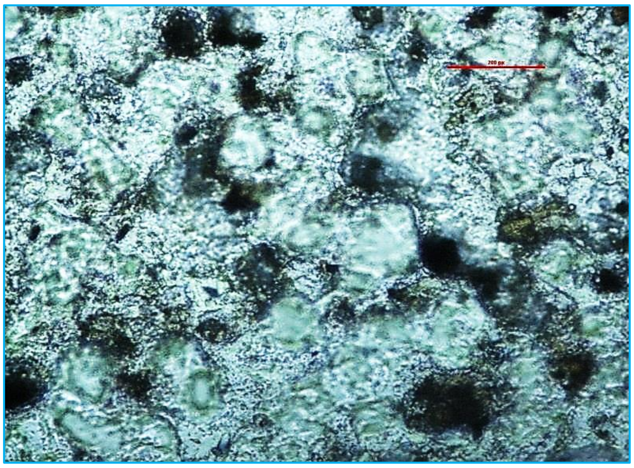
Padina tetrastromatica Hauck

Plate I Plant samples

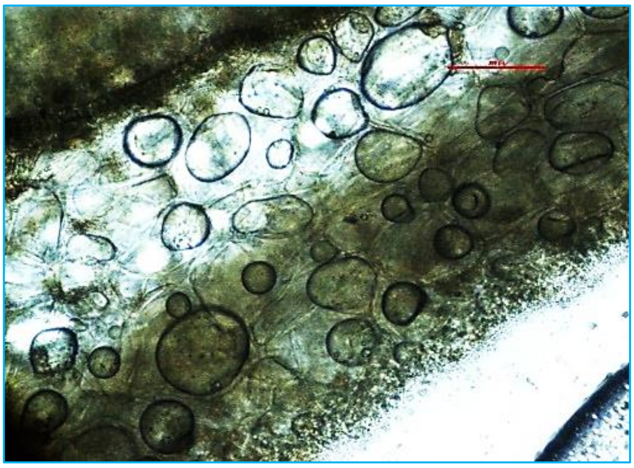
In (Plate –II) *Padina tetrastromatica* with Iodine Potassium Iodide (10x-1) shows bluish colour appearance shows the starch granules. Padina tetrastromatica with Mercuric bromophenol Blue (10x-1) dark blue colour appearance shows the presence of protein oxalates. *Padina tetrastromatica* with toluidine blue (10x-1) shows dark violet colour shows the presence of starch. *Padina tetrastromatica* with Acid fuschin (10x-1) shows bluish violet colour shows the presence of starch. *Padina tetrastromatica* with calcium chloride (10x-1) shows bluish salt crystals shows the presence of calcium oxalates. *Paina tetrastromatica* with tannic acid ferric chloride (10x-1) dark and light brown colour shows the presence of phenol and sulphated starch. These staining reactions help identify the biochemical components present in *Padina tetrastromatica*.



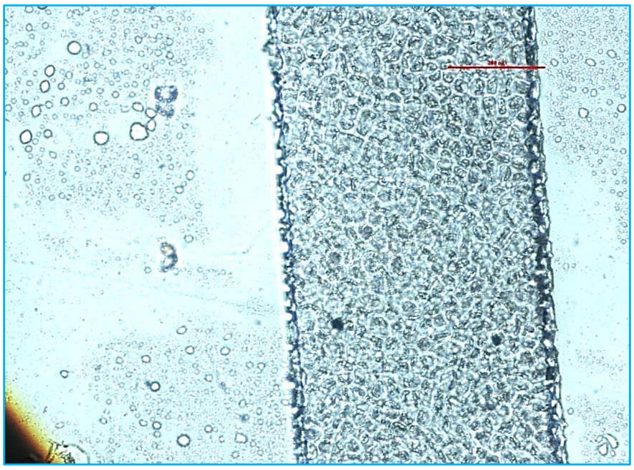
Sargassum iodine potassium iodide 20x-1



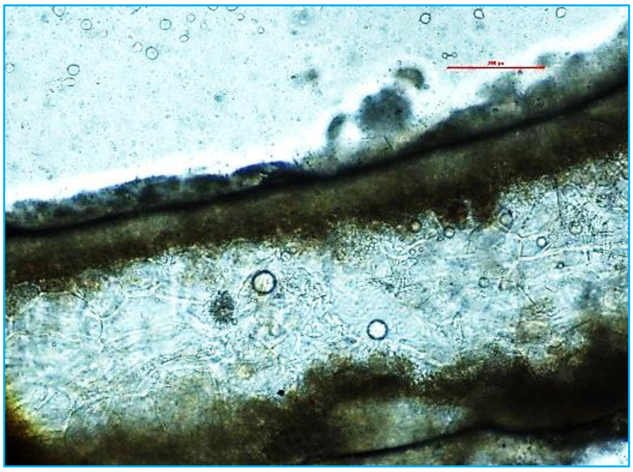
Sargassum mercuric bromophenol blue 40x-1



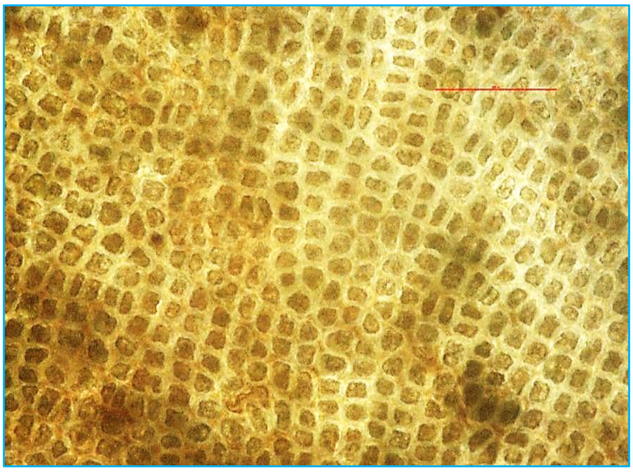
Sargassum toluidine blue 20x-1



Sargassum acid fuschin 20x-1



Sargassum calcium chloride 20x-1



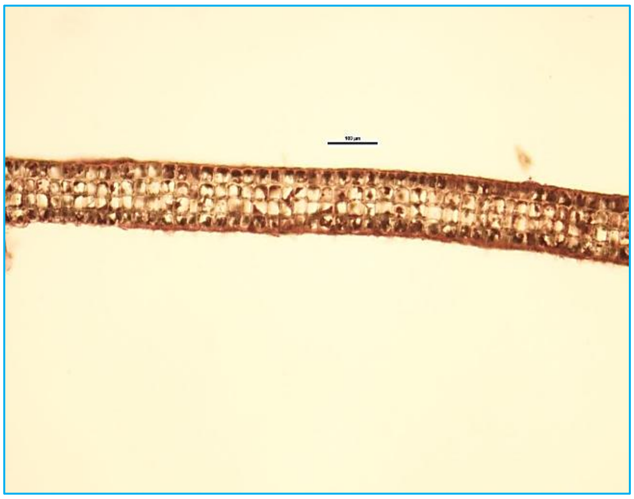
Sargassum tannic acid ferric chloride 40x-1

Plate - II

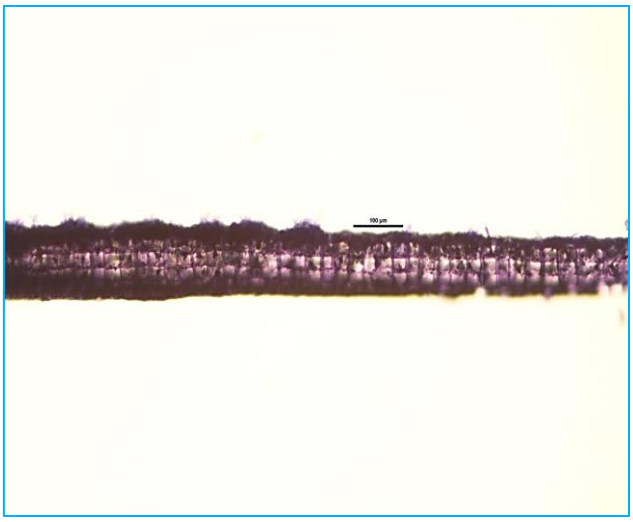
Padina shows an alternation of isomorphic generations and is isogamous. Unlike many brown algae. Padina forms spores in clusters of four, similar to the patterns seen in red algae. According to the observation of Rengasamy [16] and Allender [17], the sporophytic phase is considerably more common than the gametophytic phase with as much as 86% sporophytes against 14% gametophytes in the field. The predominance of diploid sporophytes may be explained partly by tetraspore formation by apomeiosis as documented by Gaillard [18].

Normal pancreatic architecture was observed in control group with a well-defined Langerhans islets consisting normal beta cells. Untreated diabetic animals showed necrotic and degenerative changes to β cells in Langerhans islets. Some of the beta cells showed pyknotic nuclei with dark eosinophilic cytoplasm. Congo red staining was performed to assess the A β accumulation in the cerebral cortex and hippocampus. This staining method is commonly used to identify amyloid deposits due to its specificity for amyloid fibrils. Congo red binds to the amyloid plaques, and under polarized light, the stained amyloid exhibits apple-green birefringence, which is a characteristic feature of amyloid deposits [19].

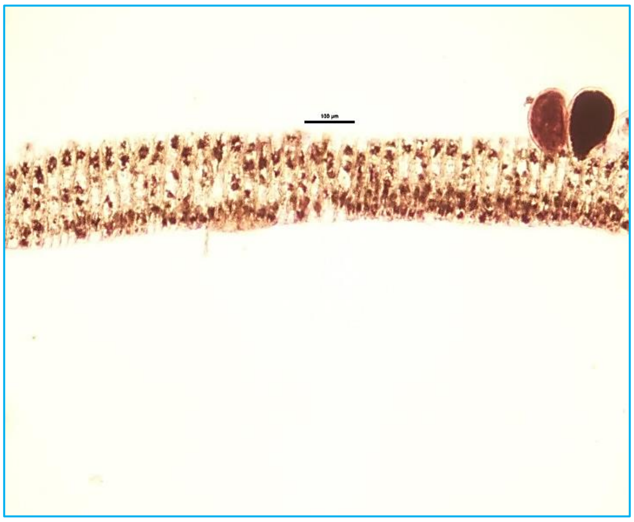
Padina iodine potassium iodide 10x



Padina mercuric bromophenol blue 10x

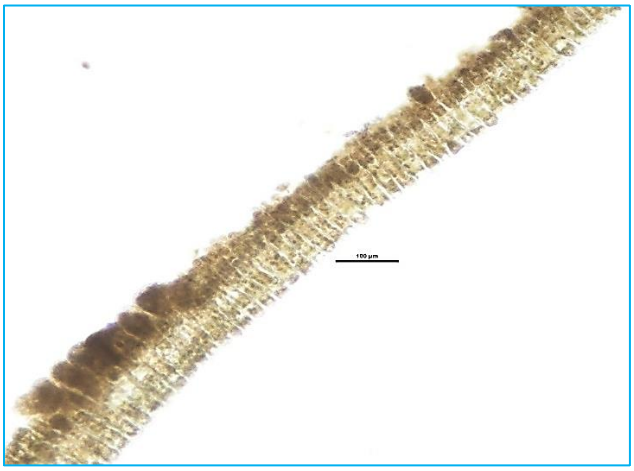


Padina toluidine blue 10x



Padina acid fuschin 10x

Padina calcium chloride 10x



Padina tannic acid ferric chloride 10x

Plate - III

CONCLUSION

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 tetrastromatica*. 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 were shown the transparent clear cellular details in all the algal specimens were observed and photographed under 10x, 40x, resolution.

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