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MICROMORPHOLOGICAL, HISTOLOGICAL AND PHYTOCHEMICAL CHARACTERIZATION OF SEAGRASSES CYMODOCEA SERRULATA AND SYRINGODIUM ISOETIFOLIUM OF PALK BAY REGION, TAMILNADU

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Abstract: *Rapidly expanding scientific knowledge on seagrasses has led to a growing awareness that seagrasses area valuable coastal resource. The present study is aimed to validate the micro-morphometric parameters and phytochemical profiling of two sea grasses Cymodocea serrulata and Syringodium isoetifolium through histochemical localization and molecular analysis. The preliminary phytochemical studies of both the seagrasses explored variety of biologically active compounds such as flavonoids, terpenoids, alkaloids, phenols, tannins, glycosides and coumarin. The micro-morphometric parameters like fiber bundles, air lacunae, branching pattern and hairs in root have significant role in taxonomic identification and delimitation of Cymodocea & Syringodium from other sea grass genera of Palk Bay. The histochemical localization coincided with qualitative phytochemical analysis and also confirmed the sites of production or accumulation of secondary metabolites in the tissues. Antioxidant activity of 88.4 percent was observed in the leaf extract of C. serrulata with the IC_{50} value of 115.7 µg/ml and 86.3 percent in S. isoetifolium with IC_{50} value of 122.42 µg/ml. The present findings on the strong antioxidant properties of both the seagrasses significantly correlated with the total phenolic content. The FTIR analysis showed absorption peaks assigned to various functional groups related to flavonoids, phenols and coumaric acid. Further research studies are needed to characterize the phytochemicals of these seagrasses at molecular level using HPLC and GCMS technique and those results can be extrapolated to clinical studies.*

Key words: Seagrass, Anatomy, Histochemistry, FTIR, DPPH, Phytochemistry

Introduction

Seagrasses are marine angiosperms found in all coastal areas of the world except in Antarctic (Duarte and Gattuso, 2010). Seagrasses continue to decline at a global rate of approximately 7 percent (Waycott *et al*. 2009), and the losses may be more acute in the Southeast Asian region due to the pressure from increasing coastal populations and developments, and the lack of data on seagrass resources (Thangaradjou *et al*., 2008). Seagrass flora of India is represented by 6 genera and 14 species, Gulf of Mannar and Palk Bay harbour the maximum species followed by Andaman and Nicobar and Lakshadweep islands. Seagrasses provide a wide range of ecosystem services that directly or indirectly benefit human needs. But recent research has shown that there are still many gaps in our comprehension of seagrass ecosystem service provision (Nordlund *et al*., 2016). Despite being a key component of marine ecosystems, seagrass meadows are a prime example of a habitat that is largely understudied and under documented in the Southeast Asian region (Ooi *et al*., 2011). The previous works reflects only the conditions of seagrass meadows, seagrass phenology, morphology and their distribution but not the importance of seagrasses and their great nutritional value.

Seagrass of the Gulf of Mannar biosphere were found to be endowed with nutraceutical characteristics and proved to be a novel source of antioxidants and literature revealed lack of adequate scientific investigation on vitamins and minerals, amino acid and fatty acid profile of Indian seagrass (Jeevitha *et al*., 2013). Among the 72 species of seagrass present worldwide, only a few have been explored for their natural products and their determinant role (Subhashini *et al*., 2013). The acquisition and allocation patterns of biochemicals have been extensively studied in many terrestrial plants, but little has been done on the submerged aquatic vegetation such as sea grasses (Mbaga and Mvungi, 2019). Recent research increases the search on novel compounds from seagrass, since they have high contents of antioxidants like polyphenols, terpenoids, flavonoids, tannins, and saponins (Ansari and Ghanem, 2019). The compounds present in seagrasses are highly antioxidative in nature, and under stress conditions, they produce secondary metabolites as defense mechanism (Amudha *et al.*, 2017; Subhashini *et al.*, 2013). The search for medicinal plants with therapeutic utility is a very old practice, which currently plays an important role in modern medicine. Though, few studies have been conducted to ensure the quality of the raw material, the microscopic study is essential to the standardization of plants used as medicines (Moreira et al., 2014). According to Upton *et al*. (2011), the preliminary set of pharmacogenetic tools used for quality assessment of medicinally valuable plant parts is macro- and micro-anatomy. In view of bioprospecting coastal resources, the present study was aimed to study the anatomical, histological and phytochemical characteristics of two dominant sea grasses *Cymodocea serrulata* (R. Brown) Ascherson & *Syringodium isoetifolium* (Ascherson) Dandy from the Palk Bay coastal zone.

Materials and Methods

Monthly sea grass samplings were carried out from different hotspots along the Palk Bay *viz.,* Kattumavadi (10° 13‘ N; 79° 23‘ E), Kodiyakarai (10° 16‘ N; 79° 49‘ E), Manamelkudi (10°02‘ N; 79°15‘ E), Kottaipattinam (09° 59‘ N; 79°13‘ E), Mimisal (09° 54‘ N; 79° 08‘ E), S. P. Pattinam (09° 51‘ N; 79° 05‘ E), Karankadu (09° 64‘ N; 78° 96‘ E) and Thondi (Lat.09°44‘N; Long.79°01‘E) from April 2020 to March 2021. Seagrass biomass was assessed from the sites during low tides by the use of $1m^2$ quadrate and taxonomic identification was carried out with the help of seagrass manual (Kannan and Thangaradjou, 2006). Collected sea grasses were thoroughly washed, separated into root, rhizome, leaves and then dried under shade at 30 ± 2 ºC for about 10 days.

Anatomical Studies and Histological Localization

Free hand cross sections of root, stem and leaf were taken from the fresh material fixed in FAA 50 percent (Johansen, 1940), washed in distilled water and stained according to the technique described by Bukatsch (1972), with safranin. Similarly, the sections were also treated with respective reagents used in qualitative phytochemical analysis to localize specific components, viz alkaloids, flavonoids, phenol, lignin and tannin in the tissues. Presence of cutin was confirmed by the appearance of red colour when fresh sections were treated with saturated solution of Sudan IV prepared in 70 percent ethanol (Margolena, 1932). Presence of starch grains were detected by appearance of blue colour when treated with potassium iodide solution, which was made by dissolving 3 gm of iodine and 1.5 gm of potassium iodide in 100 ml of water. Lignin was tested by occurrence of red colour after treating lignified portion with phloroglucinol solution followed by 1-2 drops of 2.5 percent hydrochloric acid (Sass, 1951). To test the presence of tannin, fresh sections were placed in 10 percent aqueous ferric chloride solution with a pinch of sodium carbonate and blue colour of tannin substances was observed under the microscope. Dragondorff's reagent was used to visualize alkaloids and toluidine blue was used for localizing various polysaccharides (O'Brien *et al*., 1964). The sections were treated with ferric chloride and Sudan III to localize phenolic (Reeve, 1951) and lipophilic compounds (Foster, 1949). These reagents were chosen based on their specific application in histochemistry and chromatography techniques for the detection of the respective compounds. All sections were viewed under the light microscope (Zeiss Axioscope 2*plus* ® Germany) and photographed (ULTRA Scope image 9.0V).

Screening of Phytochemicals

The air-dried plant parts were pulverized to a coarse powder in a mechanical grinder, passed through a 40-mesh sieve and extracted in a soxhlet extractor with ethanol, water and ethyl acetate (Sunita *et al*., 2012). The extract was decanted, filtered with Whatman No. 1 filter paper and concentrated at reduced pressure below 40°C through rota-vapor to obtain dry extract then subjected to further analysis. The extracts were subjected to qualitative phytochemical analysis for the presence of tannins, flavonoids, alkaloids, terpenoids, coumarin, glycosides and phenols using standard procedures. The preparations of the reagents are given in Table 1. Chlorophyll content (mg g^{-1} fresh weight) was determined through organic solvent (80 percent acetone) extraction method as described by Arnon (1949). Total phenols were quantitatively estimated using the method of Lin and Tang (2007).

Anti-Oxidative Radical Scavenging Activity

The anti-oxidative activity was assessed on the basis of the radical-scavenging effect of the stable DPPH (*1-Diphenyl-2-Picryl Hydrazine*)free radical. The concentration of DPPH will be kept at 300 µM in MeOH. 1 mg of the extracts dissolved separately in 1 mL of MeOH, from which different concentrations (0-200 µg/mL) will be prepared. 10 µL of each extract solution will be allowed to react with 200 µL DPPH at 37°C for 30 min in a 96-well microliter plate. After incubation, decrease in absorption for each solution will be measured at 490nm using a microplate reader. Ascorbic acid will be used as reference (Mensor, 2001).

FTIR Analysis

Crude sample for Fourier Transform Infrared spectroscopy (FT-IR) analysis was prepared by KBr pellet technique. IR analysis of crude ethanolic extract of *C. serrulata* and *S. isoetifolium* was carried out in 400–4,000 cm−1 range and spectra were recorded.

RESULTS & DISCUSSION

Diversity, Growth and Biomass

In the present study, a preliminary survey was made to document the distribution of available sea grasses in the sampling sites over the seasons. Five seagrass species under 3 genera were recorded in the sampling sites and the major groups of seagrasses found in all the

sampling sites are *Cymodocea serrulata* and *Syringodium isoetifolium* (Gokulakrishnan and Ravikumar, 2016). *C. serrulata* was found to be dominant in Kodiyakarai whereas rarely *Halophila ovalis* was recorded. Seagrass biomass values varied highly with respect to the species in different seasons, whereas within the sites the values are insignificant. The biomass of *S. isoetifolium* varied between 106.2 and 441.7g FWT m^{-2} registering the minimum at Thondi and the maximum at Kodiyakarai. Similarly, the minimum biomass of 204.7g FWT m^{-2} of *C. serrulata* was recorded at Karankadu and the maximum of 744.3g FWT m^{-2} was recorded at Kodiyakarai during post monsoon (Jan – Feb. 2021). Generally, the below -ground biomass was higher than above–ground biomass, which might indicate the survival strategies of seagrasses to minimize anthropogenic pressure, exposure to desiccation at low tide and to increase stability when exposed to high tides. Similar to our results, Govindasamy *et al*. (2013) reported maximum biomass for *C. serrulata* (1429 g FWT m^{-2}) during post monsoon in the Palk Bay region. Earlier reports found the distribution of seagrasses up to 3-meter depth and the present study found the distribution even beyond that, extending up to 5-meter depths. Temporal variability in *C. serrulata* and *S. isoetifolium* abundance has been found to occur in response to either anthropogenic or natural disturbances (Green and Short 2003). Coles *et al*. (2011) reported that many environmental biophysical parameters, the availability of seeds and vegetative fragments, and anthropogenic inputs can determine the presence of seagrasses along coastal areas and regulate the growth and morphology of seagrasses.

Anatomical Characterization

Seagrasses are monocotyledonous angiosperms, generally have a similar external morphology of other flowering plants with a well-developed creeping rhizome. The findings of the present study was supported by another study, which showed that *Thalassia testudinum* had the highest number of leaves per shoot, high shoot density and the greatest biomass due to nutrient and sediment input from the nearby mangrove community (Arrelano-Mendez *et al*., 2011).The ability of seagrass to occupy an area is also inseparable from the rhizome ability to develop its root system on different types of substrates and indicates morphological plasticity (Balestri *et al*. 2015). The expansion of the rooting system and its penetration into the substrate are essential for the growth of new seagrasses to overcome physical disturbance and the low availability of nutrients.

In *C. serrulata*, at rhizome nodes, there is a short erect shoot bearing several foliage leaves with serrate margin, each with a sheath at the base. Each leaf consists of a basal leaf sheath and a distal leaf blade, these leaves usually are long linear and have a similar adaxial and abaxial surface in cross section whereas *S. isoetifolium* has terete leaves (Kuo *et al*., 2018). The leaf cuticle was very thin (0.1 – 0.2µm) in both the species but *C.serrulata* showed subcuticular cavities. A fiber bundle usually consists of several fiber cells, associated only with the hypodermis adjacent to the longitudinal vascular bundles are observed in *C. serrulata* and they are absent in *S. isoetifolium*. The fiber bundles and the thickened walls of the epidermal cells provide tensile strength but retain a high degree of flexibility that allows the strap-like leaf blades to withstand vigorous wave action (de los Santos *et al*., 2016). The strap-like leaves of *C. serrulata* have several parallel longitudinal vascular bundles, which are connected by smaller transverse bundles. The longitudinal vascular bundles in both species are separated by prominent regularly arranged air lacunae of varying size. The wall of bundle sheath cells is thin, lignified in *C. serrulata* and thick, suberized in *S. isoetifolium*. The number and size of xylem elements in the vascular bundles of seagrasses, asin other aquatic plants, are much reduced in comparison with those in vascular land plants (Kuo *et al*., 2018).

Table 01: Preparation of Reagents and Qualitative Tests for Phytochemicals

Compound	Reagents	Test	Appearance	References
Alkaloids	Mayer's reagent	1ml of the extract was measured into a watch glass and little amount of dilute HCl and reagents were added	Yellow colour	Sofowara, 1993
	Dragendroff's test		Orange colour	
	Wagner's test		Reddish brown	
Coumarin	Sodium Hydroxide	1.5 ml of the extract was mixed with few drops of alcoholic sodium hydroxide	Yellow colour	Yves-Alain *et al*., 2007
Flavonoids	Magnesium ribbon	1.3 ml of the extract was mixed with 0.5 g of magnesium turnings	Orange to red colour	Sofowara, 1993
Phenols	Ferric chloride (1 percent) in 50 percent aqueous methanol	A few drops of ferric chloride solution were added to 2ml of the extract	Bluish green colour	Cook and Samman, 1996
Tannins	Ferric chloride (0.1 percent)	To 2 ml of extract, 2-3 drops of 0.1 percent ferric chloride solution was added	Blue black colour	Chung *et al*., 1998
Terpenoids	Chloroform	5 ml of each extract mixed in 2 ml of Chloroform and 3 ml conc. H_2SO_4	A reddish brown colour at the interface	Aryakrishna *et al*., 2016
Anthraquinone	10 percent Ammonia solution	5 ml of chloroform to 0.5 g of extract in a clean dry test tube. This was shaken for 5 min and the extract thereafter filtered. The filtrate was then mixed with equal volume of 10 percent ammonia solution.	Formation of a rose-pink color in the ammoniacal layer (lower layer)	Sorescu *et al*., 2018
Cardiac glycoside	Glacial acetic acid	0.5 g of extracts dissolved in 2 ml of glacial acetic acid, 1 drop of ferric chloride, 2ml of conc. sulphuric acid	Brown ring was formed at interphase indicated the presence of cardiac glycoside.	Harborne, 1998
Sterols, Triterpenoids	Chloroform	The extract added with a few drops of chloroform and few drops of acetic anhydride and mixed well. 1 ml of con. H2SO4 was added from the sides of the test tube	Appearance of red ring at the junction of two layers- triterpenoids. Appearance of a brown ring at the junction of two layers - sterols.	Bruneton, 2009

The rhizomes of *C. serrulata* and *S. isoetifolium* are herbaceous, cylindrical with scars at irregular interval (Kendrick *et al*., 2012). The internodes of rhizomes and erect shoots appear similar in transverse section. The outermost layer is the epidermis covered by a distinct cuticle contained tannin. The bulk of rhizome and stem is cortical tissue, the structure of which showed great variations. In *S. isoetifolium* the cortex has large lacunae with parenchymatous septa. In both the species, the cortex has distinct thick-walled hypodermis, the outer cortical parenchyma cells with thick, lignified walls and the inner one having thin, non-lignified walls. The innermost layer of cortical cells is the endodermis that have thickened and lignified walls which surrounds the central stele. A central stele contains one or more larger central protoxylem elements surrounded by sieve tubes; the pericycle is not distinct.

The roots of both seagrasses are adventitious as in all monocotyledons, and arise from the ventral side of the rhizome nodes. The external morphology of roots often has characteristic features in different taxa, with adaptation to different substrates, though the internal anatomy is generally similar (Kiswara *et al*., 2009). *C*. *serrulata* and *S. isoetifolium* have at each rhizome node, one or more moderately branched **roots** with few root hairs. The branching pattern of root and the presence of root hairs have sometimes been used for the taxonomic delimitation of *Cymodocea* & *Syringodium* from other genera, i.e., *Amphibolis* (no root hairs) and *Thalassodendron* (Rarely branched) (Kuo *et al*., 2018). The mature root has an epidermis, overlaying cortical parenchymatous tissue that encloses air lacunae and a central stele. The epidermal cells usually have thin, undignified walls and below the epidermis there is a distinct exodermis one or more cells thick, each of which has thickened but undignified walls. The cortex of root showed an outer layer with larger cells, large air lacunae separated by radial partitions at the middle cortex and an inner cortex consisting of compact, small cells in a regular, concentric arrangement. As similar to monocotyledons, the stele is surrounded by a layer of small, compact endodermal cells with thin to moderately thick walls, non-lignified (de los Santos *et al*., 2016). Anatomical characterization is an inherent part of practically all pharmacopoeias and is one of the primary identification tests necessary for pharmacopoeia compliance. The individual structural elements are comparatively frequent within the same plant organs, but the ways in which the tissues, elements and cells are set with in a plant organ permits diagnosis to be performed and lend support to the quality assessment of herbal drugs (Upton *et al*., 2011).

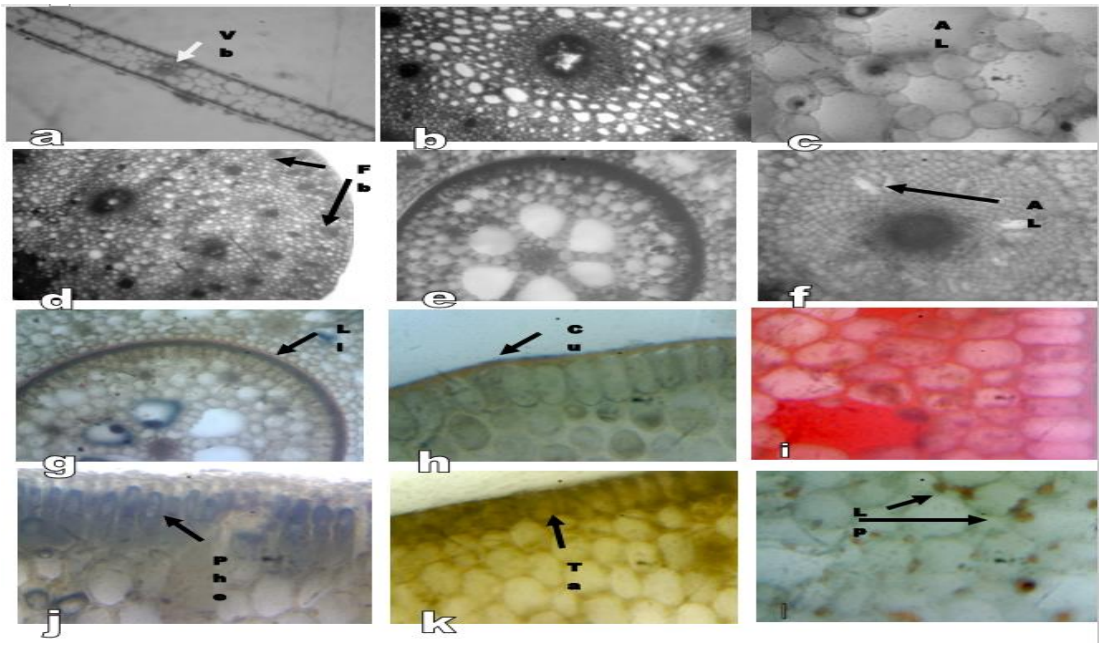


Fig 1. Anatomy and histochemical localization (a) *C. serrulata* leaf (b-c) *C. serrulata* stem showing central stele and air lacunae (d) *C. serrulata* rhizome showing hypodermal fiber bundles (e) *S. isoetifolium* leaf (f) *S. isoetifolium* rhizome central stele and air lacunae (g) Suberized endodermis of *S. isoetifolium* rhizome (h) Cutin layer of *S. isoetifolium* rhizome (i) lignified walls of fiber bundles of *C. serrulata* rhizome (i-k) Phenols & Tannin in blade epidermis and rhizome of *C. serrulata* (l) Lipid droplets in the cortex of *S. isoetifolium* leaf

Histochemical Localization

The thin-walled mesophyll parenchyma cells and the cortical parenchyma cells of rhizome of *C. serrulata* showed the presence of small starch grains (Kuo *et al*., 2018). Tannin was observed in the cuticle of rhizome and walls of blade epidermal cells (Fig. 1k). The Sudan III-stained endodermal cells at the stellar region and bundle sheath cells showed the presence of lignin in both the species. The cell walls of hypodermis and cortical parenchyma cells of the rhizome and stem stained with T blue showed purple colour which confirmed the presence of polysaccharides. Sudan III-stained lipids (Fig. 1l) which were observed as reddish granules in the upper mesophyll cells of leaf, in the outer cortical cells of rhizome of *C. serrulata* and very few in the inner cortical region of rhizome of *S. isoetifolium*. Alkaloids were noticed in the cross sections of rhizome of *C. serrulata*, but not in leaf and root. Thin layer of cutin was observed as red layer in the rhizome of *S. isoetifolium* (Fig. 1h). Cutin is the main component of the cuticle and isa lipophilic polymer that is deposited in and the top of the Outerwall epidermal cells (Upton *et al*., 2011). In the present study, phenolic compounds reacted positively with ferric chloride and found as brownish or bluish black in the hypodermal and cortical regions of the rhizome in both the species.

The histochemical test was possible to demonstrate the sites of production or accumulation of some metabolites of the plant. The phenolic compounds in epidermal cells may help the plant in different ways, as against pathogens attacks and even protection against ultraviolet radiation (Simões *et al*., 2010). Lipids and fatty acid composition of seagrass might be responsible for their thermal stability (Goncharova *et al*., 2000). Studies on histochemical localization of both the sea grasses indicated that tannins are copiously present in rhizome and leaf and were localized as dark brown contents in parenchyma cells bordering the vascular bundles. In the sub-hypodermal region of rhizome, flavonoids were located as a distinct yellow layer. Similar results on histochemical localization are also confirmed in *Excocaria agallocha* and in *Barleria lupulina* (Mandal and Mandal, 2014)

Phytochemical Screening

The study of the phytochemical properties of seagrasses is essential because they were reported to have significant nutraceutical values (Mani *et al.*, 2012). In the present study, the preliminary phytochemical tests were done in ethanol, ethyl acetate and water extracts of leaf and *C. serrulata*. Both ethanol and ethyl acetate extracts confirmed the presence of important active chemical constituents such as flavonoids, tannin, terpenoids, lignin and phenols in different parts of both the seagrass that reportedly have antibacterial (Ragupathi Raja Kannan *et al*., 2010), antialgal, antifungal, antiviral (Rowley *et al*., 2002), antiprotozoal (Orhan *et al*., 2006), anti-inflammatory (Hua *et al*., 2006), and antidiabetic (Gokce *et al*., 2008) activities. Tannins and phenols were dominantly recorded in both leaf and rhizome of *C*. *serrulata* whereas, alkaloids and polyols were traced in leaf only. The results of the phytochemical analysis are compiled in Table.1. The presence of phenolics and flavonoids in the leaf extracts of *C. serrulata* and *S. isoetifolium* extract could be attributed to the defense mechanism of the plant against the epiphytes (Pradheeba *et al*., 2011). Similarly, coumarin was detected in the ethanolic extracts of leaf and rhizome of both the sea grasses but anthraquinone was detected only in the leaf extract of *C. serrulata*. These results were supported by the earlier reports of Ergene *et al*.(2006) and Sangeetha & Asokan (2016) who revealed the presence of tannins, saponins, proteins, resins, reducing sugar, acidic compounds, alkaloids, cardiac glycosides and terpenoids in the phytochemical analysis of *C. rotundata*, *Cymodocea serrulata, Halophila ovalis and Halodule pinifolia.* The present results on the phytochemical screening were similar to that of Saranya *et al*. (2017) who reported that *Thalassia hemprichii* methanolic extract showed the presence of alkaloids, flavonoids, saponins, phenolics, tannins, steroids and triterpenoids.

Table 02: Phytochemical Localization of Secondary Metabolites in Leaf, Rhizome and Root of *C. serrulata* and *S. isoetifolium*

Phytochemicals	*C. serrulata*						*S. isoetifolium*					
	Leaf		Rhizome		Root		Leaf		Rhizome		Root	
	E	EA	E	EA	E	EA	E	EA	E	EA	E	EA
Starch	+	+	+	-	-	-	+	-	+	-	-	-
Lignin	+	+	-	-	+	-	+	-	+	-	-	-
Tannin	+	+	-	+	+	+	+	-	+	-	+	+
Alkaloids	+	-	+	-	-	-	-	-	-	-	-	-
Polysaccharides	+	+	+	+	+	+	+	+	+	+	-	-
Lipids	+	-	+	-	-	-	+	-	-	-	-	-
Cutin	+	-	+	-	+	-	+	-	+	-	-	-
Terpinoids	+	-	+	-	-	-	+	-	-	-	-	-
Phenolics	+	+	+	+	+	+	+	+	+	+	+	+
Coumarin	+	-	+	-	+	-	-	-	-	-	-	-
Steroids	-	-	+	-	+	-	+	-	-	-	-	-

E – Ethanol extract; **EA** – Ethyl acetate extract

The qualitative phytochemical screening of *C. serrulata* was in agreement with the works of Amudha *et al*. (2017). Rengasamy *et al.*, (2013) tested six seagrass species such as *Syringodium isoetifolium*, *Enhalus acoroides*, *Halodule pinifolia*, *Thalassia hemprichii*, *Cymodocea serrulata*, *Cymodocea roundata* for their antioxidant property and found high content of phenolic, tannin, and vitamin E content in *S. isoetifolium* and high flavonoid levels *in C. serrulata*. Subhashini *et al*. (2013) and Arsianti *et al*. (2017) reported that phenolic acids such as *p*-coumaric acid and gallic acid are predominantly found in *Halophila ovalis*, *Thalassia hemprichii*, *Halodule* spp., *Cymodocea* spp., *Enhalus acoroides*, *Syringodium isoetifolium* and other seagrass species. The array of phenolic acids produced by seagrasses is dependent upon the species, population, and tissue examined.

Photosynthetic Pigments

Table 2 indicated that *C*. *serrulata* growing in sandy shoreline of Kodiyakarai showed significantly higher chlorophyll contents as compared to other sampling sites; the chlorophyll a being significantly higher than chlorophyll b. The chlorophyll a content was found maximum (0.357 mg g^{-1}) in the leaves of *C. serrulata* and (0.313 mg g^{-1}) in the leaves of *S. osietifolium* during early summer (March) while it was lesser in pre-monsoon (August) (0.331 mg g^{-1}). Chlorophyll b was found less during all the seasons in the range of 0.218 to 0.292mg g^{-1}. The magnitude of chlorophyll a/b ratio was found higher during summer and it has very low carotenoid content (0.076 mg g^{-1}). Bharatharathna and Santhanam (2019) have recorded a very low content of chlorophyll a (6.78µg/g FWT) and chlorophyll b (5.13 µg/g FWT) in *S. isoetifolium* from Gulf of Mannar during wet season. The nutrient composition of sea water has an important influence on the production of photosynthetic pigments compounds by marine species. The present results are in agreement with earlier reports on other marine angiosperms (*Enhalus acoroides*, *Halophila ovalis*, *H. beccarii, Cymodocea*) which indicated that there is a clear seasonal variation in pigments concentration by registering the minimum value during the monsoon season when the water column was more turbid and the light availability to the seagrasses was limited causing light stress (Kolsi *et al*., 2017). In general, the pigments contents can be affected by several environmental factors largely influenced by the availability of the light and morphology of the seagrass leaves and the depth in which the plants are growing (Pradheepa *et al*., 2011).

Total Phenol Content

Phenolic compounds present high level in *S. isoetifolium* which has high antioxidant activity that scavenges the toxic free radicals and reactive oxygen species such as superoxide radical (O2), hydroxyl radical (OH), peroxide radical (ROO) and nitric oxide (NO) radicals (Girija *et al.*,

2013). Seasonal changes had significant effects on the concentrations of total phenol in *C. serrulata* than *S. isofolium*, being higher during the dry season than in wet season. Likewise, significant effect of geography (sampling sites) on the concentration of total phenol was evident where the highest concentration (4.36 mg g-1 dry wt.) was recorded at Kodiyakarai during summer. Even though, there was no significant effect in allocation of phenol between the leaf and rhizome, higher concentrations were frequently observed in leaves with mean values ranging from 2.96 to 4.36 mg/g-1 dwt in *C. serrulata*, and from 2.56 to 4.27 in *S. isoefolium*.

Table 03: Photosynthetic Pigment Contents and Total Phenol Content (TPC) of *C. serrulata* and *S. isoetifolium*

Sampling	C. serrulata				S. isoetifolium			
	Chlorophyll a (mg g^{-1})	Chlorophyll b (mg g^{-1})	Carotenoid (mg g^{-1})	TPC (mg g^{-1})	Chlorophyll a (mg g^{-1})	Chlorophyll b (mg g^{-1})	Carotenoid (mg g^{-1})	TPC (mg g^{-1})
SW Monsoon (June-Sep)	0.337 ± 0.04	0.218 ± 0.09	0.062 ± 0.12	4.17 ± 0.03	0.257 ± 0.04	0.208 ± 0.09	0.058 ± 0.12	4.03 ± 0.06
NE Monsoon (Oct-Dec)	0.326 ± 0.07	0.232 ± 0.11	0.058 ± 0.11	2.96 ± 0.11	0.286 ± 0.09	0.227 ± 0.12	0.052 ± 0.13	2.56 ± 0.13
Winter (Jan-Feb)	0.341 ± 0.06	0.257 ± 0.07	0.052 ± 0.09	3.11 ± 0.09	0.301 ± 0.05	0.277 ± 0.03	0.053 ± 0.11	2.91 ± 0.05
Summer (Mar-May)	0.357 ± 0.11	0.292 ± 0.13	0.076 ± 0.05	4.36 ± 0.07	0.317 ± 0.12	0.283 ± 0.13	0.066 ± 0.07	4.27 ± 0.11

Values are Mean ± SE

The presence of phytoconstituents such as phenols, flavonoids, tannin, coumarin in *C. serrulata* and *S. isoetifolium* indicated a possible role that its extracts have antioxidant activity. Similarly, some earlier reports reveal that seagrasses especially their polyphenols have the antioxidant activity (Gokce *et al*., 2008; Ragupathi Raja Kannan *et al*., 2010). Athiperumalsami *et al*. (2010) have reported highest antioxidant activity in the methanolic extract of *Halophila ovalis* than *H. pinifolia* tested by the NO scavenging method. Ragupathi Raja Kannan *et al*. (2010a) have reported antioxidant activity of 11.77 mg ascorbic acid equivalent/g of the leaf of *Enhalus acoroides* collected from Chinapallam, Gulf of Mannar, India.

Figure 02: Antioxidant activity of *C. serrulata* Leaf Extract

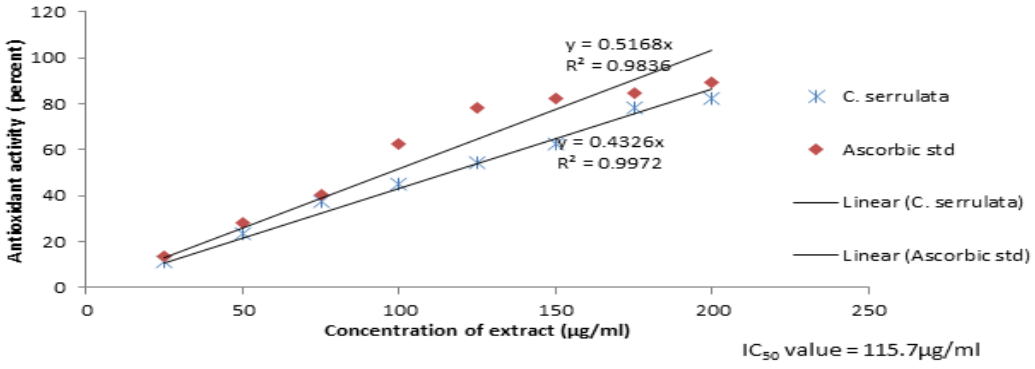
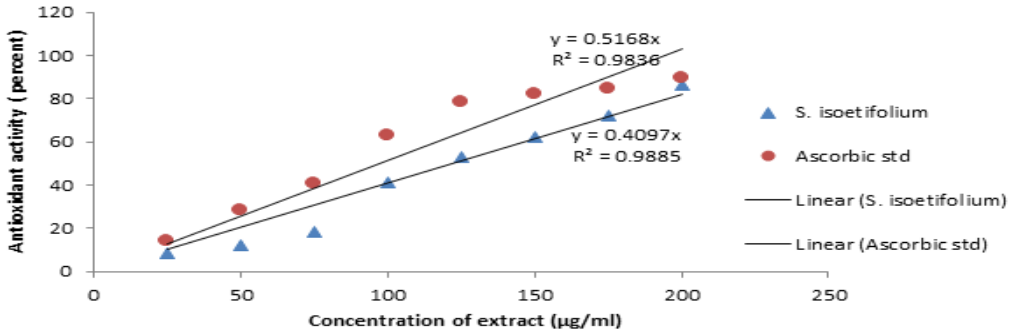


Figure 03: Antioxidant activity of *S. isoetifolium* leaf extract



Antioxidant Activity

More recently, reports have revealed that seagrasses are rich sources of antioxidant compounds (Gokce *et al*., 2008; Ragupathi Raja Kannan *et al*., 2010). The presence of phytoconstituents such as phenols, flavonoids and tannin in seaweeds and seagrass indicated a possible role that its extracts may have antioxidant activity. The results of the in vitro antioxidant activities of *C*. *serrulata* and *S. isofolium* are shown in Fig. 2 & 3. In the present study, the DPPH scavenging ability of ethanolic extract of both the seagrasses was concentration dependent and it was comparable to the ascorbic acid. DPPH radical was scavenged by antioxidant through donation of hydrogen to form a stable DPPH molecule. The concentration of antioxidant needed to decrease the initial DPPH concentration by 50 percent (IC_{50}) is a parameter widely used to measure antioxidant activity. As the IC_{50} value of the extract decreases, the free radical scavenging activity increases. In the present study, the investigated leaf extract of *C*. *serrulata* expressed the ability to scavenge the stable DPPH free radical reaching 50 percent of reduction with an IC_{50} value 115.7µg/ml conformed to vit. C.

Similarly, *S. isoetifolium* showed a maximum antioxidant activity of 86.4 percent with IC_{50} value of 122.2 µg/ml. According to Bharathi *et al*. (2016) *Syringodium isoetifolium* shows a wide range of pharmacological activities such as antibiotic, antihemolytic, cytotoxicity, antibacterial, and antifungal activity. The present findings are in agreement with those described for other extracts from five species of seagrasses and six species of seaweeds which were collected from the Gulf of Mannar, on the southeastern coast of India (Athiperumalsami *et al*., 2010). In contrast to our findings, Jayaprakash *et al*. (2016) reported a high radical scavenging activity of ethyl acetate extract in *S. isoetifolium*, *C. rotundata* and *H. uninervis*

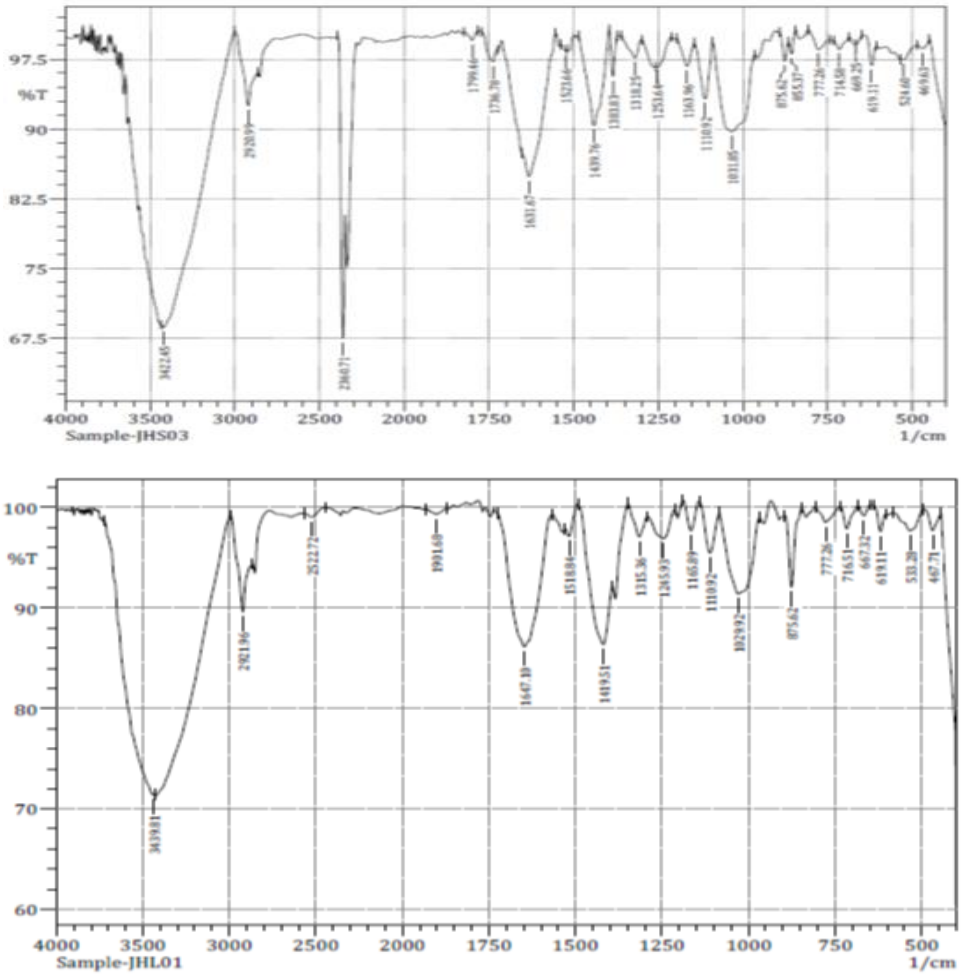
Molecular Characterization of Phytochemicals

The chemical nature and functional groups present in the dried leaf powder of *C. serrulata* and *S. isoetifolium* was predicted using FTIR. The bonds were determined by interpreting the infrared absorption spectra (Fig 4). Strong bonds were found at 3432 cm−1, 1631.92 cm−1 and 1419 cm−1, while the others varied from weak to medium. These results demonstrated the presence of hydroxyl groups, lipids, alkanes, amino acids and phosphorus compounds. The sharp absorption peak at 1631 – 1736 cm-1 are assigned to C=O stretching vibration in carbonyl compounds which may be characterized by the presence of high content of flavanoids in the leaf of *C*. *serrulata*. The FTIR spectral analysis confirmed the presence of phenolic compounds in the aqueous methanolic extracts of both the seagrasses investigated in the present study. The FTIR identified that the leaf extract contained strong C-H out-of-plane bending (oop bend) vibration for substituted benzene ring, indicating the presence of phenols and flavonoids in the crude *S. isoetifolium* extract also. The wavelength numbers of FTIR spectra for tannic acid at 669, 855, 1163, 1518 were observed in both the seagrasses.

The peak around 1734–1745 cm−1 was assigned as C=O ester, which may be related to chlorophyll (Li *et al*., 2018). The FTIR spectrum at 1654 cm−1 was assigned as the C=O conjugate. The conjugated double bond in carotenoids has been reported as the structure responsible for light absorption (Mezzomo and Ferreira, 2016). A previous study stated that a peak at 1654 cm−1 is due to chlorophyll and protein content (Kushwaha *et al*., 2014). Based on the phytochemical compounds discovered in the FTIR analysis, *C. serrulata* has the potential to be used for biomedicine applications, as phenolic compounds, tannins, flavonoids, chlorophyll and carotenoids are known to have antioxidant activities (Lachowicz *et al*., 2018). A strong broad absorption band around 3422 cm-1 was found in *C. serrulata* and 3439 cm-1 in *S. isoetifolium* may be due to the presence of hydrogen bond N-H stretching, characteristic of amino acids. The absorption band at 2921 cm-1 and 2920 cm-1 are corresponding to C-H stretching of the CH_2 groups respectively, indicates the presence of various amino acids, these

bands may also be characteristic for the presence of aliphatic CH groups in these compounds. Bands at 1518 cm-1 and 1523 cm-1 indicates the presence of benzene ring in aromatic compounds. The absorption band at 1031 cm-1 and 619 cm-1 respectively are due to the stretching vibration of C-O group of esters and phenols (Valchos *et al*., 2006).

Figure 04: FTIR Spectral Analysis of Methanol Leaf extract of (a) *C. serrulata* (b) *S. isoetifolium*



The hydroxyl groups both alcohol and carboxylic acids are observed at the 3392 cm-1 stretching band. Aromatic C-H stretching is identified by the following stretching bands 2921, 1031, and 1110. The vinyl carbons are identified by the stretching band around 1631 cm-1. The aromatic C=C are assigned by the band around 1439, 1518, 1523 cm-1. The C-O stretching or C-OH are assigned by the band at 1245 cm-1 was observed in *C. serrulata* and 1253 cm-1 in *S. isoetifolium*. The methanolic extracts showed all the above bands which are evidence for the presence of p-coumaric acid in the seagrasses (Pharmawati and Wrasiati, 2020). The identification of compounds *via* FTIR spectrophotometry supported the findings from the phytochemical screening, which detected the presence of phenols and flavonoids. The amines, imines, alkanes and phenols present were considered the major functional groups of bioactive compounds (Pradeepa *et al*., 2015).

Conclusion

Seagrasses can be assertively recognized as one of the important coastal ecosystems and it requires greater attention for its conservation, monitoring and management. The results of the present study may increase the search on novel compounds from seagrass, and they have high contents of antioxidants like polyphenols, terpenoids, flavonoid, tannins, and coumarin. Generally, the trend observed in this study indicated that accumulation of phytochemicals would differ depending on the plant organ, season, and the conditions of the environment where they grow. Further work on the purification of individual groups of bioactive components

may reveal the exact potential of the seagrass to develop a novel broad spectrum herbal antimicrobial formulation in the future. Further purification of active compounds and structural elucidation can be used for drug discovery.

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