Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(3): 742-747
Received: 23-03-2017
Accepted: 24-04-2017

Libin Baby
School of Marine Sciences, Cochin University of Science and Technology, Foreshore Road, Cochin, Kerala, India

Sankar TV
Kerala University of Fisheries & Ocean Studies, Panangad, Cochin, Kerala, India

Chandramohanakumar N
School of Marine Sciences, Cochin University of Science and Technology, Foreshore Road, Cochin, Kerala, India

Correspondence
Sankar TV
Kerala University of Fisheries & Ocean Studies, Panangad, Cochin, Kerala, India

Changes in phenolic compounds in seagrasses against changes in the ecosystem

Libin Baby, Sankar TV and Chandramohanakumar N

Abstract
Phenolic compounds in plants plays important role in pigmentation, growth, reproduction, resistance against pathogens, defense mechanism as well as protecting plants from deleterious effects of ultraviolet radiation and oxidants. Comparatively higher contents of phenolics and flavonoids were noticed in seagrasses from Palk Bay than Gulf of Mannar irrespective body parts. The leaves of *C. serrulata* contained phenolics ranging from 41 to 298 mg/100g gallic acid equivalents and flavonoids 13 to 146 mg/100g quercetin equivalents where as in the roots and rhizomes phenolics varied from 29 to 292 mg/100g and flavonoids 9.1 to 75 mg/100g. The tannins varied from 0.20 to 5.02 mg/100g.Chlorophyl a, in seagrasses ranged from 115 to 850 µg/g, Chlorophyl b from 11 to 1029 µg/g and pheophytin from 41 to 204 µg/g. These results indicated variations in the phenolic compounds with reference to the ecosystem and the presence of other plants in the meadows.

Keywords: Seagrasses, phenolics, flavonoids, chlorophylls, variation in ecosystem

1. Introduction

Seagrasses, the submerged marine angiosperms that grow abundantly in the tidal and sub tidal coastal ecosystem [1], are known as a source of secondary metabolites which plays an important role in the marine ecosystem [2-3]. Phenolic compounds in plants contribute to pigmentation, growth, reproduction, resistance against pathogens. Further they also act as defensive mechanisms against other aquatic lives as well as protecting the plant from ultraviolet radiation and oxidants [4-5] besides contributing to growth and development related to biotic stress [6-7].

Phenolic compounds usually exhibit antioxidant, antimutagenic, antiviral, antibacterial algicidal, antifungal, insecticidal, estrogenic and keratolytic activities irrespective of their origins. Hydroxyl groups present in phenolics act as hydrogen donors and they scavenge reactive oxygen species and inhibit generation of new radicals [8]. Phenolics of seagrasses are resistant towards wasting disease, a decrease leads to rapid population declines and its resistant levels are related to level of phenolics presented in leaves as well as shoots [9]. Flavonoids present in the plants help them to interact with other organisms [10]. The highly unstable poly unsaturated fatty acids present in the seagrasses are protected by flavonoids by donating the hydrogen atom there by quenching of lipid peroxy radicals formed [11]. Tannins are used for the treatment of burns, and also facilitate ruminants digestion [12]. Condensed tannins extracted from seagrasses are functioned as deterrents against herbivore feeding and also against fungal as well as bacterial invasion [13].

Environmental factors affecting on the concentrations of phenolic compounds in seagrasses include soil composition, temperature, rainfall and ultraviolet radiation incidence [14]. Variations in phenol metabolism occur due to gene duplication, mutation, subsequent recruitment, and adaptation to specific functions [15]. The production and accumulation of these compounds in seagrasses are used as indicators of stress due to both inter and intra specific competition, variations in the nutrients levels, pollution by heavy metals and temperature changes [16]. An increase in the dissolution of atmospheric carbon dioxide in seawater leading to ocean acidification also reported to influence the level of phenolic compounds [17-18]. In this background this study addresses to characterize the level of phenolic compounds and chlorophylls in seagrass species with respect to the associated flora and fauna in the seagrass beds as well as physical and chemical alteration occurring in the surrounding environments of Gulf of Mannar and Palk Bay, Southwest coast of India.

2. Materials and Methods

Seagrasses were collected from four stations [Thonithurai (area where seaweed farming is going on) and Chinnappalm (fishermen living area with plenty of fishing activities) situated on the Gulf of Mannar] during November 2010 and [Munaikkadu (geographically near to a lagoon and presence of few seaweeds) and Mathacovil (where seaweeds and corals coexists) on the Palk Bay side] during June 2011. The location of stations are range from 9^0 16' to 9^0 18 N' latitude and 79^0 08 to 79^0 13' E in longitude (figure 1). During south west monsoon, high waves affected the habitat at Gulf of Mannar while Palk Bay showed high waves during north east monsoon [19]. Sampling of seagrasses was not possible due to strong waves during north east monsoon at Palk Bay side and south west monsoon at Gulf of Mannar side.

Seagrasses were collected from the stations during low tides by the use of a $0.25m^2$quadrate [20]. Collected seagrasses were washed in seawater and carried to the laboratory in chilled condition. Each experiment consisted of three sampling of seagrasses from different seagrass meadows of same region and each sample was treated as separate. Seagrasses were washed with tap water followed by distilled water to remove sand particles and epiphytes. Seagrasses were dried in an air oven at 50^0Cfor 12 hours [21], powdered and kept in a desiccators, pending analysis.10g of the dried seagrasses were extracted for 24hour in 100ml of methanol under dark and filtered through Whatmann No: 1 filter paper. The residue was extracted again and the pooled extract was concentrated nearly to dryness using a rotary flash evaporator [22] and made up to a known volume with methanol. Methanolic extracts were analysed as per Li *et al* (2008) [23] and Chang *et al* (2002) [24] respectively for total phenolic and flavonoids. Acetone extracts of healthy seagrasses were measured at 647, 664 and 725 nm for fresh chlorophylls estimation [25]. The extract was acidified with 0.1N HCl to estimate the amounts of pheophytins [26]. Tannin in seagrasses was extracted using 0.05M NaOH at 60°C for 90 minutes and estimated spectrophotometrically [27]. For the deduction of colour interference of the extracts, sample blanks were also done and analysis was done in triplicates and average values were reported with standard deviation. The statistics (ANOVA TWO without replication) was performed to make the differences among the seagrasses using MS Office Excel (2013).

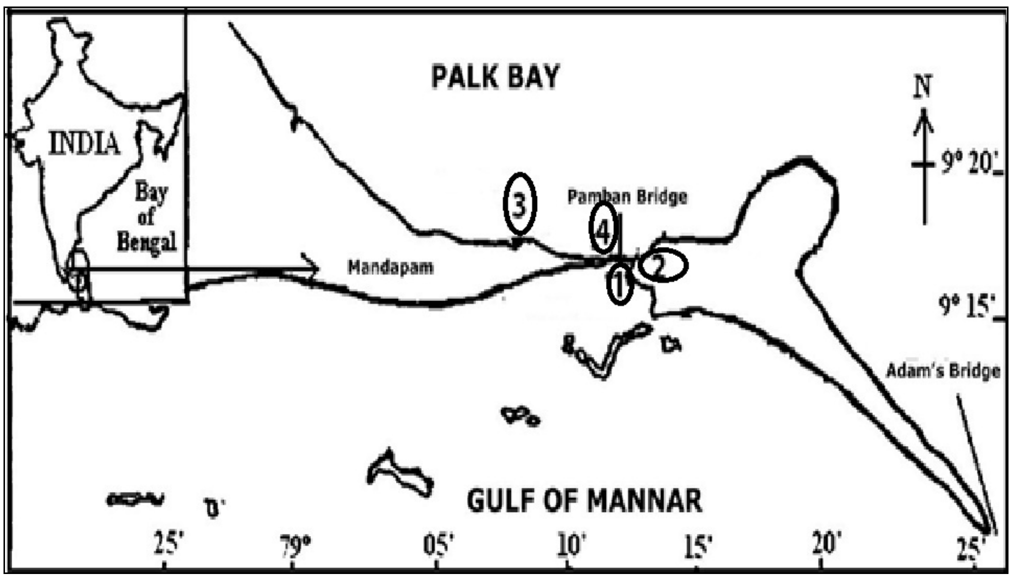

Fig 1: Geographical representation of the study area Thonithurai (1), Chinnappalam (2), Munaikkadu (3) and Mathacovil (4)

3. Results

Secondary metabolites in seagrasses

Cymodocea serrulata leaves and roots and rhizomes recorded very high contents of phenolics and flavonoids at Mathacovil and Munaikkadu compared with Chinnappalm and Thonithurai (Table 1).The phenolic content in the leaves of *C. serrulata* ranged from 41 to 298 mg/100g gallic acid equivalents and flavonoids 13 to 146 mg/100g quercetin equivalents where as in the roots and rhizomes the phenolics varied from 29 to 292 mg/100g and flavonoids 9.1 to 75 mg/100g respectively. The results showed that the phenolics in the leaves as well as roots and rhizomes of *C. serrulata* are comparable (p<0.05) except at Chinnappalam; while flavonoids in *C. serrulata* leaves generally recorded a predominance over roots and rhizomes. *Syringodium isoetifolium* of Mathacovil contained high phenolics and flavonoids compared to Thonithurai. The roots, rhizomes and leaves of *Enhalus acoroides* contained relatively high contents of phenolics and flavonoids in leaves compared to roots and rhizomes. Concentrations of phenolics in *Thalassia hemprichii* (whole) were higher than seagrasses species from Gulf of Mannar and that of flavonoids were higher than that of seagrass species from Gulf of Mannar and roots and rhizomes of *C. serrulata* from Palk Bay. Among the seagrasses, tannin varied from 0.20 to 5.02 mg/100g between the sampling areas. The leaves of *C. serrulata* from the sampling points from Palk bay reported higher content (>3.5 mg/100g) of tannin among the samples of seagrasses.

Chlorophylls

Among the seagrasses chlorophylls (chl) a and b and pheophytin showed relatively higher abundance at leaves. Remarkably higher content in the chl and pheophytin were noticed in *S. isoetifolium* collected from Thonithurai in the Gulf of Mannar. The chlorophyll content in the seagrass leaves from Gulf of Mannar was comparatively higher than Palk Bay while a reverse trend was noticed in the case of pheophytin. Chla in seagrasses ranged from 115 to 850 µg/g (leaves and whole), chlb from 11 to 1029 µg/g and pheophytin 41 to 204 µg/g (Table. 2).

Table 1: Secondary metabolites in seagrasses

Species *	Location	Station	Phenolics (mg.100g-1)	Flavonoids (mg.100g^{-1})	Tannin (mg.100g^{-1})
C. serrulata (leaf)	Gulf of Mannar	Thonithurai	55±0.42	18±0.11	1.42±0.22
C. serrulata (leaf)	Gulf of Mannar	Chinnappalam	41±0.32	13±0.09	1.21±0.14
C. serrulata (R&R)	Gulf of Mannar	Thonithurai	50±0.38	16±0.11	1.34±0.18
C. serrulata (R&R)	Gulf of Mannar	Chinnappalam	29±0.25	9.1±0.08	1.11±0.22
S. isoetifolium (W)	Gulf of Mannar	Thonithurai	21±0.17	7.0±0.06	0.20±0.08
E. acoroides (leaf)	Gulf of Mannar	Chinnappalam	108±0.56	34±0.21	1.82±0.28
E. acoroides (Rh)	Gulf of Mannar	Chinnappalam	27±0.24	9.3±0.06	1.32±0.21
E. acoroides(Rt)	Gulf of Mannar	Chinnappalam	17±0.20	5.2±0.07	1.14±0.25
C. serrulata (leaf)	Palk Bay	Munaikkadu	281±0.70	104±0.44	4.26±0.18
C. serrulata (leaf)	Palk Bay	Mathacovil	298±0.88	146±0.54	5.02±0.16
C. serrulata (R&R)	Palk Bay	Munaikkadu	285±0.62	69±0.27	4.51±0.12
C. serrulata (R&R)	Palk Bay	Mathacovil	292±0.81	75±0.30	3.70±0.10
S. isoetifolium (W)	Palk Bay	Mathacovil	277±0.66	58±0.17	0.78±0.09
T. hermprichii (W)	Palk Bay	Munaikkadu	227±0.51	85±0.22	1.21±0.10

* Three sampling in each case. ***P*<0.01 (between columns) and *P*<0.05 (between rows).

Table 2: Chlorophylls and pheophytin in seagrasses

Species*	Location	Station	Chlorophyll a (µg.g^{-1})	Chlorophyll b (µg.g^{-1})	Pheophytin (µg.g^{-1})
C. serrulata (leaf)	Gulf of Mannar	Thonithurai	817±3.20	669±2.80	150±0.78
C. serrulata (leaf)	Gulf of Mannar	Chinnappalam	822±3.28	354±1.20	41±0.32
C. serrulata (R&R)	Gulf of Mannar	Thonithurai	22±0.12	18±0.11	7±0.08
C. serrulata (R&R)	Gulf of Mannar	Chinnappalam	11±0.08	8±0.08	0
S. isoetifolium (W)	Gulf of Mannar	Thonithurai	850±3.32	1029±4.40	196±0.84
E. acoroides (leaf)	Gulf of Mannar	Chinnappalam	431±1.40	184±0.78	59±0.38
E. acoroides (Rh)	Gulf of Mannar	Chinnappalam	53±0.18	22±0.10	0
E. acoroides(Rt)	Gulf of Mannar	Chinnappalam	10±0.06	23±0.09	12±0.10
C. serrulata (leaf)	Palk Bay	Munaikkadu	127±0.66	29±0.25	204±1.10
C. serrulata (leaf)	Palk Bay	Mathacovil	115±0.62	30±0.31	134±0.77
C. serrulata (R&R)	Palk Bay	Munaikkadu	2.19±0.08	1.10±0.05	0
C. serrulata (R&R)	Palk Bay	Mathacovil	2.19±0.06	1.10±0.06	0
S. isoetifolium (W)	Palk Bay	Mathacovil	211±0.88	11±0.08	23±0.18
T. hermprichii (W)	Palk Bay	Munaikkadu	136±0.69	69±0.51	109±0.96

* Three sampling in each case. ***P*<0.05 (between columns) and *P*<0.01 (between rows).
R&R- Roots &Rhizomes,W- Whole, Rh- Rhizomes and Rt- Roots.

4. Discussion

Total Phenolic compunds

Phenolic compounds act as potential antioxidants as well as free radical scavengers [1]. In contrasts to the results relatively higher concentrations of phenolics were reported in the ethyl acetate fraction in *Z. marina* leaf extract [3]. Comparatively higher total phenol content was noticed at summer than winter season at the rhizomes of *P. oceanica* [28] and these findings were comparable to the present study. Higher concentrations at summer season was attributed to disturbing conditions affecting the meadow viz. turbidity, metal contamination, pollution, ocean acidification, competition with invasive seaweed, infection by *Labyrinthula* etc. [28]. A comparison between leaves and rhizomes of seagrasses indicated long life span for rhizomes and are less affected by the common changes in the physiological process because of their limited exposure to the environment compared to leaves [29]. Phenol content in the leaves as well as rhizomes of seagrasses from Palk Bay indicated species-wise variation as well as a positive correlation between phenol and tannin contents. Relatively higher concentrations of phenol in leaves followed by root and rhizomes were reported in *E. acoroides* of Mandapam coast [30] and similar observation was also recorded in the present study. One of the earlier studies indicated low total phenolic content in seagrasses of Mandapam coast in *E. acoroides* and maximum in *H. stipulacea* [31]. In contrast in this study, the total phenolic content in seagrasses of Gulf of Mannar was found to be minimum in *S. isoetifolium* and maximum in *E. acoroides* and a positive correlation with tannin content (Table 1). Similar positive correlations were noticed in the species in Palk Bay and higher phenol contents in leaves could be related to the defensive needs of seagrass leaves against epiphytes. Most of the terrestrial plants including angiosperms exhibit higher phenol content in rhizomes than leaves due to longer life span [32] and this study also reported similar trend.

There are reports indicating comparatively higher phenolic contents in the methanol extract from *H. pinifolia* and lower in *H. ovalis* hexane extract but in *S. isoetifolium* variations were not observed between solvents [2]. Seagrasses of Indonesia demonstrated a comparatively higher phenolic contents in the methanol extract followed by ethyl acetate and n-hexane, exhibiting solubility in polar as well as semi polar solvents depending on the nature of the species [33]. Total phenolic content was as high as 0.611 mg/g were reported in *H. stipulacea* [34] whereas this study observed variations were from 0.43 to 2.98 mg/g and 0.17 to 2.92 mg/g in leaves and roots respectively. Lower levels of phenolics in the seagrasses of Gulf of Mannar were reported [12] but contrast to the report higher phenol content were noticed in *C. serrulata* than *S. isoetifolium* from both Gulf of Mannar and Palk Bay in the present study. The variations in phenolic acids in seagrasses and vegetative propagules could depend on the abiotic and biotic factors including depth, environmental parameters, interactions of herbivores, competition between species etc. [35-36].

Seasonal wise variations were absent in the adult leaves of *P. oceanica* but with interactions with *Caulerpa taxifolia* and *C.*

*racemosa*in the meadows, adult leaves exhibited increased phenolic content [16]. A similar trend was noticed in the present study with respect to *C. serrulata* in Gulf of Mannar showing comparatively higher concentration phenolics in seagrasses from Thonithurai (seaweed culturing area) than Chinnappalam while a slight increase was observed at Mathacovil (where seaweeds and corals coexisted) than Munaikkadu of Palk Bay. Higher phenolic levels in the intermediate leaves might be due to higher metabolic rate and among these compounds; caffeic acid predominated in the adult as well as intermediate leaves [16]. Phenolic content in seagrasses of Gulf of Mannar followed the order *H. pinifolia*> *C. serrulata*> *C. rotundata*> *S. isoetifolium*> *T. hemprichii*> *E. acoroides* [10]. Total phenolic content in seagrasses showed that higher concentrations in at *H. pinifolia* and lower content in *E. acoroides* [1] while *E. acoroides* of Gulf of Mannar were recorded higher concentration than other seagrass species from the same region in the present study.

Flavonoids present in plants help to interact with other organisms [10]. The aqueous crude extract of *Z. noltii* showed higher flavonoids content with Apigenin 7-sulfate contributing to 71.3-82.7% of total flavonoids and Diosmetin 7- sulfate to 85.1-92.9% [37]. Flavonoids were concentrated at the leaf surfaces and extracellular cuticular layer in the epidermal cells. No significant differences in total flavonoid content were noticed in *H. johnsonii* between intertidal and sub tidal while relatively lower concentrations in *H. decipiens* [38]. Flavonoids localized in the cytoplasm and cuticle of leaf tissue of *H. johnsonii* showed no remarkable differences due to the effect of salinity and light/shade during a period of one day to 21 days [39]. Comparatively higher concentrations of flavonoids reported in *C. serrulata* and lower at *H. pinifolia* and wide variations were observed between species [10]. In the present study *C. serrulata* predominated over other species in flavonoid content and species wise flavonoid level in seagrasses of Gulf of Mannar followed the order *E. acoroides*> *C. serrulata*> *S. isoetifolium* while a different results reported in the seagrasses from the same region earlier [10].

Relatively higher concentrations of tannin were noticed in the leaves than roots and rhizomes, and also comparatively high content of tannin found at *C. serrulata* and lower at *S. isoetifolium* in this study. Tannin content showed species to species and seasonal variation and the class of compounds in leaves usually play an active rate in the defensive mechanism against epiphytes [32]. Tannin content in seagrasses of Gulf of Mannar followed the order *E. acoroides*> *C. serrulata*> *S. isoetifolium* and a comparable result was obtained earlier [12]. Comparatively higher concentrations of tannins were found in *H. pinifolia* and lower at *E. acoroides* and wide variations were noticed among species and location. Tannins are reported to play an important role against chronic diseases [10]. Further there are reports indicating the role of condensed tannins from seagrasses as deterrents against herbivore feeding, antifungal and antibacterial agents [13].

Cholorophylls

Total pigments as well as chla content in seagrass of *P. oceanica* varied from 472-595mg/m^2 and 282 to 334 mg/m^2 respectively indicating wide variations between control and salt –stressed plants [40]. Daudi *et al* [41] reported the absence of significant differences in chl concentration, species and locations wise in *E. accordies*, *T. hemprichii* and *T. ciliatium*. Both species wise and station wise variations in chla and b contents were observed in this study. Relatively higher concentrations of chls were found during spring and lower in winter. Chl content in seagrass varied with changes in temperature and light intensity [42]. Relatively higher concentrations of chl a and b obtained in seagrasses of Thonithurai and Chinnappalam of Gulf of Mannar and it might be due to reductions in light intensity as a result of turbidity [43-45].

Analysis of chl content in seagrasses *C. nodosa* and *Ruppia cirrhosa* were revealed higher concentrations during summer which could be related to the presence of thick layer of epiphytic algae and the presence of epiphytes in the seagrasses [46]. Chl a content in the leaves of *C. nodosa* showed seasonal variations and highest concentrations of chla and b were obtained during the month of September (169 mg dw/m^2) [47]. Total chl content in the leaves of *P. oceanica* increased from summer to winter and also chl a/chl b ratio as well as total chl/soluble proteins ratio. The low chl content during summer might be due to its degradation related to the surrounding environmental factors such as temperature, light, stress etc. [28]. The lower concentrations of chl during winter might be due to the reduction in the intensity of light and an increase in chl a with respect to chl b related to the capacity for excitation energy transfer in comparison with that of capture light [48].

Pradheeba *et al* [32] reported that chl a predominated over chl b in leaves of seagrasses of Palk Bay and was similar to the findings in the present study. Chl content in seagrasses were affected by the availability of light and morphology of the seagrass leaves. The highest concentrations of chlorophyll pigment were reported during summer season and lowest at monsoon. During monsoon season, the increased turbidity of the water column decreased the light availability to seagrasses. Species to species variations in chl content was observed which were directly related to the depth of the water column and surface areas of the leaves. Seagrasses with cylindrical leaf of *S. isoetifolium* showed higher concentrations than *C. serrulata* and *E. acoroides* at Gulf of Mannar [32] while the order was *S. isoetifolium* followed by *T. hemprichii* and *C. serrulata* at Palk Bay in this study. The accumulation of trace metals of Zn, Cd and Cu during a period of 10 days in the tissue of *T. hemprichii* affected the photosynthetic pigments as shown by a decrease in the concentrations of chl a and b [49]. Screening of photosynthetic pigments in the leaves of three seagrasses showed variations in chla and b [50] and chl content ranged from 0.701 (*H. stipulacea*) to 1.424 mg/g (*H. pinifolia*) while this study demonstrated a much higher variation (0.018 to 1.879 mg/g (Gulf of Mannar) and 0.003 to 0.205 mg/g (Palk Bay)). Enhanced levels of chlorophyll pigments in seagrasses correspond to high photosynthetic activity and productivity [50].

5. Conclusion

Secondary metabolites in seagrasses revealed that they are potential sources of phenols, flavonoids and tannins. Phenolic content was comparatively higher in the leaves than roots and rhizomes in seagrasses of Gulf of Mannar and Palk Bay. Relatively higher phenolics were noticed in *C. serrulata* and lower in *S. isoetifolium*. Phenolics in seagrasses of Gulf of Mannar and Palk Bay were comparable but lower than similar studies reported earlier from the regions. Species to species variations in the concentrations of tannin content were noticed both at Gulf of Mannar and Palk Bay, and relatively higher concentrations of tannin found in leaves than roots and rhizomes. Chl content in seagrasses of Gulf of Mannar and

Palk Bay showed species wise as well as station wise variations and comparatively higher contents of chl a and b obtained from Gulf of Mannar. Chl content in leaves were much higher than roots and rhizomes. Seagrasses with cylindrical leaf of *S. isoetifolium* showed higher concentrations than non – cylindrical leaves (*C. serrulata* and *E. acoroides*). Chl contents in seagrasses were comparable to similar work carried out at Gulf of Mannar and Palk Bay.

6. Acknowledgements

The authors are thankful to the Director, School of Marine Sciences, the Dean, Faculty of Marine Sciences, Head Department of Chemical Oceanography, Cochin University of Science and Technology for their support and facilities provided. We are also thankful to Dr. M. P. Prabhakaran, Assistant Professor, Kerala University of Fisheries and Ocean Studies, for the help in the identification of seagrasses.

7. References

1. Kannan RRR, Arumugam R, Anantharaman P. *In vitro* antioxidant activities of ethanol extract from *Enhalusacoroides* (L.F) Royle. Asian Pacific Journal of Tropical Medicine. 2010a; 898-901.
2. Girija K, Parthiban C, Hemalatha A, Saranya C, Anantharaman P. Evaluation of antioxidant activities and preliminary phytochemical analysis of seagrass *Halodul epinifolia*, *Halophil aovalis* and *Syingodium isoetifolium*. Journal of Phytochemistry Photon. 2013; 114:181-187.
3. Choi HG, Lee JH, Park HH, Sayegh FAQ. Antioxidant and antimicrobial activity of *Zostera marina* L. extract. Algae. 2009; 24(3):179-184.
4. Carletti P, Masi A, Wonisch A, Grill D, Tausz M, Ferretti M. Changes in antioxidant and pigment pool dimensions in UV-B irradiated maize seedlings. Environmental Experimental Botany, 2003; 50:149-157.
5. Kutchan TM. Ecological Arsenal and Developmental Dispatcher. The Paradigm of Secondary Metabolism. Plant Physiology, 2001; 125:58-60.
6. Logemann E, Tavernaro A, Schulz W, Somssich IE, Hahlbrock K. UV light selectively co induces supply pathways from primary metabolism and flavonoid secondary product formation in parsley. Proceeding of the National Academy of Sciences. 2000; 97:1903-1907.
7. Kolb CA, Käser MA, Kopecký J, Zotz G, Riederer M, Pfündel EE. Effects of Natural Intensities of Visible and Ultraviolet Radiation on Epidermal Ultraviolet Screening and Photosynthesis in Grape Leaves. Plant Physiology. 2001; 127:863-875.
8. Castellano G, Tena J, Torrens F. Classification of Phenolic Compounds by Chemical Structural Indicators and Its Relation to Antioxidant Properties of *Posidonia oceanica* (L.) Delile. MATCH Communications in Mathematical and Computer Chemistry. 2012; 67:231-250.
9. Arnold TM, Targett NM. Marine Tannins: The importance of a mechanistic frame work for predicting ecological roles. Journal of Chemical Ecology. 2002; 28(10):1919-1934.
10. Kannan RRR, Arumugam R, Thangaradjou T, Anantharaman P. Phytochemical constituents,antioxidant properties and P-coumaric acid analysis in some seagrasses. Food Research International. 2013; 54:1229-1236.
11. Hamid AA, Aiyelaagbe OO, Usman LA, Ameen OM, Lawal A. Review-Antioxidants: Its medicinal and pharmacological applications. African Journal of Pure and Applied Chemistry. 2010; 4(8):142-151.
12. Athiperumalsami T, Kumar V, Jesudass LL. Survey and phytochemical analysis of seagrasses in the Gulf of Mannar, South east coast of India. Botanica Marina, 2008; 51:269-277.
13. McMillian C. The condensed tannins (paranthocyanidins) in seagrasses. Aquatic Botany, 1984; 20:351-357.
14. Monteiro JM, Albuquerque UP, Lins Neto EMF, Araújo EL, Albuquerque MM, Amorim ELC. The effects of seasonal climate changes in the Caatinga on tannin level. Brazilian Journal of Pharmacognosy. 2006; 16:338-344.
15. Jutta Papenbrock. Review Article on Highlights in Seagrasses' Phylogeny, Physiology, and Metabolism: What Makes Them Special International Scholarly Research Notices, ISRN Botany 2012, 15. Article ID 103892.
16. Dumay O, Coasta J, Desjobert JM, Pergent G. Variations in the concentrations of phenolic compounds in the seagrass *Posidonia oceanica* under conditions of competition. Phytochemistry. 2004; 65:3211-3220.
17. Arnold T, Mealey C, Leahey H, Miller AW, Hall –Spenar JM, Milazzo M *et al*. Ocean acidification and the loss of phenolic substances in marine plants. PLoS ONE. 2012; 7(4):1-10.
18. Jin P, Wang T, Liu N, Dupont S, Beardall J, Boyd PW *et al*. Ocean acidification increases the accumulation of toxic phenolic compounds across trophic levels. Nature Communications, 2015; 6:8714.
19. Gowthaman R, Sanil Kumar V, Dwarakish GS, Mohan SS, Jai Singh, Ashok Kumar K. Waves in Gulf of Mannar and Palk Bay around Dhanushkodi, Tamil Nadu, India. Current Science. 2013; 104(10):1431-1435.
20. Lewis FG, Stoner AW. An examination of methods for sampling macro-benthos in seagrass meadows. Bulletin of Marine Science. 1981; 31:116-129.
21. Khamsah SM, Akowah G, Zhari I. Antioxidant activity and phenolic content of Orthosiphon stamineusbenth from different geographical origin. Journal of Sustainability Science and Management. 2006; 1:14-20.
22. Adomi PO. Antibacterial activity of aqueous and ethanol extracts of the stem bark of *Alstonia boonei* and *Morinda lucida*. Scientific Research Essay. 2006; 1(2):50-53.
23. Li HB, Wong CC, Cheng Ka W, Chen F. Antioxidant properties *in vitro* and total phenolic contents in methanol extracts from medicinal plants. LWT, Food Science and Technology. 2008; 41:385-390.
24. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis. 2002; 10:178-172.
25. Dennison WC. Chlorophyll content In: RC Philips, CP McRoy (Eds). Seagrass Research Methods, Unesco, Paris. 1990.
26. Plante-Cuny MR. Evaluation par spectrophotométrie des teneurs en chlorophyl-a fonctionelleer en phaeopigments des substrates meublesmarins. O.R.S.T.O.M. Nosy-Bé, 1974, 45.
27. Nair SM, Balchand AN, Nambisan PNK. On the determination and distribution of hydroxylated aromatic compounds in estuarine waters. Toxicology and Environmental Chemistry. 1989; 23:203-213.
28. Rotini A, Belmonte A, Barrote I, Micheli C, Pierane A, Santos RO *et al*. Effectiveness and consistency of a suite of descriptors for assessing the ecological status of

seagrass meadows (*Posidonia oceanica* L. Delile). Estuarine, Coastal and Shelf Science. 2013; 130:252-259.
29. Migliore L, Rotini A, Randazzo D, Albanese NN, Giallongo A. Phenol content and 2-D electrophoresis protein pattern: a promising tool to monitor *Posidonia* meadows health state. BMC Ecology. 2007; 7:6.
30. Kannan RRR, Arumugam R, Meenakshi S, Anantharaman P. Thin layer chromatography analysis of antioxidant constituents from seagrasses of Gulf of Mannar, Biosphere Reserve, South India. International Journal of Chem Tech Research. 2010b; 2(3):1526-1530.
31. Rangasamy RRK, Rajasekharan A, Micheline GD, Perumal A. Antioxidant activity of the seagrasses of the Mandapam coast, India. Pharmaceutical Biology. 2012; 50(2):182-187.
32. Pradheeba M, Dilipan E, Nobi EP, Thangaradjou T, Sivakumar K. Evaluation of seagrass for their nutritional value. Indian Journal of Geo–Marine Sciences. 2011; 40(1):105-111.
33. Santoso J, Anwariyah S, Rumiantin RO, Putri AP, Ukhty N, Yoshie-Stark Y. Phenol content, antioxidant activity and fitness profile of four tropical seagrasses from Indonesia. Journal of Coastal Development. 2012; 15(2):189-196.
34. Abd El-Hady HH, Hamed ER, Shehata AN. Molecular identification, Antimicrobial and antioxidant activities of the tropical seagrass *Halophila stipulacea* grown in El-Bardaresil Lake, Egypt. Australian Journal of Basic and Applied Sciences. 2012; 6(12):474-481.
35. Gringnon-Dubois M, Rezzonico B, Alcoverro T. Regional scale patterns in seagrass defenses: Phenolic acid content in *Zosteranoltii*. Estuarine, Coastal and Shelf Science. 2012; 114:18-22.
36. Spencer DF, Ksander GG. Phenolic acid content of vegetative propagules of *Potamogeton* spp. and *Hydrilla* vetricilletta. Journal of Aquatic Plant Management. 1994; 32:71-73.
37. Grignon-Dubois M, Rezzonico B. First phytochemical evidence of chemotypes for the seagrass *Zosteranoltii*. Plants. 2012; 1:27-38.
38. Gavin NM, Durako MJ. Localization and antioxidant capacity of flavonoids from intertidal and subtidal *Halopila johnsonii* and *Halopila decipens*. Aquatic Botany. 2011; 95:242-247.
39. Gavin NM, Durako MJ. Localization and antioxidant capacity of flavonoids in *Halopila johnsonii* in response to experimental light and salinity variation. Journal of Experimental Marine Biology and Ecology. 2012; 416-417:32-40.
40. Marin-Guirao L, Sandoval-Gil JM, Bernardeau-Esteller J, Reuz JM, Sanchez –Lizaso JL. Responses of the Mediterranean seagrass *Posidonia ocaeanica*to hyper saline stress duration and recovery. Marine Environmental Research. 2013; 84:60-75.
41. Daudi LN, Lugomela C, Uku JN, De Toroch M. Effect of nutrient enrichment on seagrass associated marine fauna in Tanzania. Marine Environmental Research. 2012; 82:49-58.
42. Lee KS, Dunton KH. Production and carbon reserve dynamics of seagrass *Thalassia testudinum* in corpus Christi Bay, Texas, USA. Marine Ecology Progress Series. 1996; 143:201-210.
43. Dennison WC, Alberte RS. Role of daily light period in the depth distribution of *Zostera marina* (eelgrass). Marine Ecology Progress Series. 1985; 25:51-61.
44. Abal EG, Loneragon N, Bowen P, Perry CJ, Udey JW, Dennison WC. Physiological and morphological responses of the seagrass *Zostera capricorni* Aschers to light intensity. Journal of Experimental Marine Biology and Ecology. 1994; 178(1):113-129.
45. Lee KS, Dunton KH. Effects of in site light reduction on the maintenance, growth and partitioning of carbon resources in *Thalassia testudinum* Banks ex Koning. Journal of Experimental Marine Biology and Ecology. 1997; 210:53-73.
46. Abd El–Hady HH, Daboor SM, Ghoniemy AE. Nutritive and antimicrobial properties of some seagrasses from Bardawil Lake, Egypt. Egyptian Journal of Aquatic Research. 2007; 33(3):103-110.
47. Zavodnik N, Travizi A, De Rosa S. Seasonal variations in the rate of photosynthetic activity and chemical composition of the seagrass *Cymodoceano dosa* (Ucr).Asch. Scientia Marina. 1998; 62(4):301-309.
48. Lichtenthaler HK, Babani F. Light adaption and senescence of the photosynthetic apparatus. Changes in pigment composition, chlorophyll fluorescence parameters and photosynthetic activity. In: Papageorgiou G. C., Govindjee (Eds), Chlorophyll fluorescence: a signature of photosynthesis. Springer, Dordrecht. 2004, 713-736.
49. Lei L, Xiaoping H, Borthakur D, Hui N. Photosynthetic activity and antioxidative response of seagrass *Thalassia hemprichii* to trace metal stress. Acta Oceanologica Sinica. 2012; 31(3):98-108.
50. Kannan RRR, Arumugan R, Hemalatha A, Anantharaman P. Phytochemical analysis of seagrass from Mandapam coast, Tamilnadu. Seaweed Research Utilization. 2010c; 32(1-2):171-175.