



ANALYSES OF PHYTOCHEMICAL, BIOCHEMICAL, PIGMENTS AND ANTIOXIDANT ACTIVITY OF SEAGRASS *SYRINGODIUM ISOETIFOLIUM*

P. Bharatharathna and P. Santhanam*

Marine Planktonology & Aquaculture Lab., Department of Marine Science, School of Marine Sciences, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India

*Corresponding author: santhanamcopepod@gmail.com

ABSTRACT

Seagrasses are valuable food source and contains significant quantities of proteins, lipids, vitamins and minerals etc. In the present study, seagrass *Syringodium isoetifolium* was subjected to biochemical, phytochemical and pigments analysis. The study indicated that the *S. isoetifolium* showing high photosynthetic pigments and carbohydrate. Likewise acetone and methanol extracts of *S. isoetifolium* showed positive activity with phytoconstituents such as steroids, protein, glycosides, alkaloids and phenolic compounds but tannin and terpenoids showed negative activity. The study concluded that the *S. isoetifolium* could be a good candidate in food, feed and biomedical field as it contains rich biochemical profile.

Keywords: Seagrass, *Syringodium isoetifolium*, Pigments, Phytochemicals, Biochemical

1. INTRODUCTION

Seagrasses have been identified as an important habitat linked to the productivity of our abundant fisheries [1]. Approximately 40 percent of all primary energy production, or photosynthesis, occurs in the seas. In process, oceanic plants (phytoplankton, seaweeds, and seagrasses) take up carbon dioxide and convert it into organic carbon (primarily sugars) and oxygen using light from the sun as an energy source. Though not true grass-like plants they are termed “seagrasses” because they grow in highly variable saline environments. Seagrasses can influence the nature and depth of their own sediment bed by trapping and binding sediment particulars associated with damping wave and tidal energy [2]. They are a highly productive, faunally rich, and ecologically important habitat. Their physical structure stabilizes sediments and prevents the resuspension of particulate matter, thus helping to maintain water transparency or clarity [3].

Each seagrass species can occur as a monotypic seagrass bed or can be found intermixed with the other species. In India, 14 species of seagrasses have been recorded so far along the east and west coasts. The Gulf of Mannar, Palk Bay, Andaman and Nicobar Islands and Lakshadweep Islands are known for their seagrass resources. Seagrasses take up nutrients from the sediments, transporting them through the plant and releasing them into the water column through the leaves.

The surface of seagrass leaves provide the substratum for attachments by a myriad of small algae and animals (*e.g.*, crustaceans, worms, sponges, bryozoans), which provide the basis for the food variety of larger seagrass-associated animals [4]. The structure of seagrass beds provides living space for a diverse assemblage of mobile and sessile organisms [5, 6]. In general, seagrasses acquire most of their required inorganic carbon from free CO₂ and assimilate nitrogen and phosphorus from the sediments via their roots and rhizomes and from the water column via their leaves. Seagrasses are direct source of food for sea turtles, geese, dugongs, and manatees. Hence researchers are focused to replace the fishmeal by seagrass meal due to presence of good source of proteins, essential amino acids, lipid, HUFA's, carbohydrate, vitamins, minerals, pigments, carotenoids, antioxidants, and antimicrobial properties. So the present study was conducted on the analysis of pigments, biochemical, phytochemical and antioxidant activity of seagrasses *Syringodium isoetifolium*. Although seagrasses contains several advantages, their use in aquafeed formulation is not much studied.

2. MATERIALS AND METHODS

2.1. Analyses of water quality parameters

Water samples were collected from study area where seagrass were collected. Atmospheric and surface water temperatures were measured using standard mercury

filled centigrade thermometer. Salinity was measured with the help of hand refractometer (ERMA, Japan) and pH measured using an ELICO grip pH meter. Dissolved oxygen was estimated by the modified Winkler's method [7]. For the analysis of nutrients, the surface water samples were collected in clean polyethylene bottles and kept in an ice box and transported immediately to the laboratory and analyzed for dissolved inorganic nutrients such as phosphate, nitrate, nitrite, ammonia and reactive silicate according to Strickland and Parsons [8]. All nutrients concentrations were expressed in $\mu\text{M/L}$. Chlorophyll 'a' and 'b' were estimated as followed by acetone method [8].

2.2. Determination of pigments in seagrasses

A known quantity of seagrass *S. isoetifolium* was homogenized with a mortar and pestle in a dark room and extracted repeatedly with acetone. Chlorophyll and total carotenoids contents were analyzed as per the standard procedure [9, 10] by measuring the absorbance at 470 nm for carotenoids, and 646 and 663 nm for chlorophyll using UV-Vis Spectrophotometer (Shimadzu UV-160-A). The total carotenoid content was expressed in terms of percent dry weight. The astaxanthin content was determined at 480 nm by using an extinction coefficient of 2,500 at the 1% level by the method [11].

2.3. Biochemical analysis

The biochemical constituents such as total protein, total carbohydrate, total lipid, ash and moisture were estimated in *S. isoetifolium* using the standard methods [12-16].

2.4. Phytochemical screening

Acetone, methanol, ethanol, chloroform, ethyl acetate, petroleum ether and DMSO extracts were prepared using the seagrass. Various phytochemicals such as tannins, flavonoids, terpenoids, steroids, protein, glycosides, alkaloids and phenolic compounds were estimated as described [17].

2.5. Preparation of extract

The dried seagrass *S. isoetifolium* powdered samples (5 g) were extracted for 24 h in 100 ml of ethanol, methanol, chloroform, acetone, ethyl acetate, DMSO, petroleum ether and aqua's water at room temperature under dark condition. The extraction was twice repeated and filtered using Whatmann No. 1 filter paper. The solvent from the extracts were evaporated to dryness and stored in a refrigerator at 4 °C for further use.

3. RESULTS AND DISCUSSION

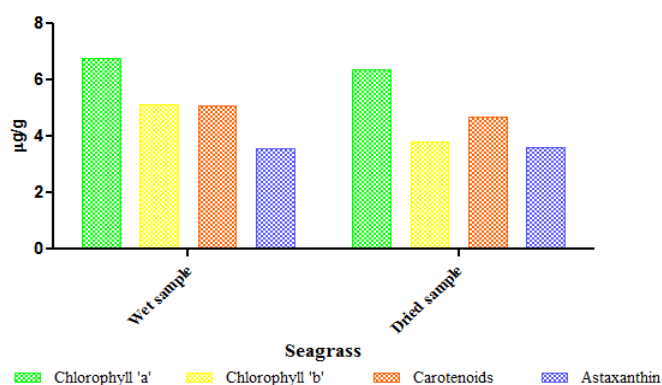
The entire life on the earth depends on water and therefore the hydrological study is highly essential to comprehend the relationship among its diverse trophic levels and food webs [18]. There are various sources which are responsible to change the biodiversity of a particular area. Temperature seemed to be high in the surface water due to seasonal fluctuation in the weather condition. Generally, the surface water temperature is influenced by the intensity of solar radiation, evaporation, freshwater influx and cooling and mix up with recede and flow from adjoining neritic waters [19]. The recorded high pH value might be due to the influence of seawater penetration and high biological activity [20]. The recorded higher salinity value could be attributed to the low amount of rainfall, higher rate of evaporation and also due to neritic water dominance [20, 21]. It is well known that the temperature and salinity affect the dissolution of oxygen [22], which could be seen with the water quality parameters analyzed. The recorded nitrate value could be mainly due to the organic materials received from the catchment area. Nitrite values could be due to variation in phytoplankton excretion, oxidation of ammonia and reduction of nitrate and by recycling of nitrogen and bacterial decomposition of planktonic detritus [23]. One of the major dissolved nutrients are nitrogen and prosperous to play in the stimulating the primary production of phytoplankton and plant growth in the ocean. Silicate could be due to bottom sediments exchanging with overlying water. Hence the study on the physico-chemical characteristics to assess the present environmental condition could be used to analyze the environmental status of the particular region from where seagrass were collected for the present nutrition study.

The recorded level of hydrobiological parameters (Table 1) such as water temperature, pH, dissolved oxygen, nitrate, nitrite, phosphate, ammonia, silicate, chlorophyll 'a', and chlorophyll 'b' in seawater were 27 °C, 8, 30 (ppt), 5.01 mg/l, 1.5 $\mu\text{M/l}$, 0.17 $\mu\text{M/l}$, 1.4 $\mu\text{M/l}$, 0.11 $\mu\text{M/l}$, 1.5 $\mu\text{M/l}$, 2.0 mg/ml and 2.26 mg/ml respectively.

Present study was carried out on the biochemical, pigments, phytochemicals and antioxidant activities of the seagrass *Syringodium isoetifolium*. Seagrasses are the only flowering plants living under water in the photic zone of coastal areas.

Table 1: Physico-chemical parameters in sea water samples

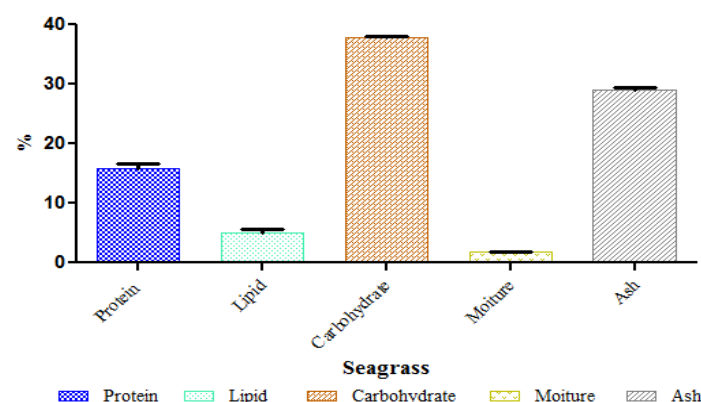
Parameters	Values
Surface water temperature (°C)	27
pH	8
Salinity (‰)	30
Dissolved oxygen (mg/l)	5.01
Nitrate (μM/l)	1.5
Nitrite (μM/l)	0.17
Phosphate (μM/l)	1.4
Ammonia (μM/l)	0.11
Silicate (μM/l)	1.5
Chlorophyll 'a' (mg/ml)	2.0
Chlorophyll 'b' (mg/ml)	2.26

**Fig. 1: Pigments in wet and dry *S. isoetifolium***

Seagrasses take up nutrients from the sediments, transporting them through the plant and releasing them into the water column through the leaves. So the photosynthetic pigments are highly present in the seagrasses. This shows that the study area has a very low amount of nutrient level in the water surface which is due to the absorption of nutrients by the seagrass present in the area. The pigments such as chlorophyll 'a' and 'b', carotenoids and astaxanthin were analyzed in the wet and dried sample of seagrass *S. isoetifolium* (Fig 1). The chlorophyll 'a' ($6.783 \pm 0.263 \mu\text{g/g}$), chlorophyll 'b' ($5.13 \pm 0.12 \mu\text{g/g}$), carotenoids ($5.077 \pm 0.027 \mu\text{g/g}$) were found highly in wet sample when compare to the dry sample of seagrass *S. isoetifolium*. The astaxanthin was noticed in eligible amount ($3.55 \pm 0.14 \mu\text{g/g}$).

Nutrients essential to fish are the same as those required by other animals. These include water, protein, lipids, carbohydrates, vitamins and minerals [24, 25]. Seagrass has been found to contain high amount of carbohydrates

hence use of carbohydrate rich plant source would be a viable option to replace protein sparing effect in the diets. Normally protein content of seagrass is lower (12-19%) than that of the animal (23%). The high level of carbohydrate was present in seagrass in the soluble form of glucose and another soluble carbohydrate is sucrose and fructose. Seagrass shows low level of lipids. The proximate constituents such as total protein, carbohydrate, lipid, moisture and ash were analyzed in dry powder of different seagrasses. In the present study, the carbohydrate (37.77 ± 0.27) and ash (29.005 ± 0.455) content were noticed higher in seagrass *Syringodium isoetifolium* when compare to the protein (15.92 ± 0.81), lipid (5.1 ± 0.6) and moisture (1.75 ± 0.15) content (Fig. 2).

**Fig. 2: Biochemical compositions of seagrass of *S. isoetifolium***

The qualitative phytochemical analysis showed that most of the phytoconstituents were obtained with methanol extraction compared to other solvents extracts. Several chemical compounds including alkaloids, flavanoides, phenols, tannins, saponins, tannins, sterols, proteins, monosaccharide's, polysaccharides and resins have been detected in the seagrass *Syringodium isoetifolium* and coincides with the results [26]. Some of these have also been detected in similar seagrasses or other plants such as *Piper umbellatum* and *Piper pellucid* [27], *Syringodium isoetifolium* [28], *Raphanus sativus* [29], *Cymodocea serrulata* [30], *Halodule pinifolia* and *Halophila ovalis* [31]. The present study qualitative test of phytochemical screening for eight different chemical compounds were tested in seven different extracts viz., acetone, chloroform, DMSO, ethanol, ethyl acetate, methanol, petroleum ether and water. Among the eight solvent the methanol extract of *Syringodium isoetifolium* revealed the maximum phytochemical constituents when compare to the other

extracts. The methanol and acetone extracts of *Syringodium isoetifolium* followed results the presence of flavonoids, steroids, protein, glycosides, alkaloids and

phenolic compounds. Tannin and terpinoids were absent (Table.2).

Table 2: Phytochemical constituents of *S isoetifolium*

Sample	Solvent Extract	Tan	Glyc	Flav	Phen	Ter	Steroi	Alka	Prote
<i>Syringodium isoetifolium</i>	H ₂ O	-	+	-	+	+	+	-	-
	Acetone	-	++	++	++	-	+++	+	+
	Chloroform	+	-	-	+	-	-	-	-
	DMSO	+	+	-	+	-	++	+	++
	Ethanol	+	-	+	+	-	+	+	+
	Ethyl acetate	-	++	++	+	-	++	-	++
	Methanol	-	++	++	+++	-	++	+	++
	Petroleum ether	-	+	+	++	-	+	+	+

*Tan – Tannin, Glyc – Glycosides, Flav – Flavonoids, Phen – Phenolic compounds, Ter – Terpinoids, Steroi – Steroids, Alka – Alkaloids, Prote – Protein

4. CONCLUSION

The proximate composition of the seagrass *S. isoetifolium* has revealed its potential with the availability of protein, carbohydrate, lipid, moisture and ash contents. Among these carbohydrates were found to be higher than other compounds as it indicate *S. isoetifolium* is a potential source of carbohydrate followed by protein. The methanol extract of *S. isoetifolium* shows the potential phytochemicals such as flavonoids, steroids, glycosides, alkaloids as more significantly. As the pigment carotenoids present in the seagrass it could be an effective source of antioxidants.

5. ACKNOWLEDGEMENTS

Authors thank the Head, Department of Marine Science and authorities of Bharathidasan University, Tiruchirappalli-24 for the facilities provided. One of the author (PB) thank UGC, Govt. of India, - for providing Rajiv Gandhi National Fellowship (2016-17 F1-17.1/2016-17/RGNF-2015-17-SC-TAM-6374).

6. REFERENCES

- Ogden J C. *3rd Int Coral Reef Symp*, 1977; **1**: 377-382.
- Burrell D C and Schubel J R. *A Scientific Perspective. Marcel Dekker, Inc., New York*, 1977; 196-232.
- Kenworthy W J and Haunert D E. *proceedings of a workshop*, 1991.
- Virnstein RW and Curran M C. *Mar. Ecol., Prog. Ser.*, 1986; **29**: 279-288.
- Harlin MM. *New York, NY*, 1980; 117-152.
- Stoner AW. *Mar. Sci.*, 1980; **30**: 537-551.
- Winkler LW. *Chem. Ber.* 1888; **27**:2843-2855.
- Strickland JDH and Parsons TR. *Fish. Res. Bored Can. Bull.* 1972; **167**:310
- Lichtenthaler, Hartmut K and Alan Wellburn R. 1983; 591-592.
- Lichtenthaler HK. *Methods Enzymol.* 1987; **148**:350-382.
- Davies A J, Khare A, Mallams A K, Massy-Westropp R A, Moss G P and Weedon BC. *J. Chem. Society, Perkin Transactions 1*, 1984; 2147-2157.
- Lowry OH, Rosebrough NJ, Farr AL and Randall RJ. *J. Biol. Chem.*, 1951; **193**:265-275
- DuBois M, Gilles KA, Hamilton JK, Rebers PA and Smith F. *Anal. Biochem.*, 1956; **28**(3): 350-356.
- Folch J, Lees M and Sloane-Stanley GH. *J. Biol. Chem.*, 1957; **97**:383-394.
- Marsham S, Scott G W and Tobin M L. *Food chemistry*, 2007; **100**(4):1331-1336.
- AOAC (*Association of Official Analytical Chemicals*). 1995; 832.
- Sanjeet K, Kabi M and Kumari M. *Emerging Science*. 2010; **2**:5.
- Soundarapandian P, Premkumar T and Dinakaran GK. *Res. J. Biol. Sci.*, 2009; **1**(3):102-105.
- Govindasamy C, Kannan L and Jayapaul A. *J. Environ. Biol.*, 2000; **21**:1-7.
- Balasubramanian R and Kannan L. *Int. J. Ecol. Environ. Sci.*, 2005; **31**:265-271
- Sridhar R, Thangaradjou T, Senthil Kumar S and Kannan L. *J. Environ. Biol.*, 2006; **27**:561-566.

22. Saravanakumar A, Rajkumar M, Sesh Serebiah J, and Thivakaran GA. *J. Environ. Biol.*, 2008; **29**:725-732
23. Asha P S and Diwakar K. *J. Mar. Biol. Ass. India*, 2007; **49**:7-11.
24. Abovei JFN, Ekurbo, AT. *Br. J. Pharmacol toxicol.*, 2011; **2**:179-191
25. Royes JAB and Chaman, FM. *Cir*; 2009; 97.
26. Mani, Aswathi Elizabeth V, Bharathi and Jamila Patterson. *Inte. J. Micro Rese.*, 2012; 99-103.
27. Mensah J K, Ihenyen J O and Okhiure M O. *J. Nat. Prod. Plant Resour*, 2013; **3(1)**:8-14.
28. Mani A E, Bharathi V and Patterson J. *Inter. Jour MicroRese.*, 2012; **3(2)**:99-103.
29. Janjua S, and Shahid M. *Advancement in Medicinal Plant Research*, 2013; **1(1)**:1-7.
30. Ravikumar S, Ali MS, Ajmalkhan PAM and Dhinakaraj *In Jour Sci and Tech*, 2011; **4(2)**:98-100.
31. Qi SH, Zhang S, Qian PY and Wang BG. *Botanica Marina*, 2008; **51(5)**:444-447.