Comparative Histochemical Studies on Two Seaweeds Collected from Mandapam Coast in Tamil Nadu

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Abstract- Seaweeds are multicellular photosynthetic eukaryotic thallus. They have high concentration of essential vitamins, trace elements, proteins, lipids, polysaccharides, enzymes and minerals compared to terrestrial foodstuffs. This present study was focused on the occurrence and distribution of chemical constituents of the two seaweeds *Solieria robusta* (red algae) and *Lobophopra variegata* (brown algae) using histochemical analysis. It was used for evaluation of therapeutic efficiency of herbal drugs and where histologial and histochemical techniques are of great use. The histochemical tests were made on the fresh sections of the thallus treated with the chemical reagents to identify the presence and absence of metabolites like alkaloids, protein, starch, tannins and lipids. The stained sections were observed under microscope and photographed. All the reagents showed positive effects on both the seaweeds. In *S.robusta* (red algae) protein and starch were stained profusely in the epidermis and medullary parts. While alkaloids stained in epidermis and cortex region, tannins stained abundantly in the cortex part and lipids stained throughout the thallus like patches. In *L.variegata* (brown algae) all the reagents showed main presence mostly in the peripheral layer and cortex layer, while in the medulla it has meagerly present. Hence this study offer a base for both the seaweeds as herbal alternative for the synthesis of active compounds.

Keywords: *Solieria robusta*, *Lobophora variegata*, Histochemistry, Secondary Metabolites.

1. INTRODUCTION

Seaweeds are multicellular photosynthetic eukaryotic thallus without the roots, stems and leaves (Akrong *et al*.,2021).They have high concentration of essential vitamins, trace elements, proteins, lipids, polysaccharides, enzymes and minerals as compared to terrestrial foodstuffs. Seaweed are one of the important marine living resources and are excellent source of vitamins (A,B,B12,C,D&E), riboflavin, niacin, pantothenic acid and folic acid as well as minerals such as Ca, P, Na and K. Seaweed dietary fibres perform varied range of functions such as antioxidant, antimutagenic, anticoagulant, antitumor etc (Dargalkar *et al*., 2005).They are divided into three groups based on their pigments such as Chlorophyta, Rhodophyta and Phaeophyta. Indian coastal communities have therapeutically utilized marine plants since prehistoric times (Makkar & Chakraborty, 2017).For evaluation of therapeutic efficiency of herbal drugs certain special and routine histological and histochemical techniques are of great use in development and use the understanding mechanisms inherited in bioresources in response to various biotic and abiotic environmental factors and the potential and useful functions of these mechanisms. This gives more opportunities to find the solutions for the taxonomical and pharmacognostical problems. Over 2400 natural products have been isolated from seaweeds (mainly from the divisions Rhodophyta, Phaeophyta, and Chlorophyta), the majority of which come from subtropical and tropical populations (Munro *et al*., 1999; Faulkner *et al*., 2001).

The genus *Lobophora* includes 28 species and distributed in tropical and subtropical oceans worldwide (Camacho *et al*., 2019). *L.variegata* is common species of brown seaweed. *L.variegata* variety of bioactive compounds in accordance with their maturity. The amount of these biocompounds vary within species seasonally and annually. The general composition of *L.variegata* includes; phycobiliproteins, carotenoids, pigments, terpenes, polyphenols, phlorotannins, polysaccharides and minerals (Adaikkalam *et al*.,2020; Rodriguez *et al*., 2018). Among the marine macroalgae, the Phaeophyta members constitute a significant group of seaweeds having a wealthy source of potential drug like compounds.

Solieria, the type genus of the commercially important red algal family Solieriaceae (Gigartinales), contains seven or eight species and are found in tropical and subtropical regions throughout the world and are an extremely rich source of secondary metabolites with diverse structural features. They are shown to be composed predominantly of carrabiose 2, 4'-disulphate units (the repeating unit of l-carrageenan) and a significant proportion of 4', 6'-pyruvated carrabiose 2- sulphate units (Anthony chiovitti *et al*., 1999). In this study the secondary metabolites were localized by using histochemical analysis morphology and anatomy of the two seaweeds were investigated.

2. MATERIALS AND METHOD

The seaweeds *S.robusta* and *L.variegata* were collected from Rameshwaram coast, Tamil nadu. The collected samples were cleaned well with seawater to remove all the extraneous matter such as epiphytes and particles, pebbles and shells. Then the Fresh materials were taken free hand sections from the thallus. Histochemical tests were made on the fresh sections of the thallus followed by the methods described by Johansen (1940), Ruthman (1970), conn (1944) and (Almeida *et al.*, 2001).The stained sections were observed under the microscope, the localization of the components such as alkaloids, proteins, starch, tannins and lipids in the thallus were photographed.

3. RESULTS AND DISCUSSION

3.1 Morphological characters of the Seaweeds

The red algae (S.robusta) thallus was red to yellowish brown in colour, upright, fleshy, branched up to 20-25cm tall, branching trichotomous, irregularly branched at 1-4cm, umbellate, opposite and pinnate, numerous primary axes arising from the upper part of the thallus. Holdfast fibrous, branched, 1-2cm across, with several fronds.

The morphological characters of *L.variagata* shows thalli crect, dark brown (or) reddish brown, fan shaped thalli, margins undulated and slightly curved, lobes not present, complete, attenuated base, surface smooth, concentric rows of sporangia present, thalli feel hard to touch, leathery, lover portion dark coloured as compared to apper portion, dark coloured transverse & horizontal lines Present on the surface of the thalli (Fig: 1A&1B). Fig: 1A& 1B: Morphology of the seaweeds

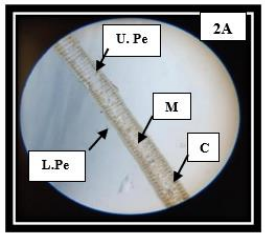
SOLIERIA ROBUSTA LOBOPHORA VARIEGATA

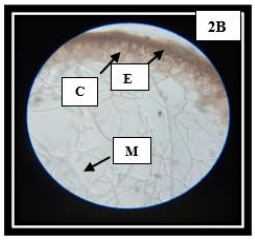
3.2. Anatomical Features

The red algae *S.robusta* transverse section shows a single layered epidermis, pseudoparenchymatous cortex 5-6 cells thick, inner cells (medulla) irregularly ovoid, oblong, rounded with numerous secondary pits, 30-80µm in diameter, connected with subcortical layer, loosely arranged.

The thallus of *L.variegata* consists of 8-9 layers, such as upper and lower peripheral layers, 2-3 layered Cortex on the both sides of medulla and Single layered medulla present at the Centre part. The peripheral cells are small, cubical, thin walled, dark brown with dense phaeoplats, the cortical cells are large, vertically elongated, thin walled arranged in irregular tires, the single layered medulla consists of large horizontally elongated, thick walled cells with dense phaeoplast, the cells are variable in size, some cells are broader than others (Fig; 2A&2B).

Fig: 2A&2B Anatomy of the seaweeds





T.S. of *L.variegata* T.S. of *S.robusta*

C-Cortex, E- Epidermis, M-Medulla, L.Pe –Lower Peripheral layer, U.Pe-Upper peripheral layer.

3.3. HISTOHEMICAL ANALYSIS

Histochemical ananlysis is a study that concerning the identification and distribution of the chemical constituents of tissues by means of stains, indicators and microscopy. The both red and brown seaweeds were tested for histochemical analysis for the deposition and distribution of major storage

compounds such as alkaloids, starch, tannins, protein and lipids and thus, showed the following results.

3.3.1. ALKALOIDS

Alkaloids are novel algal biochemically active compounds attracting significant drug research interest due to their complex and diverse structure and biological activity. Marine alkaloids are obtained from marine organisms, such as more algae and macroalgae (Saadaoui *et al*., 2020). Algal alkaloid extracts presented interesting bioactivities against a wide range of microorganisms (Alghazeer et al., 2013) in addition to demonstrating antioxidant (Rehman *et al*.,2017, Liu *et al*., 2020), anticancer (Gross *et al*.,2006), (Nijampatnam *et al*.,2015), anti- inflammatory (Saudagar *et al*., 2019), neuromodulatory and neurotransmitory functions (Guvn *et al*.,2010, Luqman *et al*., 2018).

The Alkaloids were determined by two methods namely; Mayer's method and Wagner's method. In Mayer's method (Fig: 3A&3B), the red algae *S.robusta* showed the presence of alkaloids in the epidermal layer only and in brown algae *L.variegata* it was stained in the upper and lower peripheral layer and slightly in the cortex part. In Wagner's method (Fig: 4A&4B), the alkaloids were stained in the epidermal layer and cortex region, and the patches present in the medullary part.

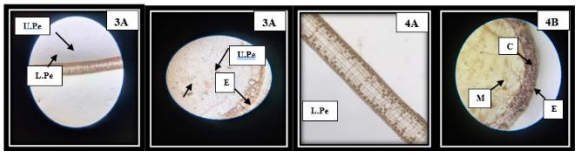


Fig: 3A, 3B, 4A&4B Histochemical staining with alkaloids in seaweeds

Fig: 3A. T.S of *L.variegata* stained with Alkaloid (Mayer's method), Fig: 3B.T.S of *S.robusta* stained with Alkaloid (Mayer's method), Fig; 4A. T.S of *L.variegata* stained with Alkaloid (), Fig: 4B. T.S of *S.robusta* stained with Alkaloid (Wagner's method).

3.3.2. STARCH

Starch is a major storage carbohydrate in various species of seaweeds (R.E.Cian *et al*., 2015). It is a completely biodegradable polysaccharide and one of the most abundant renewable resources. It has been considered as an excellent candidate to partially substitute synthetic polymer in packaging and other low-cost applications due to its abundance, biodegradability and low cost (Sangwan *et al*., 2014). In red algae the starch was stained profusely in the epidermis and medulla part, while in the brown algae it was stained in the upper and lower peripheral layer and cortex region (Fig:5A&5B).

Fig: 5A &5B Histochemicalstaining with Starch in seaweeds

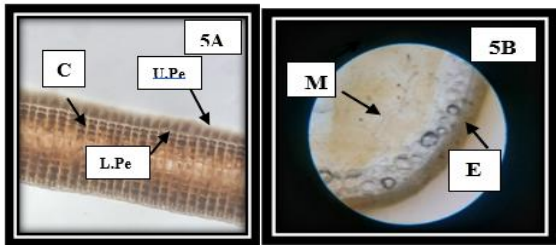


Fig: 5A. T.S of *L.variegata* stained with Starch
Fig: 5B. T.S of *S.robusta* stained with Starch

3.3.3. TANNINS

Tannins were used therapeutically as antiviral, antibacterial, antiulcer and antioxidant agents. Many tannin containing drugs are used in the treatment of piles, inflammation, burns and as astringent (Kolodziej *et al.*, 2005). In red algae showed the presence of tannins in the cortex part, while in brown algae showed the presence in upper and lower peripheral layer (John,2014) (Fig:6A&6B).

Fig: 6A &6B Histochemical staining with Tannin in seaweeds

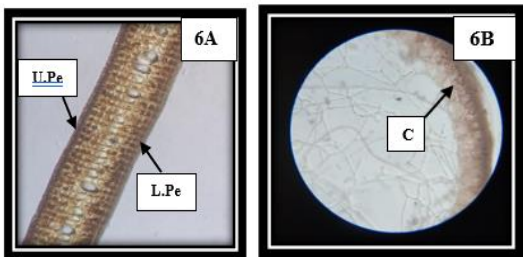


Fig: 6A. T.S of *L.variegata* stained with Tannin
Fig: 6B. T.S of *S.robusta* stained with Tannin

3.3.4. PROTEIN

Seaweeds are rich source of protein and can contain up to 47% on the dry weight basis (Maehre *et al*., 2015). The protein content of seaweeds differ according to species. Generally, the protein fraction of brown seaweed is low compared with that of the red seaweeds (Joel *et al*., 1999). The protein stained in the red algae profusely in epidermis and cortex region where as the brown algae stained in the upper and lower peripheral layer (Fig :7A&7B).

Fig: 7A &7B Histochemical staining with Protein in seaweeds

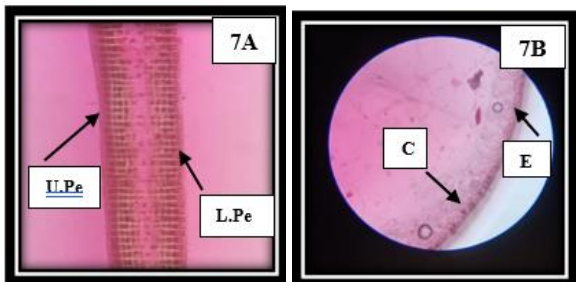


Fig: 7A. T.S of *L.variegata* stained with Protein
Fig: 7B. T.S of *S.robusta* stained with Protein.

3.3.5. LIPIDS

In recent years, lipid composition in marine algae has raised considerable interest due to their high content of PUFAs, specifically α-linolenic, octadecatetraenoic, arachidonic and eicosapentaenoic acids. This class of acids was considered as essential nutritional components in humans and animals. For example, they play an important role in the prevention of cardiovascular diseases, osteoarthritis, diabetes, and it possesses anti-microbial, antiviral, anti-inflammatory and anti-tumoral properties (Dawczynski *et al*., 2007) (Newton *et al*., 1996). In this studies of histochemical showed the presence of lipids in the red seaweed was seen in the medulla part and in the brown algae it was present in the upper and lower peripheral layer and megarly in the cortex part (Fig:8A&8B).

Fig: 8A &8B Histochemical staining with Lipids in seaweeds

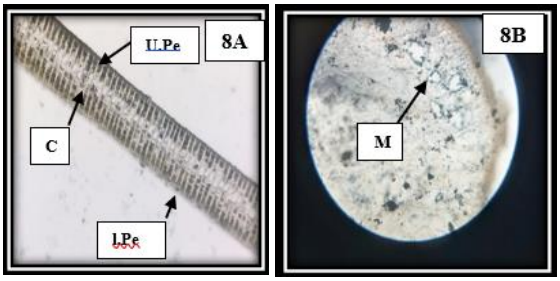


Fig: 8A. T.S of *L.variegata* stained with Lipids
Fig: 8B. T.S of *S.robusta* stained with Lipids.

CONCLUSION

The two seaweeds (*S.robusta* and *L.variegata*) from the mandapam coast of Rameshwaram Tamil nadu were used to see the morphology, anatomy and identify the major components in it such as alkaloids, starch, protein, tannins and lipids by using histochemical analysis. This study offer a base for both the seaweeds as herbal alternative for the synthesis of active compounds and considered as a potential source of various metabolites that could use in the food, pharmaceutical and cosmetic industries.

REFERENCE

1. Akrong, M.O., Anning, A.K., Addico, G.N.D., deGraft-Johnson, K.A.A., Adu-Gyamfi, A., Ale, M., Meyer, A.S. 2021.Spatio-temporal variations in seaweed diversity and abundance of selected coastal areas in Ghana Reg. Stud. Mar. Sci.
2. Dhargalkar, V.K and Neelam Pereira.2005. Seaweed: Promising plant of the Millennium. Science and Culture,Vol. 71, Nos.3-4: 60-66.
3. Chakraborty, K., Thilakan, B., Raola, V.K., and Joy, M. 2017. Antibacterial polyketides from Bacillus amyloliquefaciens associated with edible red seaweed *Laurenciae papillosa*, Food. Chem, 218 427-434.
4. Munro, M.H.G., Blunt, J.W., Dumdei, E.J., Hickford, S.J.H., Lill, R.E., Li, S.X., Battershill, C.N., Duckworth, A.R. 1999.The discovery and de velopment of marine compounds with pharmaceutical potential. J. Biotechnol, 70:15–25.
5. Faulkner, D.J., 2001. Marine natural products. Nat. Prod. Rep., 18: 1-49.
6. Camacho, O., Fernández-García, C., Vieira, C., Gurgel, C. F. D., Norris, J., Freshwater, D. W., and Fredericq, S. 2019. The systematics of Lobophora (Dictyotales, Phaeophyceae) in the western Atlantic and eastern Pacific oceans: eight new species. J. Phycol. 55: 611–24.
7. Adaikkalam, A., Sankaran, S., Rajan, S. S., and Subramanian, P.2020. Biological clock and carcinogenesis: Role of Lobophora variegata extract on liver cancer. Biological Rhythm Research, 51(1), 139-150.
8. Rodriguez, T. G., Juarez Postilla, C., Olivares Banvelos, T., and Zepeda, R. C. 2018. Anticancer activity of seaweeds. Drug Discovery Today, 23, 434-447.
9. Anthony Chiovitti., Anthony Bacic., Gerald T. Kraft., David, J., Craik and Ming-Long Liao. 1999. Pyruvated carrageenans from *Solieria robusta* and its adelphoparasite *Tikvahiella candida*. Hydrobiologia, 398/399: 401-409.
10. Johansen, D.A., 1940. Plant Micro technique. Tata Megrew hill Publ Co Ltd., New Delhi.

11. Ruthman, A.C., 1970. Method in Cell Research. Cornell Univ Press, Ithaca, New York.
12. Conn J., 1997. Biological Stains, 9th ed., Baltimore; Williams. Wilkins Co.
13. Almeida, M., Filipe, S., M. Humanes, M., Maia, M.F., Melo, R., Severino, N., J. da Silva, J.A.L., Fraústo da Silva, J.J.R., Wever, R. 2001. *Vanadium haloperoxidases* from brown algae of the Laminariaceae family.
14. Saadaoui, I., Rasheed, R., Abdulrahman, N., Bounnit, T., Cherif, M., Al Jabri, H., Mraiche, F.,2020.Algae-derived bioactive compounds with anti-lung cancer potential. Mar. Drugs 18, 197.
15. Alghazeer, R., Whida, F., Abduelrhman, E., Gammoudi, F., Azwai, S., 2013.Screening of antibacterial activity in marine green, red and brown macroalgae from the western coast of Libya. Nat. Sci. 5, 7–14.
16. Rehman, S., Khan, H., 2017. Advances in antioxidant potential of natural alkaloids. Curr. Bioact. Compd. 13, 101–108.
17. Liu, M., Xu, H., Lin, X., 2020.Pharmacological profile of bromophenols derived from marine organisms. Encycl. Mar. Biotechnol. 3, 1619–1639.
18. Gross, H., Goeger, D.E., Hills P., Mooberry, S.L., Ballantine, D.L., Murray, T.F., Valeriote, F.A., Gerwick, W.H., 2006. Lophocladines, bioactive alkaloids from the red alga *Lophocladia sp*. J. Nat. Prod. 69, 640–644.
19. Nijampatnam, B., Dutta, S., Velu, S.E., 2015. Recent developments in the isolation, synthesis, and bioactivities of bispyrroloquinone alkaloids of marine origin. Chin. J. Nat. Med. 13, 561.
20. Saudagar, R.B., Saokar, S., 2019. Anti-inflammatory natural compounds from herbal and marine origin. J. Drug Deliv. Ther. 9, 669–672.
21. Güven, K.C., Percot, A., Sezik, E., 2010. Alkaloids in marine algae. Mar. Drugs. 8, 269–284.
22. Luqman, A., Nega, M., Nguye, M.T., Ebner, P., Götz, F., 2018. SadA-expressing staphylococci in the human gut show increased cell adherence and internalization. Cell Rep. 22, 535–545.
23. Sangwan, P., Petinakis, E., & Dean K. 2014. Effects of Formulation, Structure, and Processing on Biodegradation of Starches. Starch Polymers, 357-378.
24. Kolodziej, H. and Kiderlen, A.F. 2005. Antileishmanial activity and immune modulatory effects of tannins and related compounds on Leishmania parasitised RAW 264.7 cells. Phytochemistry. 66(17):2056-2071.
25. John petter paul, J. 2014. Histochemistry and fluorescence analysis of *Turbinaria ornata* (Turner) J.AG.-An important brown seaweed (phaeophyceae). Indian J. Plant Sci. 2319-3824, vol.3(1).
26. Dawczynski, C., Schubert, R., Jahreis, G. 2007. Amino acids, fatty acids, and dietary fiber in edible seaweed products. Food Chem.103:891–899. doi: 10.1016/j.foodchem.2006.09.041.
27. Newton, I.S., 1996. Long chain fatty acids in health and nutrition. J. Food Lipids. 3:233–249. doi: 10.1111/j.1745-4522.1996.tb00071.