Species Profiling and Biochemical Characterizations of selected seagrass species of Gulf of Mannar and Palk Bay, India

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by

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Ph.D. Thesis under the Faculty of Marine Sciences

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Certificate

This is to certify that the thesis entitled **"Species Profiling and Biochemical Characterizations of selected seagrass species of Gulf of Mannar and Palk Bay, India"** is an authentic record of research work carried out by Mr. Libin Baby (Reg. No.3663), under our supervision and guidance at the Department of Chemical Oceanography, School of Marine Sciences, Cochin University of Science and Technology, Cochin - 682016, in partial fulfilment of the requirements for Ph.D. degree of Cochin University of Science and Technology and no part of this has been presented for the award of any other degree in any University. All the relevant corrections and modifications suggested by the audience and recommended by the doctoral committee of the candidate during the pre-synopsis seminar have been incorporated in the thesis.

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Declaration

I hereby declare that the thesis entitled **"Species Profiling and Biochemical Characterizations of selected seagrass species of Gulf of Mannar and Palk Bay, India"** is an authentic record of research work carried out under the supervision and guidance of Dr. T. V. Sankar, Director of Research, KUFOS, Panangad and Dr. N. Chandramohanakumar, Emeritus Professor, Department of Chemical Oceanography, School of Marine Sciences, Cochin University of Science and Technology, Cochin, and that no part of it has previously formed the basis for award of any degree, diploma, associated fellowships or other similar recognition in any University or Institution.

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Dedicated to

My beloved grandparents.....

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Preface

Seagrasses are aquatic angiosperms noticed at near shore environments of fresh water, estuarine, marine and hyper saline conditions. Seagrasses have leaf, root, rhizome and stem as important and among them rhizome is important for anchoring while roots transport oxygen and nutrients. They are influenced by physical, chemical and biological environments of coastal waters. Seagrasses are reported to be distributed at South East Pacific Reefs and Islands, South America, Southern Atlantic, Indian Ocean Islands and West African coast except Antarctica. Anthropogenic activities as well as natural processes destroy or degrade the seagrass meadows and it leads to a decrease in seagrass functions and values to the coastal ecosystems. The rehabitation of seagrass meadows in World are done through transplantation, improvement of water quality, restrictions on boating activity, fishing and aquaculture, and also by protection of existing meadows through law and environmental policy. Physical parameters like temperature, salinity, waves, currents, depths, substrate and day length have crucial role in regulating the physiological functions of seagrasses while natural phenomena which retard the photosynthetic activity of seagrasses are light, nutrients, epiphytes, diseases, anthropogenic inputs of nutrient and sediment loading. Even though strict environmental laws exist seagrass meadows are declining due to anthropogenic activities, urban and industrial waste disposal, bottom trawling fisheries etc., forcing several countries to initiate transplantation of seagrasses, even though the process is costly and offers less yield.

There are 72 species of seagrasses under 14 genera and six families grow both at shallow sub tidal and inert tidal environments in Worldwide. In India, there are 15 species of seagrasses under seven genera and two families. *Cymodocea*, *Halodule*, *Halophila*, *Enhalus*, *Syringodium* and *Thalassia* are the six genera from marine waters and genera Ruppia belongs to fresh water seagrass. The dominant species in India are *Cymodocea*

serrulata, *Thalassia hemprichii*, *Halodule uninervis* and *Halodule pinifolia*. The distribution of seagrasses were higher in Tamil Nadu (15 species) followed by Andaman and Nicobar Islands (9 species). In Tamil Nadu, seagrass populations are higher at Palk Bay and Gulf of Mannar and among these Gulf of Mannar contained 11 species.

The importance of the natural wealth in the Gulf of Mannar region is through shelter to marine organisms (fishes, sponges, dugongs, turtles etc), protecting the coastline (coral Reefs, sand dunes, mangroves and seagrass meadows against beating waves, storms and cyclones), regulating the local climate, producing food (through photosynthesis by tropical dry mixed evergreen forests, tropical scrublands, mangroves, coral reefs, macro and micro algae and seagrass meadows), preventing floods (mud in mangroves), preventing erosion (seagrass meadows, mangroves and mud flats), trapping carbon dioxide (tropical dry mixed evergreen forests, tropical scrublands, mangroves and seagrasses as carbon sinks), maintaining soil productivity (seagrass meadows, mangroves and mud flats) and supporting traditional livelihoods (mangroves, coral Reefs and seagrass meadows support traditional fisheries). Palk Bay is also rich in biodiversity and important groups associated includes sponges and gorgonids (275 species out of 31 endemic form), corals (63 species out of 22 genera), stony corals (128 species out of 43 endemic form), fisheries (elasmobranches, squids, lobsters, crabs, cephalopods and pearl oyster culture contain 580 species), turtles (five species), birds (61 species), crustacea (651 species out of 159 endemic form), mollusca (733 species out of 26 endemic form), mammals (11 species including dolphins and dugongs), algae and seagrasses.

Seagrasses are one of the specialized groups of marine flora which are poorly known in India compared to seaweeds and mangroves especially for bioactive potential studies. People living along the coastal areas use seagrasses as folk medicine against fever, muscle pains, wounds, skin diseases, stomach problems, heart diseases and blood pressure. Antioxidants

in biological systems have the ability to prevent cell damage caused by the action of reactive oxygen species. Secondary metabolites especially phenolic compounds are responsible for antioxidant, antibacterial, and antifungal activities in seagrasses. They are also rich source of macro and trace metals which are essential for humans. Nowadays, in medical health treatments many countries have incorporated traditional medicines. Antioxidant and antimicrobial activities exhibiting secondary metabolites are generated in seagrasses due to their interactions with micro and macro algae, epiphytes and other organisms in the habitat. Studies showed that an interaction of *Caulerpa taxifolia* and *Caulerpa racemosa* with *Posidonia oceanica* leaves exhibited an increase in phenolic content, due to their defensive mechanism against others. Antioxidant and antimicrobial activities in seagrasses are decreased with an increase in the content of pollutants. In this aspect, antimicrobial and antioxidant studies are more important if samples are obtained from an unpolluted environment.

The investigations on seagrasses in India are scarce compared to mangroves and seaweeds. Little seagrass research is focused on biology, density, distribution, taxonomy (normal as well as using DNA), utility of products from seagrasses, microbiology and eco biology. Seagrasses play a major role in biogeochemistry of the coastal environment, provides ecological, medicinal, commercial uses to humans and other related organisms. Although a variety of secondary metabolites are produced by seagrasses which have pharmaceutical significance, only very few studies were carried out to characterize these compounds.

The thesis is divided into six chapters. Chapter 1 is the introduction which details the distribution of seagrasses in Worldwide as well as India, importance of seagrasses in the ecosystem and human life and reviews on the biochemical compositions and profiles as well as bio potentials of seagrasses. It also deals with aim and scope of the present study.

Chapter 2 provides the details of the seagrasses collected for the study, sampling stations and the analytical methodology adopted.

Chapter 3 gives the environmental characteristics of the related ecosystems (water and sediment quality) and biochemical evaluation of seagrasses (biochemical, phenolics, pigments, elemental and mineral compositions and trace metals distributions) of Gulf of Mannar and Palk Bay in order to evaluate the basic chemical nature of the seagrasses with respect to their surrounding environment.

Chapter 4 is concerned with fatty acids, amino acids and carbohydrate profile of seagrasses of Gulf of Mannar and Palk Bay. Fatty acid profile details the level of essential fatty acids and its variations with respect to station as well as body parts while amino acid profile gives the concentrations of essential amino acids while the carbohydrate profile describes the dominated individual carbohydrates.

Chapter 5 deals with the bio potential of seagrasses of *Cymodocea serrulata* and *Syringodium isoetifolium* of Gulf of Mannar grown at low salinities and is assessed on the basis of antioxidant and antimicrobial activities. The level of activities indicates whether these species can be used as a source of nuetraceuticals for humans.

In chapter 6 the salient features of the present investigation are summarised at the end of the thesis.

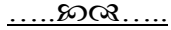
All chapters have a brief introduction, discussion and conclusions sections. Respective references are appended at the end of each chapter.

Contents

List of Abbreviations

AE	Ascorbic acid equivalents
Chl	Chlorophyll
CS R	*Cymodocea serrulata* roots and rhizomes from Thonithurai
CS W	*Cymodocea serrulata* whole from Thonithurai
CSLC	*Cymodocea serrulata* leaf from Chinnappalam
CSLF	*Cymodocea serrulata* leaf from Farm pond
CSLM	*Cymodocea serrulata* leaf from Mathacovil
CSLT	*Cymodocea serrulata* leaf from Thonithurai
CSRC	*Cymodocea serrulata* roots and rhizomes from Chinnappalam
CSRF	*Cymodocea serrulata* roots and rhizomes from Farm pond
CSRM	*Cymodocea serrulata* roots and rhizomes from Mathacovil
CSRT	*Cymodocea serrulata* roots and rhizomes from Thonithurai
DGDG	Digalactosyldiacylglycerol
DGTS	Diacylglyceryltrimethylhomo-Ser
DO	Dissolved oxygen
DPPH	2, 2-diphenyl-1-picrylhydrazyl
EAA	Essential amino acid
EALC	*Enhalus acoroides* leaf from Chinnappalam
EALRHC	*Enhalus acoroides* rhizomes from Chinnappalam
EARTC	*Enhalus acoroides* roots from Chinnappalam
EFA	Essential fatty Acid
FA	Fatty acid
FRAP	Ferric reducing antioxidant power
GAE	Gallic acid equivalents
H_2O_2	Hydrogen peroxide
HCl	Hydrochloric acid
HNO_3	Nitric acid
MGDG	Monogalactosyldiacylglyceride
MIC	Minimum inhibitory concentration
MUFA	Mono unsaturated fatty acid
ND	Not detected

NEAA	Non essential amino acid
NH_x	Reduced nitrogen
NSP	Non starch polysaccharides
OC	Organic carbon
OH^-	Hydroxy radical
PC	Phosphatidylcholine
PCA	Principal component analysis
PE	Phosphatidylethanolamine
PG	Phosphatidylglycerol
PP	Phenylpropenoid
PUFA	Poly unsaturated fatty acid
QE	Quercetin equivalents
R&R	Roots and rhizomes
Rh	Rhizomes
Rt	Roots
SA	Shikimic acid
SAFA	Saturated fatty acid
SI W	*Syringodium isoetifolium* whole from Thonithurai
SIWM	*Syringodium isoetifolium* whole from Mathacovil
SIWT	*Syringodium isoetifolium* whole from Thonithurai
SQDG	Sulfoquinovosyldiacylglyceride
TAA	Total antioxidant activity
THWF	*Thalassia hemprichii* whole from Farm pond
TN	Total nitrogen
TOC	Total organic carbon
TP	Total phosphorus
WSC	Water soluble carbohydrates



Chapter 1

INTRODUCTION

Contents

1.1 Seagrasses

Seagrasses are marine flowering plants which complete the life cycle in water and usually seen at near shore environments of most of the continents. They have the ability to survive in fresh water, estuarine, marine and hyper saline conditions. Seagrasses are included under the family *Hydrocaritaceae* and *Zosteraceae* evolved from single monocotyledons flowering plants between 70 million and 100 million years ago (Sulochanan, 2012). Seagrasses cover 0.1% of the ocean floor and it is <0.02% of the angiosperm flora (Thangaradjou et al., 2015). These are paraphyletic group of marine hydrophilus angiosperms have leaf, root, rhizome and stem, and pollination takes place underwater with specialized pollen whereas seeds are dispersed by both biotic and abiotic agents. Roots and rhizomes have importance in the

transportation of oxygen and nutrients in seagrasses. Besides seagrasses, its litter and bio films play important role in the cycling, bio transfer and biogeochemical deposition in coastal ecosystem (Hosokawa et al., 2016). *Posidonia oceanica* from Mediterranean Sea acts as a source of transparent exopolymer (Iuculano et al., 2017). Compared to other marine plants, seagrasses act as a source of carbon to surrounding ecosystem through particulate and dissolved organic matter and its carbon sequestrated extends from nearby non-vegetated sediments to deep sea (Duarte and Krause-Jensen, 2017). They are influenced by physical, chemical and biological environments of coastal waters (Papenbrock, 2012). Seagrasses are reported to be distributed at South East Pacific Reefs and Islands, South America, Southern Atlantic, Indian Ocean Islands and West African coast except Antarctica (Short and Coles, 2001; Sulochanan, 2012; Rotini et al., 2013). Comparatively lower species distribution is observed in regions where seagrasses grow greater than 10m below mean sea level (Lee Long et al., 1996; Thangaradjou et al., 2015). Anthropogenic activities as well as natural processes destroy or degrade the seagrass meadows and it leads to a decrease in seagrass functions and values to the coastal ecosystems (Short and Coles, 2001; Connell et al., 2017; Shelton et al., 2017).

1.2 Global Distribution of Seagrass

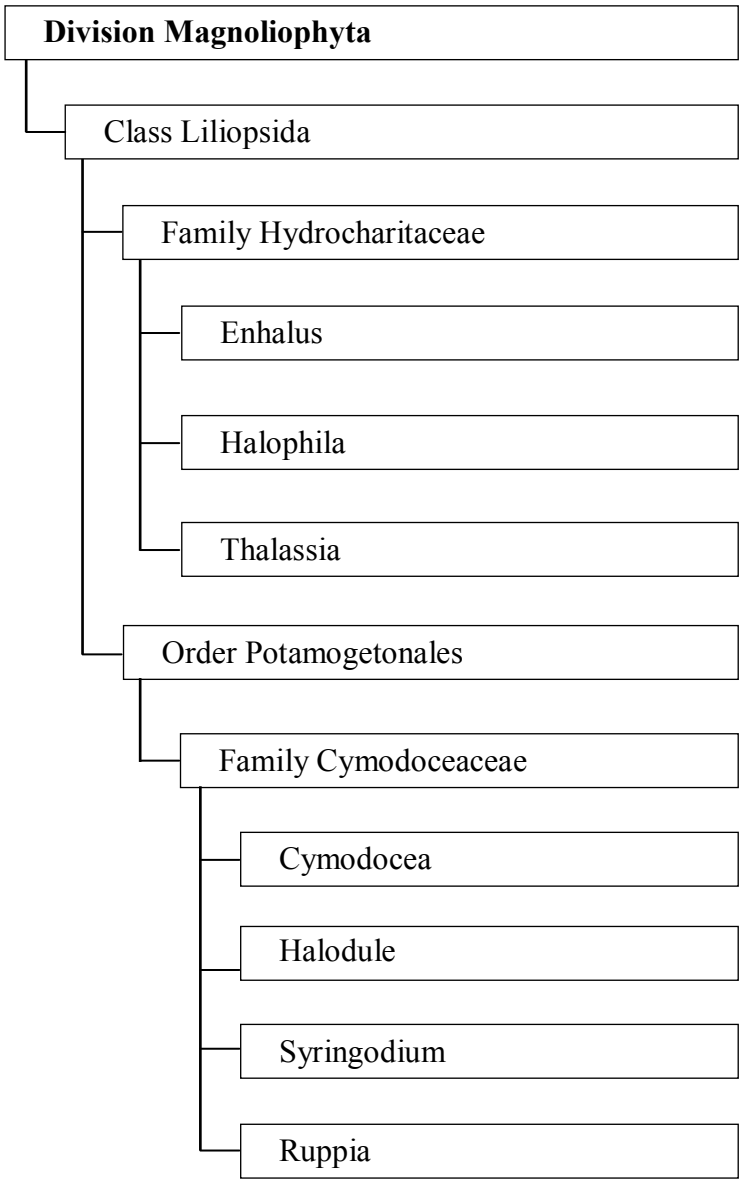
Worldwide, 72 species of seagrasses under 14 genera and six families grow both at shallow sub tidal and inert tidal environments (Table 1.1). They are not related to terrestrial grasses under the genera of *Poaceae*. Seagrasses population all over the world are classified into two based on their habitat. Generas of *Amphibolis*, *Hetrozostera*, *Phyllospadix*, *Posidonia*, *Pseudalthania*, *Lepilaena* and *Zostera* belongs to temperate seas while

Cymodocea, *Enhalus*, *Halophila*, *Halodule*, *Syringodium*, *Thalassia* and *Thalassodendron* belong to tropical seas (Short and Coles, 2001; Kannan and Thangaradjou, 2015).

Table 1.1 Distribution of seagrasses throughout the World (Short and Coles, 2001)

Sl No:	Region	Species name	Total species
1	North Pacific (Japan to baja California)	*Zostera marina*, *Phyllospadix iwatensis*, *P. japonicus*, *Ruppia maritima*, *Z. asiatica*, *Z. caespitosa*, *Z. caulescens*, *Z. japonica* , *P. scouleri*, *P. serrulatus*, *P. torreyi*, *Halodule* spp., *H. decipiens* (Mexico) and *Halophila ovalis* (Japan).	14
2	Chile	*Heterozostera tasmanica.*	1
3	North Atlantic (North California to Spain) and Europe	*Z. marina*, *R. maritime*, *Z. noltii* and *H. wrightii* (North California).	4
4	Caribbean (Florida to Brazil)	*Thalassia testudinum*, *Syringodium filiforme*, *H. wrightii*, *Halodule spp.* complex, *H. beaudettei*, *H. ciliata*, *H. bermudensis*, *H. emarginata*, *Halophila baillonis*, *H. decipiens*, *H. engelmanni*, *H. johnsonii* and *R. maritima*.	13
5	South West Atlantic	*R. maritima.*	1
6	Mediterranean (Black, Caspian and Aral Seas and NW Africa)	*Posidonia oceanica*, *Cymodocea nodosa*, *R. cirrhosa*, *R. maritima*, *Z. marina*, *Z. noltii*, *Halophila stipulacea*, *H. wrightii*, *H. decipiens* and *Potamogeton pectinatus*.	10
7	South East Atlantic region	*H. wrightii*.	1
8	South African region	*H. ovalis*, *Ruppia spp.*, *Z. capensis*, *H. uninervis*, *S. isoetifolium* and *Thalassodendron ciliatum.*	6
9	Indo – Pacific Region (East Africa & South Asia to Eastern Pacific)	*H. uninervis*, *H. ovalis*, *H. stipulacea*, *S. isoetifolium, C. rotundata*, *C. serrulata*, *Enhalus acoroides*, *H. decipiens*, *H. minor*, *T. hemprichii*, *T. ciliatum*, *H. pinifolia*, *H. beccarii*, *R. maritima*, *H. ovata*, *H. spinulosa*, *Z. japonica*, *H. hawaiiana*, *H. capricorni*, *H. tricostata*, *Z. capricorni*, *C. angustata* and *Z. capensis*.	23
10	Southern Australia	*Amphibolis antarctica*, *H. australis*, *H. decipiens*, *H. ovalis*, *H. tasmanica*, *Lepilaena cylindrocarpa*, *P. australis*, *R. megacarpa*, *Z. capricorni*, *Z. mucronata*, *Z. muelleri*, *Z. novazelandica*, *A. griffithii*, *L. marina*, *P. sinuosa*, *P. angustifolia*, *P. ostenfeldi* complex, *R. tuberosa*, *S. isoetifolium* and *T. pachyrhizum*.	20

Taxonomic classification of Indian seagrasses



1.3 Seagrass Distribution in India

In India, there are 15 species of seagrasses under seven genera and two families (Jagtap et al., 2003; Thangaradjou et al., 2015). *Cymodocea*, *Halodule*, *Halophila*, *Enhalus*, *Syringodium*, *Thalassia* and *Ruppia* are the seven generas among these Ruppia belongs to fresh water seagrass. The dominant species in India are *Cymodocea serrulata*, *Thalassia hemprichii*, *Halodule uninervis* and *Halodule pinifolia* (Short and Coles, 2001).

Table 1.2 Distribution of seagrasses in India (Japtap et al., 2003).

Sl. No:	States/Union Territories	No: of Species
1	Gujarat	4
2	Maharashtra	2
3	Goa	2
4	Karnataka	3
5	Kerala	1
6	Lakshadweep	7
7	West Bengal	6
8	Orissa	3
9	Andhra Pradesh	7
10	Tamil Nadu	15
11	Andaman & Nicobar Islands	9

1.4 Seagrasses of Gulf of Mannar and Palk Bay

In India, seagrass populations are relatively higher at Palk Bay and Gulf of Mannar and later showed 11 species (Figure1.1) (Sulochanan, 2012).

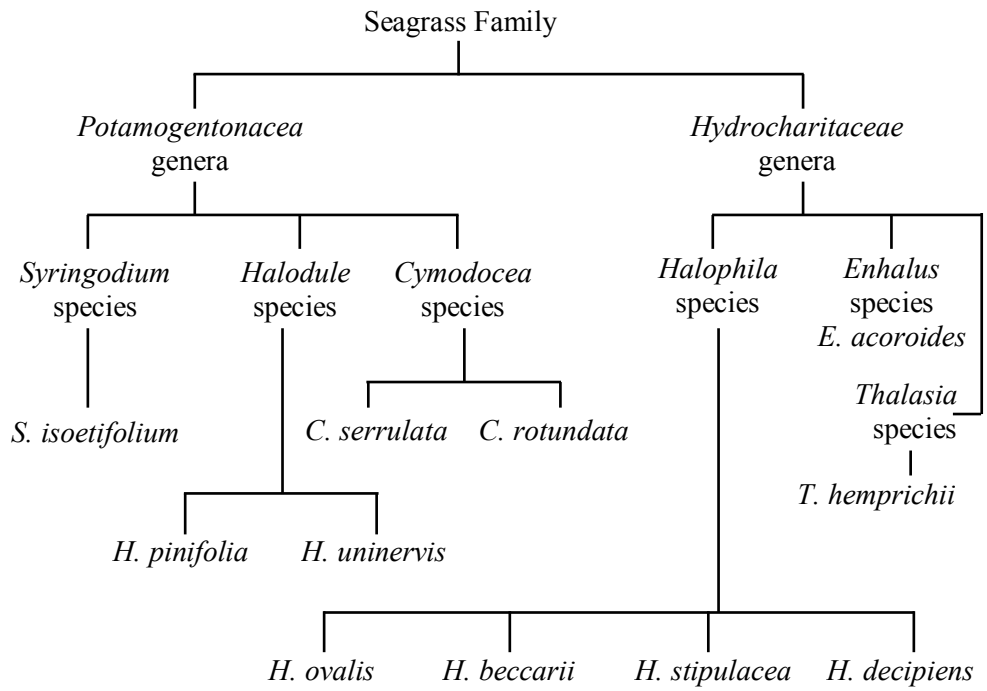


Figure 1.1 Seagrasses of Gulf of Mannar and Palk Bay

1.5 Ecological Importance of Seagrasses

In the presence of sunlight, seagrasses convert carbon dioxide and water to oxygen and carbohydrate, and the generated oxygen are pumped through roots into the sediments (Mckenzie, 2008). It is estimated that approximately 10 litres of oxygen is produced per day from one square meter (Mckenzie, 2008). The fact is that seagrasses distributed only 0.1% in the marine environment contribute to 12% of the organic carbon in the ocean, thereby regulating the global carbon cycle (Borum et al., 2004; Mckenzie, 2008; Rotini et al., 2013). Seagrass beds act as a carbon sink due to its absorbing capacity of carbon dioxide from atmosphere which leads to a decrease in the effects of global warming (Hemminga and Duarte, 2000).

Seagrasses provide the nursery feeding grounds for marine organisms including fishes, mollusks, prawns, crabs, sea horses, green turtles, dugongs, sea urchins, etc. (Jagtap et al., 2003;de la Torre-Castro and Ronnback, 2004; Mckenzie, 2008; Choi et al., 2009; Mani et al., 2012a). It is also reported that more than 40 times marine organisms interact with seagrasses meadows than on bare sand (Borum et al., 2004; Mckenzie, 2008) besides feeding seagrasses directly as well as its detritus. Seagrasses reduce the sediment erosion (Jagtap et al., 2003;de la Torre-Castro and Ronnback, 2004; Mckenzie, 2008; Choi et al., 2009) and increase the clarity of water by settling the suspended materials to the bottom (Borum et al., 2004; Mckenzie, 2008; Rotini et al., 2013). It improves the water quality by uptake of nitrogen and phosphorous in excess from coastal run off, which otherwise lead to algal blooms and acts as an indicator of human pollution in the marine environment (Mckenzie, 2008). Seagrass meadows are responsible for an increase in the primary productivity (Jagtap et al., 2003; Borum et al., 2004) and biomass of the coastal zones and its primary productivity ranged from 0.1 to 8.5gC/day (Jagtap et al., 2003). Seagrasses posses the ability to reduce the speed of waves and change the directions of currents; thereby contribute to decrease in damage intensity at coastal zones due to the action of strong waves (de la Torre-Castro and Ronnback, 2004; Mckenzie, 2008).

The ecological role played by seagrasses includes shelter for marine organisms as well as recycling of nutrients (de la Torre-Castro and Ronnback, 2004; Mani et al., 2012a). Seagrass beds contribute to establishment of biodiversity and habitat diversity of coastal water (Jagtap et al., 2003; Borum et al., 2004). Marine organisms observed in seagrass blades have more than 153 species of microalgae, 359 species of

macroalgae and 178 species of invertebrates as epiphytes and epizooties (Ravikumar et al., 2011). Marine invertebrates are bryozoans, chidarian, sponges and tunicates and these organisms are competing for space with algae (de la Torre-Castro and Ronnback, 2004; Mani et al., 2012a). The three dimensional structure of seagrasses provides hiding places for marine organisms to escape from their predators (Borum et al., 2004; de la Torre-Castro and Ronnback, 2004). Decomposition of seagrasses occur slowly and a major part of carbon is retained for a long periods in sediments. The ecological importances of seagrasses are doubled when both periphytic and benthic algae habitat in seagrasses make seagrasses ecosystem as marine productive system than many agricultural crops and forests on land (Borum et al., 2004).

Migratory birds of brent geese, wigeons and pintails use seagrass meadows for resting and feeding during their travel while others feed on associated fauna (Borum et al., 2004). The particle trapping capacity of seagrasses occur through filter feeding and active capture by the organisms living on the leaves as well as direct attachment to the mucus covered seagrass surface leads to the transparency of the water column. Seagrass meadows have the ability to improve the quality of coastal waters by controlling the suspended dead particles, phytoplankton cells, nutrients, etc. in the water column. Roots and rhizomes of seagrasses provide structural frame network to reduce the resuspension of the sediment by waves and currents (Borum et al., 2004; Haznedaroglu and Zeybeck, 2007). Seagrasses play an important role in erosion of coastal line and protection of the shoreline (Borum et al., 2004; Thangarajou and Kannan, 2007; Mckenzie, 2008; Sridhar et al., 2008). Seagrasses manage the sulfur cycle in the coastal

waters through decomposition process and buffering action of leaves through sedimentation of organic and inorganic materials (Newmaster et al., 2011).

Seagrasses function as natural fish aggregating devices (FAD) and have created ideal fishing grounds. Habitat importance for fishes shows that seagrasses have top priority and followed the order; seagrass>coral Reef > mangroves>seaweed farms. Major fish families in habituating on seagrasses are *Scaridiae*, *Mullidac*, *Labridae* and *Lathrinidae* (de la Torre-Castro and Ronnback, 2004). Seagrass meadows are rich sources of food and serve epiphytes, detritus materials and organisms living on them (de la Torre-Castro and Ronnback, 2004; Rotini et al., 2013). Fishermen often used seagrasses as substrate for bait a mixture of seagrasses and gozi (seagrass sponge mixed with cyanobacteria and diatoms). Also, used as a substrate for seaweed farming, both for positive and negative effects. Seagrass functions as good fertilizers for *Euchema*. Seagrasses are also used for navigation purposes including direction of currents, intensity of currents and indicators of seasons (de la Torre-Castro and Ronnback, 2004).

1.6 Medicinal Aspects of Seagrasses

Seagrasses are a source of antioxidants, antibacterial agents, minerals and anticancer compounds. The medicinal uses of seagrasses are categorized into maintenance of general health, mental disorder, heart diseases, dermatological problems, infections and gastro intestinal diseases (Newmaster et al., 2011). Seagrasses are rich in secondary metabolites which possess antibacterial, antiviral, antifungal and antialgal activities (Jensen et al., 1998; Qi et al., 2008; Kannan et al., 2010a; Marhaeni et al., 2011; Ravikumar

et al., 2011; Mani et al., 2012a & b). *E. acoroides* roots are used as remedy against stings of rays (fishes of *Scorpaenidae* and *Siganidae* spp.) as a tranquillizer for babies (as soothing during pregnancy), against cough and malaria. *Halophila* spp. is reported as effective against malaria and skin diseases and more effective towards early stages of leprosy (Newmaster et al., 2011). Seagrasses of *Thalassia hemprichii* and *Halophila* spp. are used against fever and malaria while *Thalassia ciliatium* used for the treatment of small pox and a mixture of *Thalassia* and *Cymodocea* against fever and skin diseases (de la Torre-Castro and Ronnback, 2004). Seagrasses are used by Seri Indians of Mexico for the treatment of diarrhoea (Felger and Moser, 1985). Seagrasses (leaf litter) are giving good health through prevented respiratory infections during sleeping, alleviation of skin diseases (acne) and pain due to varicose veins (Terrados and Borum, 2004). Newmaster et al. (2011) noticed that seagrasses are used for multiple ailments. In India, fishing communities of Cuddalore and Nagapattinam districts of Tamil Nadu used *H. ovalis* as medicine for the treatment of skin diseases, burns and boils (Kannan et al., 2010b). Chemical constituents isolated from seagrasses include antibiotic flavones glycoside from *T. testudinum*, diterpenes and sugar derivative from *Ruppia maritima*, phenolic compounds from *P. oceanica* and steroids from *Zostera japonica* (Qi et al., 2008). Zosterine polysaccharide, isolated from *Zostera marina* is comparable to two antioxidant drugs (Choi et al., 2009). Phenolic compounds in foliar tissues of seagrasses are used as an indicator of stress level in plants which have application in protecting plants from predators (Agostini et al., 1998).

The leaf extracts of *Enhalus acoroides* exhibited antimicrobial and anticancer activities (Ismail et al., 2012). In India, peel of fresh rhizome skin of *E. acoroides* with a cup of seawater is used against heart disease and low blood pressure (Newmaster et al., 2011). The inhibitory compound zosteric acid isolated from *Zostera* shows biological activity with various commercial applications. L-chiro–inosital (cyclitol) isolated from *Syringodium isoetifolium* is used for nutraceutical ortherapeutic applications due to its hypoglycaemic action (Papenbrock, 2012). *E. acoroides* used against muscle pains, wounds, stomach problems and fever (in the form of mafusho was a smoke of a mixture of plants and herbs) (de la Torre-Castro and Ronnback, 2004). Seagrasses are often used as herbal remedies for rheumatism and skin ailments (Karleskint et al., 2009). Seagrasses are rich source of dietary fiber contents. High dietary fiber foods are significant in low prevalence of colon cancer, ischemic heart diseases, diabetes mellitus, gallstones, hemorrhoids and hiatus hernia (Papas, 1999; Elleuch et al., 2011).

1.7 Commercial Importance and Economic Value of Seagrasses

The awareness of the economic value of seagrasses to humans increased towards twentieth century and it was 1583 ha^{-1} Y^{-1}, two orders of magnitude higher than crop lands (Borum et al., 2004). In 19^{th} (end) and 20^{th} (beginning) century dried flotsam of *Z. marina* is used for making mattress, padding material, erosion protection mat and insulation (Papenbrock, 2012). The global value of seagrasses for their nutrient cycling was US$19,004 $ha^{-1}Y^{-1}$ (average) during the year 1994 and it was more valuable than mangroves as well as coral Reefs (Mckenzie, 2008). Nutrient cycling functions in seagrass beds are valued at US$3.8 trillion and it is the second highest among all the ecosystem values listed (Preen et al., 1995). A value of 2-6.3 million baht

(Thai Bhat) per year in terms of fishery yield (net benefit) was determined for one seagrass bed in Southern Thailand. The economic value of seagrases in South China has been estimated as US$ 16,640-18,385 per hectare. In Vietnam one village surveyed obtained revenue of US$ 23,000 in a six month period from harvesting seagrasses for sale to the agricultural sector (UNEP, 2004).

Seagrasses are transplanted at dredging areas to stabilize the sediments after the dredging (McRoy and Helfferich, 1977). *Z. marina* (dried leaves) is used to make dolls (Felger and Moser, 1973) and also used for cultured studies and lives for more than two years. Dried seagrass is used for house insulation and also in sound-proof radio studios of UK and USA due to its non-flammable (high silicon content), thermal and sound proofing properties (Hurley, 1990). People living on the coast are used seagrasses as fertilizer for coconut and tobacco plantations (de la Torre-Castro and Ronnback, 2004; Newmaster et al., 2011). Leaves of *Cymodocea serrulata* and *T. hemprichii* are used by fishermen as insulation to keep stored ice and fish. Also, seagrasses are used as green manure for gardens (Newmaster et al., 2011). Seagrasses are used to bind clay and soil in embankments and an erosion resistant mat for the purpose of seed germination in sand (Walker, 1977). *Z. marina* is used as packing material in stuffing pillows, mattresses and upholstery instead of horse hair, while in crab industry (Chesapeake Bay) for exporting crabs from the region (Hurley, 1990). Seagrass fibres enhance water and oil retention as well as improve emulsion and oxidative stability (Elleuch et al., 2011). Seri Indians living along the Gulf of California harvested carbohydrate rich flour from seeds of *Z. marina* and used in different dishes (Hemminga and Duarte, 2000).

P. oceanica along with lime and phosphate are used as a meal for feeding poultry in Mediterranean countries and South Australia. Seagrasses are used as a source of boron, salt (soda minerals) and garden mulch (Stewart and Mills, 1975). During seventeenth century, fibres of seagrasses are used for leaks in ship hulls (Hurley, 1990). Fibres of seagrasses are used for basket weaving, coir mats, paper making, rugs sold and substitute for nitrocellulose (during Second World War in Germany). *Phyllospadix iwatensis* is used for making wet weather gear by fishermen of Japan up to 1930 (McRoy and Helfferich, 1980). Seagrasses has importance in traditional beliefs and used for both positive and negative activities. Conflict resolution, love problems and work problems under positive while, negative is to harm someone. Also used in the rituals and unes against ghosts and devils, and necklaces used for babies against nightmares. Fishermen in boats used seagrasses for knowing the direction of wind. People living along the coast used seagrasses of *T. hemprichii*, *C. serrulata* and *S. isoetifolium* as feed for animals mainly goats and pigs, along with few species which have an importance in their culture (de la Torre-Castro and Ronnback, 2004). *C. serrulata* and *S. isoetifolium* have the potential for inhibiting the growth of marine biofilm forming bacteria and they can be used as alternative to commercially available metal based antifouling coatings (Marhaeni et al., 2011). In Mediterranean countries, leaves of *P. oceanica* are used as packing material for the transport of fragile materials while the straws are used for cattle bedding in stables, filling materials for mattresses and cushions and also, seagrass are used to transport fresh fish from coastal to cities (Terrados and Borum, 2004).

1.8 Importance of Seagrasses on Coastal Bio Geochemistry

Seagrass meadows create an interface between the water column and sediment in tidal as well as sub tidal environments by providing support to large biomasses below and above the ground (Duarte and Chiscano, 1999). The physical and chemical conditions of the water column and sediments are strongly influenced by the metabolic activities and structure of seagrass beds (Benner et al., 1986; Mateo et al., 2003), besides affecting the carbon and nutrient dynamics, along with level of oxygen in the water column and sediments (Borum et al., 2005). The coastal geochemistry of seagrass beds are controlled by the process of mineralization of organic matter and regeneration of nutrients (Jones et al., 1997). These processes in sediments lead to an increase in the quantity of microbial population (Gacia and Duarte, 2001). During carbon fixation process in seagrasses, a part of it is translocated from leaves to below ground organs and a fraction of it is released as dissolved organic carbon (DOC) to the sediments, the release of which vary with respect to season (Ziegler and Benner, 1999). By photosynthesis seagrasses reduce the concentration of dissolved inorganic carbon and increases dissolved oxygen in the water column, thereby regulating the two parameters in the growing coastal area (Erftemeijer and Middelburg, 1993).

The net leaf production of seagrasses of *Amphibolis antarctica* was varied with nutrient enrichment mainly with dissolved inorganic nitrogen (Connell et al., 2017). Seagrass plays an important role in sediment nitrogen cycling and production of ammonium through mineralization of alochthonous particulate organic material trapped within the meadows, microbial breakdown of dissolved organic nitrogen released from plant roots and decomposition of senescent plant material (Smith et al., 1988;

Pedersen et al., 1999; Holmer et al., 2001). Sulfate reduction process in marine sediments occurs due to high sulfate concentrations in seawater and its rate are stimulated by seagrasses (Nielsen et al., 2001; Holmer et al., 2005). High rate of sulphate reduction leads to an increase in the concentration of sulphide in pore waters (Holmer et al., 2005). Seagrass beds act as a source of sink of phosphorous and recycled within the beds during colonization and development (Pedersen et al., 1997).

1.9 Importance of Seagrasses on Fish and Fisheries

Comparatively high primary and secondary productivity of seagrass meadows support large varieties of fishes and invertebrates including both commercially and recreationally important species. Non-commercial species living on seagrasses act as a food source for commercial species and thereby forming trophic linkages between them (Bell et al., 2002). Seagrass meadows provide shelter for fish and fisheries. Juveniles are more attracted due to an increased food availability and protection from predators due to the structural complexity of seagrass beds and their distance from reef habitats (Heck et al., 1997). Seagrass beds used by fishes are categorized into three and they are permanent, rare occasions and temporary residents. Seagrass beds play an important role in feeding habitats for juvenile and adult fishes, and major prey from seagrass beds are crustaceans for both omnivores and carnivorous. In addition to this seagrass provides habitats for migratory predatory fishes (Hindell et al., 2000). Enhanced diversity, abundance of prey, increased microhabitat availability, reduced risk of predation and hydodyanamic effects on larval supply are the reasons for varieties of species and its richness in seagrass

habitats than nearby non-vegetated zone (Jordan et al., 1997; Jenkins and Hamer, 2001; Heck et al., 2003). Survival as well a growth rates of juvenile fishes and invertebrates are higher in seagrass habitats than unstructured (Heck et al., 2003).

Comparing the habitats of seagrasses with algae showed that relatively large numbers of fishes are present at seagrasses whereas the quantities of fishes at algae and seagrass meadows showed higher than non-vegetated habitats. Assemblages of animals in seagrasses and algae revealed a difference in habitats and comparison with over structured bare sand or mud, an entirely different assemblage have been observed with respect to seagrasses and algae (Guidetti, 2000). A common similarity found in seagrasses as well as algae habitats is in the levels of crustaceans (Haywood et al., 1995). Comparatively higher densities of juvenile fishes are noticed at mangrove habitats than seagrasses and also abundance in species as well as quantities are observed at seagrass beds near to mangroves than seagrass meadows alone (Nagelkerken and van der Velde, 2002; Sheridan and Hays, 2003), and noticeable differences in assemblages were obtained between seagrasses and mangroves habitats (Laegdsgaard and Johnson, 1995). Relatively higher quantities of fishes and invertebrates of different species are also found at non-vegetated habitats near seagrassses and mangroves, and it might be due to the migratory behavior of organisms from high tide to low tide (Nagelkerken et al., 2001). Different species of fishes are using salt marshes (as nursery grounds) and their levels are less than seagrass habitats (Minello et al., 2003); while no differences in the total number of fishes are noticed between salt marshes and seagrasses (Rozas and Minello, 1998) and also the survival of animals (Heck et al., 2003).

1.10 Importance of Phenolics of Seagrasses

The antioxidant activities in plants have been attributed to the presence of phenolic compounds, flavonoids, alpha-tocopherol and carotenoids. Phenolic compounds have multiple functions including protection of cells from oxidative damage, major signaling pathways of cells, etc. The oxidative damage causing species are superoxide radical, hydroxyl radical, peroxy radical and nitric acid radical, and are generated in living organisms due to excessive metabolisms which leads to age related degenerative diseases, cancer and wide range of other human diseases (Prakash et al., 2007; Bilto et al., 2012). Among the polyphenols, phenolic acids are the major component produced by plants. Phenolics in seagrasses are performed as protection of the plant against photosynthetic stress, reactive oxygen, antropogenic pressures and inter specific competition. It is reported that almost 20 compounds have been detected in *P. oceanica* belongs to phenolic and cinnamic acid derivatives. Marine ecosystems are enriched with tannins and related phenolic compounds produced by submerged vascular plants, emergent salt marsh vegetation, mangroves and brown algae as antimicrobial agents. Phenolics in seagrasses as phenolic acids, sulfated phenolic acids, phenolic acids conjugates, flavones, tannins (condensed not hydrolysable) and lignin, are varied widely and dependent upon species, population and tissue. Leaves and shoots of seagrasses accumulate significant levels of condensed tannins (proanthocyanins). Phenolic acids act as antimicrobial agents, herbivore deterrents and osmoregulatory agents. Also, phenolics of seagrasses are resistant towards wasting disease, disease leads to a rapid population declines, and its resistant levels are related to level of phenolics present in leaves as well as

shoots (Arnold and Targett, 2002). An increase in carbon dioxide level can trigger accumulation of phenolic compounds in plants such as lignins, tannins, phenolic acids and glycosides, and they are involved against defensive mechanisms as well as disease organisms (Arnold et al., 2012). Marine plants are a rich source of structurally diverse bio active compounds of pharmaceutical potential (Kannan et al., 2013c).

Most of the plant phenolics are produced through shikimic acid (SA) and phenylpropenoid (PP) pathways. SA pathways are obtained in fungi, bacteria and vascular plants, and it starts from the condensation of the carbohydrate intermediates erythrose-4-phosphate and phosphophenol pyruvate to chorismate followed by aromatic amino acids of phenyl alanine, tyrosine and tryptophan. These amino acids are either used for protein biosynthesis or poly phenolics production via pp pathways. The production of phenyl propenoids (C6-C3 compounds) arises from the deamination of aromatic amino acids which leads to the formation of cinnamic and p-coumaric acids, and from there more complex phenolic compounds are produced (Arnold and Targett, 2002). Flavonoids accumulate in epidermal cells of plant organs namely flowers, leaves, stems, seeds and fruits; usually occur as glycosides and glycones (non-glycoside) (Sakihama et al., 2002).

Phenolic compounds exhibit antioxidant, antimutagenic, antiviral, antibacterial, algicidal, antifungal, antiinsecticidal, estrogenic and keratolytic activities irrespective of their origins. Hydroxyl groups present in phenolics act as good H (hydrogen) donating antioxidants which scavenge reactive oxygen species and inhibit generation of new radicals. The radical scavenging property retards free radicals operated oxidation of lipids,

proteins as well as DNA, which leads to illness. Phenolics also break enzymes which involve in the generation of radicals (Castellano et al., 2012). Phenolic compounds act as key signaling molecules, an alternative for carbon resources (in some diazotrophs) and precursors for the synthesis of phenolic lipids (Awstini et al., 1998). Mono or polyhydroxy phenolic compounds have low activation energy to donate hydrogen, and resulting antioxidant radicals does not initiate further chain propagation and initiation due to the stability of the resulting product. Moreover, the antioxidant free radicals interact with lipid free radicals to form more stable complex which are more resistant towards oxidation. PUFAs present in phospholipids are protected by the flavonoids via donating the hydrogen atom thereby quenching of lipid peroxyl radicals (Hamid et al., 2010).

Phenolic compounds present in seagrasses in trace amounts are caffeic and gallic acid (Castellano et al., 2012). Flavonoids are involved directly in photo protection against high solar irradiance by absorbing incident photons (act as sunscreens) and it might due to its antioxidant activity. Among the different forms of flavonoids, cytosolic flavonoids served as effective antioxidants whereas cuticular, vacuolar and cell-wall bound flavonoids are important in shielding chloroplasts from excess high energy quanta, and concentrations of flavonoids show changes due to abiotic stress (Gavin and Durako, 2011). Flavonoid variations are more with respect to salinity than light quality (Gavin and Durako, 2012). Phenolic compounds act as enzymatic inhibitors and they are tannins complexed with proteins, used as an indicator of stress induced by both intra and inter-specific competition as well as abiotic factors of nutrient limitation, pollution by heavy metals, temperature changes, etc. (Dumay et al., 2004).

1.11 Review of Literature

Biochemical evaluation of seagrasses

Seagrasses are marine flowering plants usually seen at near shore environments of most of the World continents. They have the ability to survive at fresh water, estuarine, marine and hyper saline conditions. Seagrasses, included under the family *Hydrocaritaceae* and *Zosteraceae*, are evolved from single monocotyledons flowering plants between 70 million and 100 million years ago (Sulochanan, 2012). Seagrasses (<0.02% of the angiosperm flora) occupied 0.1% of the ocean floor (Short et al., 2007). Many of the key ecological and physiological attributes of natural populations, viz., rates of reproduction, recruitment, growth, energy flow, and the level of environmental stress to which organisms are exposed, and changes in biochemical and molecular properties are reflected directly in the physiology of organisms. A study on the biochemical evaluation of seagrass implies the possibility of seagrasses as a source of nutrient in the present era.

Elemental composition

The dominant role of nutrient availability, primarily nitrogen (N) and phosphorous (P), in controlling the growth and abundance of phytoplankton, macroalgae and marine and freshwater angiosperms have been established unambiguously. The elemental composition of seagrass *T. testudinum* leaf revealed deviations in P content than carbon (C) and N (Campbell et al., 2012). Comparatively higher concentrations of N and P were observed in leaves followed by roots and rhizomes in *H. uninervis* whereas in *S. isoetifolium*, N content predominated in leaves followed by rhizomes.

However, no remarkable differences were observed between leaves and rhizomes in the case of P (Udy et al., 1999). Relatively lower concentrations of C and N content obtained respectively for *S. isoetifolium* and *H. uninervis* and higher at *T. ciliatum* and *C. rotundata* (Mariani and Alcoverro, 1999). The average C, P and N content in 27 seagrass species showed that leaves were a good source of major elements with 36% carbon, 1.5% nitrogen and 0.2% phosphorous and variations were related to surrounding environmental conditions (Duarte, 1990). Total C content in rooted aquatic plants revealed that relatively higher C and N content found in these species, and species to species variations were noticed more in N content than carbon (Spencer and Ksander, 1994).

The elemental composition of seagrass *P. oceanica* leaves were reported to be abundant in P content whereas in leaf litter (banquette) C and N content are predominated (Mateo et al., 2003). Elemental composition in the leaf blades of seagrasses contained higher contents of C, P and N than leaf sheath. The roots and rhizomes contained lower C, P and N than leaf, and the differences were more in the case of P and N content. Relatively higher concentrations of these major elements were observed in seagrass species showing slow grow rate than fast growing seagrass species (Yamamuro and Chirapart, 2005). The decay kinetics of C, P and N content in scales and rhizomes in *P. oceanic*a showed that C loss with time was very lower in rhizomes and scales, while a sharp decrease was observed in P and N content in rhizomes whereas in scales remained constant (N) or increased (P) (Romeo et al., 1995). Relatively higher concentrations of P were found in *E. acoroides* from Philippines (Montano et al., 1999). Elemental components in a sulphated polysaccharide isolated from *H. pinifolia*

contained 18.25% of C and 1.77% N with a C/N ratio of 2.04 (Kannan et al., 2013c). Comparatively higher P, N and C contents were reported in *P. oceanic*a than *C. nodosa*, and variations were observed between stations as well as species (El Din and El-Sherif, 2013).

Biochemical constituents

Marine lipids are a valuable tool to measure inputs, carbon cycling, transfer of materials, loss of materials, and their heterogeneous nature makes them versatile biomarkers. Proteins present in all cells are an important source of energy and are an essential component of most biochemical processes playing key roles in important metabolic pathways, and associated with maintenance, growth, reproduction and immunity. Marine carbohydrates are most important organic molecules synthesised by photosynthetic organisms and the sulphated polysaccharide find use in cosmeceutical industry and biological applications. Protein contents in leaves of seagrasses from Palk Bay were lower in *H. beccarii* and higher at *E. acoroides*. Relatively lower protein concentrations were observed in leaves and rhizomes during monsoon and higher at summer while lipid content of seagrass leaves were lower concentrations than rhizomes. Similarly carbohydrate concentrations were lower in leaves of seagrasses in comparison with rhizomes, and both lipid and carbohydrate content variations were noticed with respect to season. Lipid also followed highest concentrations during summer and lowest at monsoon seasons (Pradheeba et al., 2011). Wide variations in the biochemical constituents of seagrasses were observed between species with higher variations in protein and carbohydrate content. In comparison with seaweeds, seagrasses contained low amount of carbohydrates and lipids while protein content predominated

over seaweeds (Athiperumalsami et al., 2008). Species to species variations were negligible in carbohydrate and lipid while slight variation was observed in the case of protein (Kannan et al., 2010a). Lipid content in seagrasses showed slow growth rate with less variation in leaf blade while leaf sheath contained relatively high quantity (Yamamuro and Chirapart, 2005).

Protein content in *T. testudinum* ranged from 0.30 to 4.12% while carbohydrate varied from 6.7 to 26.78% (Hernández et al., 2016). Crude protein level in marine plants of South Australian beaches displayed species wise variations (Torbatinejad and Sabine, 2001). *R. cirrhosa* contained higher protein concentration whereas *C. nodosa* showed higher carbohydrate and lipid content (Abd El-Hady et al., 2007). Crude protein content in macro algae and seagrasses revealed species wise variations (Serviere-Zaragoza et al., 2002). Relatively higher concentrations of protein and lipid noticed in leaves than rest of the body parts and showed variations with respect to region and time of sampling (Dawes et al., 1987). Comparatively higher concentrations of biochemical constituents were found in the seeds of *E. acoroides* and the composition of starch was comparable to common starch available in nature. *E. acoroides* seeds flour contained slightly less protein and fat and more carbohydrate than *Z. marina* (Montano et al., 1999). Biochemical compositions of the seagrasses of *C. nodosa* and *P. oceanica* followed the order: protein>lipid>carbohydrate (El Din and El-Sherif, 2013).

Phenolics

Seagrasses are a rich source of secondary metabolites (Choi et al., 2009; Mani et al., 2012 a&b; Girija et al., 2013). There are reports indicating relatively higher total phenol content during summer than winter season in the rhizomes of *P. oceanica* (Rotini et al., 2013). A comparison between leaves and rhizomes indicated that rhizomes exhibit long life time and less affected by common changes in the physiological process (Migliore et al., 2007). Evaluation of phenol content in the leaves and rhizomes of seagrasses from Palk Bay indicated relatively lower concentrations in *H. beccarii* and higher in *E. acoroides*, and a positive correlation was noticed between phenol and tannin contents (Pradheeba et al., 2011). Phenolic compounds from seagrasses varied from species to species, and higher extractions were reported in the methanolic extract in *H. pinifolia* and lowest in hexane fraction (Girija et al., 2013). Similar results indicating high extraction of phenolics in methanol followed by ethyl acetate were reported from Indonesia (Santoso et al., 2012).

It is also reported that phenolic acid content in the vegetative propagules were at higher concentrations and also species to species variations were observed in the phenolic acid content (Spencer and Ksander, 1994; Rengasamy et al., 2012). The predominated phenolic acid in *Z. noltii* fresh leaves were rosmaric acid (RA) followed by Zosteric acid (ZA) and Caffeic acid (CAF), and followed the order; RA>CAF>ZA (Grignon-Dubois et al., 2012). The total phenol content in the seagrasses of Gulf of Mannar followed the order: *C. serrulata>S. isoetifolium> H. pinifolia>H. ovalis* (Athiperumalsami et al., 2008). Further, the total phenolic content was higher in the intermediate leaves than adult at all the

stations (Dumay et al., 2004). Phenolic content in seagrasses of Gulf of Mannar followed the order: *H. pinifolia*>*C. serrulata*>*C. rotundata*> *S. isoetifolium*>*T. hemprichii*>*E. acoroides* (Kannan et al., 2013b; 2010c). However, *E. acoroides* from Mandapam coast contained relatively higher concentrations of phenol at leaf followed by root and rhizome (Kannan et al., 2010b). Total phenolic content in *H. uninervis* was 20.17µg/g (Supriadi et al., 2016). Polyphenolics in *T. testudinum* ranged from 7.19 to 58.81mg/g (Hernández et al., 2016). There are reports indicating that total phenolics in seagrasses of Palk Bay ranged from 3.77 to 15.38mgGAE/g in leaves while 2.08 to 16.26mgGAE/g in rhizomes (Jeyapragash et al., 2016).

The aqueous crude extract of *Z. noltii* showed higher flavonoid content with Apigenin 7-sulfate and Diosmetin 7-sulphate were found to be predominating (Grignon-Dubois and Rezzonico, 2012). Flavonoid level in seagrasses of Gulf of Mannar followed the order: *C. serrulata*> *C. rotundata*>*T. hemprichii*>*S. isoetifolium*>*E. acoroides*>*H. pinifolia* (Kannan et al., 2013b). Also species wise variations in total flavonoid content in seagrasses were noticed (Ramah et al., 2014). Flavonoids in *T. testudinum* ranged from 9.47 to 51.30mg/g (Hernández et al., 2016). Comparatively higher concentrations of tannin observed in leaves and rhizomes of *E. acoroides* while lower concentrations at *H. uninervis* and *H. pinifolia* respectively for leaves and rhizomes (Pradheeba et al., 2011). Similarly the tannin content also varied between species (Athiperumalsami et al., 2008; Pradheeba et al., 2011; Kannan et al., 2013b). Lower content of tannin obtained at green and fresh leaves while high at dry washed leaves (Torbatinejad et al., 2007; Torbatinejad and Sabine, 2001). Tannin content in *H. uninervis* was 1.22mg/g (Supriadi et al., 2016).

Chlorophyll pigments

Chlorophyll a and b are the major pigments reported in seagrasses. No wide variations were observed between control and salt-stressed plants in the total pigments (Marin-Guirao et al., 2013). Chlorophyll (chl) concentration in seagrasses biofilm of *E. acoroides*, *T. hemprichii* and *T. ciliatium* showed no significant differences both species and locations wise (Daudi et al., 2012). Relatively higher concentrations of total chl contents in the leaves of *T. testudinum* were reported which varied with respect to seasons (Lee and Dunton, 1996). Chlorophyll a content in *Cymodocea nodosa* and *Ruppia cirrhosa* demonstrated higher concentration in *C. nodosa* than *R. cirrhosa* (Abd El-Hady et al., 2007). Total Chl content in the leaves of *P. oceanica* increased from summer to winter and was lower during summer (Central) (Rotini et al., 2013). Seagrass with broad leaf showed higher chl content than cylindrical as well as linear leaf (Pradheeba et al., 2011). Photosynthetic pigments in the leaves of three seagrasses followed the order: *H. stipulacea*>*C. serrulata*> *H. pinifolia* (Kannan et al., 2010a). In certain other species it was reported that chl a content followed the order; H. *pinifolia*>H. *ovalis*>*C. serrulata*> *S. isoetifolium*; and while chl b followed the order *H. pinifolia*> *H. ovalis*> *C. serrulata*> *S. isoetifolium* (Athiperumalsami et al., 2008). Chl a and b in *T. testudinum* was 0.26-1.52 and 0.43-1.56µg/g DW respectively and a positive correlation of chls with protein was observed (Hernández et al., 2016). It also reported that chl a predominated over chl b in seagrass leaves collected from Palk Bay (Pradheeba et al., 2011). Total chl content was not strongly influenced by pH and temperature while chl a and b concentrations were decreased with increase in pH and temperature (Repolho et al., 2017).

Macro elements

Macro elements, in general, play an important role in metabolism of a living being; whether it is a plant or an animal. The level of macro elements in seagrasses of *H. stipulacea*, *C. nodosa* and *P. oceanica* demonstrated that their distribution pattern showed high content of Na followed by Ca, K and Mg (Malea, 1998). The leaves of *P. australis* from the West coast of Australia followed the order: Na>K>Ca>Mg (Hocking et al., 1980). *P. oceanica* leaves of Tunisia recorded the distribution of macro elements as Mg>Ca>K (Saidane et al., 1979). The Mg content in three seagrass species from Gulf of Aqaba (Jordan) revealed that its concentration in leaf is in the order of *H. stipulacea>H. ovali>H. uninervis*. Mg content in different body parts followed the order: leaves>roots>rhizomes at both *H. stipulacea* and *H. ovalis* (Wahbeh, 1984). Magnesium followed by Na, K and Ca in *H. beccarii* along the central West coast of India (Jagtap, 1983). Macro elements of Na, K, Ca and Mg in six species of seagrasses from Gulf of Mannar indicated that these seagrasses have been regarded as a good source of macro elements, and the elemental composition in seagrasses followed the order; Mg>Na>K>Ca except in *C. serrulata* and *Cymodocea rotundata* (Mg>Na>Ca>K) (Kannan et al., 2011a). There are reports indicating seasonal variations in the concentrations of minerals in different body parts of selected seagrasses of *C. serrulata*, *H. ovalis*, *H. pinifolia* and *S. isoetifolium* from Tuticorin Bay (Jeevitha et al., 2013). Among the macro elements Na, Ca, Mg and K in *Z. marina* showed seasonal variations and it was more in Na (Lyngby and Brix, 1983). Relatively higher concentration of Ca was found in the seeds of *E. acoroides* from Philippines (Montano et al., 1999). Major elements in the leaves of *C. nodosa* and *P. oceanica*

revealed that Na and Ca were predominated in *P. oceanica* while K in *C. nodosa* (El Din and El-Sherif, 2013).

Heavy metals

Greater variations in heavy metals were noticed in seagrasses due to their direct exposure from the surrounding ecosystem. The heavy metal concentrations in seagrass of *Zostera capricorni* from Illwara Lake showed that comparatively high concentrations were noticed in leaves than rhizomes (Howley et al., 2004). Heavy metals Mn, Fe, Cu and Zn in seagrass of *C. serrulata* and *S. isoetifolium* from southwest coast of India revealed that higher accumulation of heavy metals in selected stations, and *C. serrulata* contained higher quantities of metals compared to *S. isoetifolium* (Govindasamy et al., 2011). However, it is also revealed that seagrasses contained relatively lower levels of heavy metals compared to seaweeds, and among the stations, comparatively higher concentration of heavy metals noticed from Thondi station (Sudharsan et al., 2012). The heavy metal analyses of seagrass species of Lakshadweep Islands showed higher concentration of Mg and Al (Thangaradjou et al., 2013). Heavy metal dynamics in seagrasses and seaweeds in Magdalena Bay of Mexico showed that Fe, Cu and Mg were the most significant metals found in seagrasses, red and blue algae (Riosmena-Rodríguez et al., 2010). Heavy metal contents in marine macrophytes and sediments along the Mediterranean coast of Spain revealed that a bio monitoring capacity is established between the marine macrophytes and sediments except Cd (Sanchiz et al., 2000).

Accumulation pattern of metals viz; Cd, Pb and Zn between sediments, marine animals and seagrasses demonstrated relatively higher

concentration in fishes and seagrasses, and followed the order; Zn>Cd>Pb. Bioaccumulation of metals were reported in many species even though no biomagnifications processes of these metals could be identified (Ward et al., 1986). There are reports indicating significant variations in Cu, Pb, Fe and Ni content between seasons and locations with respect to sediment. It is noted that Ni and Cu content in *H. ununervis* tissues were significantly higher than sediment while a reverse trend was noticed with respect to Pb content (Al-Bader et al., 2014; Hosokawa et al., 2016). A correlation study between the heavy metals in sediments, seagrass and mussels showed that metal concentrations in *P. oceanica* leaves followed the order: Cr>Cu>Pb> Cd while it was Cu>Pb≤Cd>Cr in the tissue of mussel, *Mytilus galloprovinciallis*. Sediment correlated more with the tissue of mussel than leaves of seagrass and Cd showed higher concentration in *P. oceanica* compared to sediment. A positive correlation was observed in *P. oceanica* with sediment and the correlation level was lower than mussel. The heavy metals content in organisms living in the same environment revealed that the persistent pollutants present in their body were influenced not only by their concentration in the sediment but also strongly related with free cations as well as their rapid release into the water column (Malltezi et al., 2012).

Biomagnifications as well as bioaccumulation of heavy metals in sediment, seagrass and gastropods recorded a significant direct relation to the pollution in the ecosystem. Comparatively higher concentrations of Co, Mn, Ni and Zn obtained from the leaves of *C. nodosa* than roots and stems while in gastropods, viscera contained high content of metals than in the muscle except Cd (Nicolaidou and Nott, 1998). Effect of trace metals of Cd, Zn, Pb and Cu in *T. hemprichii* exposed to various concentrations for a

period of 10 days demonstrated a breakdown of photosynthetic parameters to a remarkable extend at higher concentrations. Chlorophyll as well as carotenoid levels decreased by a continuous exposure of Cu, and the order of depression was higher for Cu and Zn than Pb and Cd. *T. hemprichii* displayed a positive response against the antioxidant enzymes, and was due to the protection of seagrass against the stress raised by Zn, Cd and Pb (Lei et al., 2012). Metal accumulation in *P. oceanica* from Benghazi coast in Libya contained high content of Zn, Cd and Pb and the study demonstrates *P. oceanica* as bio monitors for trace metals (Benkhayal et al., 2013).

Relatively higher concentrations of heavy metals exhibited nearer to anthropogenic source and a positively significant correlation was obtained in seagrasses with the environments. Significantly higher concentrations of Cd and Zn were observed in blades compared to roots and rhizomes in *T. hemprichii* and *E. acoroides*, and also exhibited a relation with respect to season. The variation in trace metal concentrations were attributed due to the differences in the uptake of metals in different species and also to the bioavailability (Lei and Xiaoping, 2012). Trace metals in *T. testudinum* from Mexican Caribbean coast showed lower concentrations at most of the sites and its accumulation in leaves increased during rainy season (Sol´ıs et al., 2008). Trace metals in the sheaths (dead tissue) and leaves in *P. oceanica* revealed no significant temporal variations in metals except Zn, Cd and Pb which recorded a decreasing level (Gosselin et al., 2006). Trace metals content in *Z. noltii* from Sinop coast of the Black Sea followed the trend; Fe>Zn>Cu>As=Pb>Hg (Bat et al., 2016). Heavy metals content in the shoots and roots of *Z. japonica* followed the order; Mn>Zn>Pb>As> Cu>Cr>Cd>Hg (Lin et al., 2016).

Distribution of heavy metals in different body parts of *P. oceanica* exhibited comparatively higher concentrations of Hg, Pb, Sn and Cu in roots whereas the lowest concentration of metals noticed at the basal part of the leaf and Hg showed a correlation between the roots and sediments (Leo et al., 2013). A comparative study of seagrasses with sediment, water, mussel (*M. galloprovincialis*) showed that Cd, Co, Cr, Hg, Ni and Pb displayed a positive correlation between water and sediment with *P. oceanica* (Lafabrie et al., 2007). The distribution of trace metals showed differences in their concentration during the development stages of leaves (Cozza et al., 2013). Comparatively high concentrations of heavy metals were noticed in *Z. marina* roots than rhizomes of different ages. Among the rhizomes, Cr and Zn concentrations were high in youngest part than oldest, while vice versa for Pb content, and for Cd there was no variation in distribution. Cd, Pb and Zn contents increased with age of the leaves, whereas Cr content decreased with age of leaves. Also it can be noted that the heavy metal concentrations in leaves were greater than stem fraction. The decomposition of leaf materials led to an increase in the concentrations of Cr, Pb and Zn significantly in the surrounding water (Lyngby and Brix, 1989).

An investigation on the relation between sediment, seagrass and dugongs indicated higher concentrations of Cr, Fe, Mn, Ni and Pb in sediments than seagrasses while the reverse order in the case of Cd and Hg. The concentrations of heavy metals in liver of dugongs were found to be lower and most of the metals except Cr and Hg displayed a positive correlation with sediments (Haynes, 2001). Comparatively lower concentrations of heavy metals were reported in corns than leaves of *E. acoroides* and followed the order; Fe>Zn>Ca>Cd>Hg>Pb (Suwandana et al., 2011).

Heavy metal cycling in seagrass *Z. noltii* with their surrounding environments and organisms indicated that metals concentration followed the order; Fe>Mn>Zn>Cu for water, seagrasses and epiphytes while sediment followed Fe>Zn>Mn>Cu with comparatively higher concentrations of metals obtained at epiphytes than seagrasses. Cu and Fe contents were relatively higher at roots and rhizomes whereas Zn and Mn in leaves in the case of *Z. noltii* (Wasserman and Wasserman, 2002). Heavy metal contents in seagrasses of Andaman Islands showed comparatively high level of Mn, Fe and Al whereas Co, Pb, Cd and Ni were at lower concentration (Thangaradjou et al., 2010).

Biochemical profile of seagrasses

Fatty acid profile

Seagrasses in general, demonstrated a composition very similar to terrestrial plants. The chemical composition of *Z. marina* and *Z. nana* from Black Sea revealed lipids characteristic for terrestrial plants (Khotimchenko, 1993; Milkova et al., 1995) with predominating glycolipids. The fatty acid composition of seagrasses was more similar to terrestrial plants than algae (Milkova et al., 1995). PUFAs predominated over SFAs and MUFAs, and major FAs were linoleic acid and alpha-linolenic acid (Khotimchenko, 1993). Major lipid components in the leaves as well as roots of *T. hemprichii* demonstrated sterols, alcohols, hydrocarbons and fatty acids (Nichol and Johns, 1985). Major lipid classes noticed in marine macrophytes of seaweeds and seagrasses were phospho and glyco lipids, and *Z. marina* fatty acis composition showed that it as a source of PUFAs of both ω-3 and ω-6 (Popov and Krivoshapko, 2013). Constituents of crude methanolic exctract of *Zostera japanica* were palmitic acid, oleic acid and linoleic acid

and its methyl ester (Hua et al., 2006). FA profiles of *Zostera noltii* showed marginal differences with epiphytes, microphytobenthos, suspended soluble organic matter and suspended particulate organic matter during summer and winter season. The most abundant FAs in the leaves and roots were C16:0, C18:3n-3, C18:2n-6 and C18:1n-9c with considerable amount of C24:0 in roots of *Z. noltii* during winter (Lebreton et al., 2011). Major FAs present in *Z. marina* were C16:0, C18:2n-6 and C18:3n-3, and PUFAs predominated over SFAs and MUFAs (Sanina et al., 2004). PUFAs of stearidonic acid exhibit commercial importance and were concentrated in glycolipisds (Callaway et al., 1996; Hong et al., 2002; Khotimochenko, 2003). FA composition of food sources of amphipods exhibited relatively higher PUFA content in fresh leaves than litters of *P. oceanica* and major FAs were C16:0, C18:0, C20:5n-3, C20:4n-6, C16:1n-7and C18:1n-9 (Michel et al., 2015).

Major PUFAs were 18:2n-6 and 18:3n-3 whereas SFAs were C16:0 and C18:0, and C18:1n-9 was the major MUFA (Gillan et al., 1984; Dembitsky et al., 1991; Milkova et al., 1995; Gonocharova et al., 2000; Khotimchenko, 2003; Sanina et al., 2004, 2008). PUFAs and MUFAs in *Z. marina* were showed relatively lower concentration in phospholipids and higher in glycolipids while SFAs varied from glycolipids to phospholipids. Wide variations were observed in the content of C16:3n-3. The major PUFAs were C18:3n-3, C18:2n-6 and C16:3n-3 and among these C18:3n-3 dominated (Gonocharova et al., 2000). In *T. hemprichii* major differences were observed in C18:3<9>, C18:2<9>, C18:0, C16:0, C16:1<9> and C18:1, and their total content accounts for more than 80% of total fatty acids (Gillan et al., 1984). Among the marine macrophytes, ω-3 PUFAs of

α-linolenic acid predominated over C20:5n-3 and C22:6n-3 while in *Z. marina* ω-6 PUFAs of linoleic acid than γ-linolenic acid and arachidonic acid and total ω-3 PUFAs were comparatively higher in *Z. marina*. Total ω-6 PUFAs in *Z. marina* was greater than *Ulva fenestrata* and less than *Sargassum pallidum* (Popov and Krivoshapko, 2013). Major components exhibiting antioxidants as well as antimicrobial activities were n-hexadecanoic acid, 9, 12-octadeceadienoic acid, 9-octadecanoic acid methyl ester, oleic acid and phytol, along with 9, 12, 15-octadecatrienoic acid (22.83%) found in *H. pinifolia* and 21.52% of 13-octadecenal in *S. isoetifolium* (Kannan et al., 2012).

Amphibolis griffithi and *H. ovalis* PUFAs were 59% and 48% of total FAs respectively while *Posidonia sinuosa*, predominated with SFAs (51.60%). The fatty acid profile of *P. sinuosa* followed the order: SFA>PUFA>MUFA (Hyndes and Hanson, 2009). The most abundant FAs in *Z. marina* were C18:3ω-3, C16:0, C18:2ω-6 and C18:1ω-9, and comprised of 73% of total fatty acids. Variations were more observed in the case of PUFAs than MUFAs between live and detrital leaves of *Z. marina* while no wide variations observed in the case of SFAs (Harbeson, 2010). For comparison, in red and brown algae major PUFAs were C20:4n-6 and C20:5n-3 whereas in green algae C18:2n-6, C18:3n-3 and C18:4n-3, and C18:2n-6 and C18:3n-3 predominated in seagrasses (*Z. marina* and *Phyllospadix scouleri*) (Galloway et al., 2012). FAs displayed distinct variation in *Z. marina* detritus with green leaves and rhizomes (Kharlamenko et al., 2001). Abundant FAs in seagrasses of *Z. marina* and *P. iwatensis* were C16:0, C16:3n-3, C18:2n-6, C18:3n-3 and C22:0, and PUFA C18:3n-3 predominated in *P. iwatensis* while C18:2n-6 in *Z. marina* (Vaskovsky et al., 1996).

Amino acid profile

The total nitrogen content in seagrasses ranged from 1.81 to 4.22% (dry weight) (Pulich Jr., 1986). The free amino acid in plant sources provides the bio potential to the material. Generally, proline as well as total nitrogen contents increased with rise in salinity from 20 to 32*psu* and among these maximum hikes in concentration was reported in *R. maritima* and *H. wrightii* at a salinity of 20 to 47*psu*. Also, total nitrogen content increased with increase in salinity and at *Halophila* rather than proline, alanine contained high concentrations. Moreover the ammonium contents increased with an increase in salinity in most of the species (Pulich Jr., 1986). The amino acids of highest concentrations obtained in *Amphibolis antarctica* were glycine, lysine and lowest by tyrosine and methionine (Harris et al., 1994). The major essential amino acids were isoleuine and tryptophan whereas non essential amino acids were tyrosine and glutamine in *H. pinifolia*. The amino acid content followed the order; *H. pinifolia*>*H. ovalis*>*S. isoetifolium*> *C. serrulata* (Jeevitha et al., 2013). Seventeen amino acids were detected in *P. australis*, and differences were observed between dry unwashed and washed, green and fresh (Torbatinejad and Sabine, 2001).

The free extractable amino acids (FEAA) in *P. oceanica* recorded high concentrations during light than dark, and differences in concentrations were observed between young leaves, old leaves, roots and rhizomes. They followed the order; young leaves>roots>old leaves>rhizhomes. Aspartate was the major amino acid in young leaves in all the light as well as dark and roots (light). During dark condition taurine predominated in old leaves and roots whereas γ-ABA, glutamate, ornithine and alanine during light condition (Jorgensen et al., 1981). Comparatively higher concentrations of total amino

acids were obtained in *H. uninervis* than *S. isoetifolium*, and huge differences due to high contents of proline. Asparagine and glutamine were the other dominant amino acids. Fertilization with nitrogen alone and nitrogen with phosphorous in seagrasses increased the concentrations of glutamine and aspargine (Udy et al., 1999). Proline and aspargine were the predominated amino acids in *H. uninervis* (Udy and Dennison, 1997) while in *Z. capricorni* proline and glutamine (Prange and Dennison, 2000). Further it is shown that the total amino acids as well as proline concentrations recorded an increase in seagrasses during hyper saline stress (Marin-Guirao et al., 2013).

Carbohydrate profile

Carbohydrates are one of the major constituents of vascular plants and neutral sugars have more importance than other constituents. Plant nutrients have an important role in the regulation of carbon storage within seagrasses (Campbell et al., 2012). Carbohydrate composition of pollen grains of *Amphibolis antarctica* showed that predominated neutral monosaccharides were glucose, galactose and rhamnose, and along with small amounts of arabinose, xylose and mannose (Harris et al., 1994). Studies in *T. testudinum* on relationships between plant nutrient content and rhizome carbohydrate content showed an intra and inter regional variation between plant nutrients with rhizome carbohydrate. An increase in rhizome carbohydrate is related directly with higher water temperature, underwater irradiance and blade chlorophyll concentrations, and the steep decrease in carbohydrate concentration related with usage of stored carbons for tissue maintenance and new growth (Lee and Dunton, 1996). The major monosaccharides of soluble non starch polysaccharides (NSP) were mannose, glucose and galactose whereas insoluble NSP contained xylose and glucose,

comparatively higher monosaccharides observed at insoluble NSP than soluble NSP in four different forms of *P. australis* (Torbatinejad and Sabine, 2001). Structural constituents of the seagrass *P. oceanica* namely green, fresh, dry wash and dry unwashed revealed that glucose, galactose and mannose were the major monosaccharides as soluble constituents, and are significantly higher than non starch polysaccharides (Torbatinejad et al., 2007).

The dominant monosaccharides other than glucose in seagrass angiosperms were arabinose, xylose and galactose, and these species belongs to dicotyledon genus and monocotyledons genus (Knox, 1984). Cell wall composition of *Z. marina* fibers was comparable to other terrestrial fibers (Davies et al., 2007). The stability of neutral sugars depends on the source of the sugar. Photobleached *H. wrightii* showed an increase in the concentrations of glucose, rhamnose and xylose (Opsahl and Benner, 1993). Seagrasses of *Zannicheliaceae* contained glucose, fructose and sucrose and among these comparatively higher concentrations of soluble sugar noticed in *C. nodosa* and *S. filiforme* whereas sucrose was predominat in *Posidoniaceae* family. *Phyllospadix torreyi* was the only seagrass species which has four soluble sugars under the genera *Zosteraceae* and relatively high contents of glucose, fructose and sucrose (*Z. marina*). Among the seagrasses of family *Hydrocharitaceae*, *E. acoroides* have high concentrations of soluble sugars while comparatively lower in *H. decipiens* (Drew, 1983).

Under salinity stress, the total carbohydrate level in leaves increased and the hike was more on longer exposure. The non structural carbohydrate acts as an organic osmolytes against the saline stress (Marin-Guiro et al., 2013). Sucrose was the dominant carbohydrate in the total soluble

carbohydrate pool and others were glucose, fructose, apiose, arabinose, fucose, galactose, mannose, rhamnose and xylose. Seagrass species with high leaf sugar content accumulate more carbohydrates at rhizome (Touchette and Burkholder, 2000). The stability of seagrasses against the simulated grazing was in the order; *H. ovalis*>*Z. capriconi*>*C. serrulata*. Level of water soluble carbohydrates (WSC) in rhizomes were higher than leaves and wide variations observed in the WSC between the control and clippings, it was more at rhizomes of *H. ovalis* and *C. serrulata* (Kuiper-Linley et al., 2007).

Bio potential of seagrasses

Antioxidant activities

Antioxidants in biological systems have the ability to prevent cell damage caused by free radicals as well as reactive species such as superoxide radical (O_2^-, hydroxyl radical (OH^-), peroxide radical (ROO^-) and nitric oxide radical (NO^-)). Around 65-80% of the people in developing countries use traditional medicine for primary health care and most of them were consumed as plant extracts. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity of mangroves bark extract demonstrated higher activity in comparison with the proven compound butylated hydroxyl toluene (BHT) (Krishnamoorthy et al., 2011), and a positive correlation was observed with total phenolic content (Choi et al., 2009; Krishnamoorthy et al., 2011; Girija et al., 2013). Different extracts of seaweeds showed that ethyl acetate have more activity than methanol and hexane extracts of *Bryopsis plumosa* and *Dictyopteris australis* while extracts of *Gracilara pudumadamensis* followed the order; methanol>ethyl acetate>hexane

(Chejara et al., 2014). No wide variations were observed in DPPH free radical scavenging activity of brown seaweeds of Gulf of Mannar (Kayalvizhi et al., 2014). Based on the antioxidant constituents in seagrassses, relatively higher antioxidant activity was recorded in *H. pinifolia* followed by *T. hemprichii* and lower activity showed by *E. acoroides* and *S. isoetifolium* (Kannan et al., 2010c). The activity ranged from 16.93 (*H. ovata*) to 68.07% (*H. pinifolia*) (Rengasamy et al., 2012). Crude methanol as well as sub fractioned solvents of *Z. marina* displayed a maximum DPPH free radical scavenging activity towards non-polar fractions of ethyl acetate followed by n-butanol and polar methanol (Choi et al., 2009). Comparatively higher DPPH radical scavenging activity was recorded for methanol extracts of seagrasses followed by acetone and hexane extracts (Girija et al., 2013). DPPH radical scavenging activity of a fucoidan like sulphated polysaccharide crude extract of *H. pinifolia* showed highest activity of 90.94% at a concentration of 1mg/ml (Kannan et al., 2013c).

DPPH scavenging radical activity of *H. uninervis* extract was increased with an increase in the concentration of the extract (Baehaki et al., 2016). The reducing power assay was comparable in brown seaweeds from Gulf of Mannar (Kayalvizhi et al., 2014) and a positive correlation was observed with phenolic content (Krishnamoorthy et al., 2011; Kayalvizhi et al., 2014). Relatively higher ferric reducing antioxidant power (FRAP) assay was observed in *H. stipulacea* (46.29mg gallic acid/g) and lower in *E. acoroides* (3.37mg gallic acid/g). A positive correlation was not observed with FRAP assay and phenolic content except *H. stipulacea* (Rengasamy et al., 2012). The non polar fractions of ethyl acetate and n-butanol showed relatively higher reducing power than crude methanol extract as well as other

extracts (Choi et al., 2009). Among the three extracts (methanol, acetone and hexane) of seagrasses, methanol fraction predominated over rest of them in seagrass species from Tuticorin (Girija et al., 2013). Species to species variations in FRAP assay were found in seaweeds of Gulf of Mannar (Meenakshi et al., 2012). Total reducing power in seagrasses of Palk Bay revealed that maximum reducing capacity noticed in rhizomes than leaves (Jeyapragash et al., 2016). Reducing power of *H. uninervis* extract was increased with an increase in the concentration of the extract (Baehaki et al., 2016).

The crude sulphated polysaccharide effect on scavenging hydroxyl radicals records a maximum activity of 52% at a concentration of 1mg/ml (Kannan et al., 2013c). Hydroxy radical and hydrogen peroxide (H_2O_2) activities were highest in extracts from *C. rotundata* while lowest in *T. hemprichii* and *E. acoroides* (Kannan et al., 2013b). Higher H_2O_2 scavenging antioxidant activities were found in seagrasses of *H. ovalis*, *C. serrulata*, *S. isoetifolium* and *T. hemprichii* (Athiperumalsami et al., 2010). H_2O_2 activity in *S. isoetifolium* was 38 and 42% respectively for leaves and rhizomes (Jeyapragash et al., 2016). For comparison, H_2O_2 activity of brown seaweeds of Gulf of Mannar noticed that activities were higher than the standard gallic acid (Kayalvizhi et al., 2014). Comparatively higher H_2O_2 activity was observed in *H. pinifolia* over *H. ovalis* and *S. isoetifolium* (Girija et al., 2013). H_2O_2 activity increased with an increase in concentration of crude sulphated polysaccharide and at a concentration of 250µg/ml activity reached nearly to 100% (Kannan et al., 2013c). An increase in the concentration of H_2O_2 was observed in seagrass of *P. oceanica* with an epiphyte than *P. oceanica* alone (Sureda et al., 2008).

Total antioxidant activity (TAA) varied from *E. acoroides* (8.44) to *H. pinifolia* (132.38mg ascorbic acid/g) (Rengasamy et al., 2012). Total antioxidant activity was higher in methanol and acetone extracts of *H. pinifolia* while observed a reverse order in *S. isoetifolium* (Girija et al., 2013). Total antioxidant activity of a fucoidan like sulphated polysaccharide isolated from *H. pinifolia* showed a maximum activity of 125mgAE/g (Kannan et al., 2013c). In aqueous methanol extracts of six seagrasses, total antioxidant activity ranged from 3.19 (*T. hemprichii*) to 15.75mgAE/g (*H. pinifolia*) (Kannan et al., 2013b). No variations were observed in TAA of ethanol extracts from different body parts (leaf, root and rhizome) of *E. acoroides* (Kannan et al., 2010b). Total antioxidant activity in seagrasses of Palk Bay varied from 2.54 to 12.83mgAE/g and 2.20 to 17.63mgAE/g in leaves and rhizomes respectively (Jeyapragash et al., 2016).

Chemical constituents and characteristics (FTIR, UV-VIS and GCMS)

Antibacterial and antifungal activity exhibited SFAs were hexadecanoic acid methyl ester, octadecanoic acid methyl ester, oleic acid and erucic acid (Khoobchandani et al., 2010; Wagh et al., 2006). Major active compounds noticed in *S. isoetifolium* and *C. serrulata* were 2-pentadecea-none, 9, 12-octadecanoic (Z, Z) methyl ester, 9, 12, 15- octatrienoic acid methyl ester (Z, Z, Z), oleic acid, erucic acid and octadecanoic acid methyl ester (Iyapparaj et al., 2014). Common chemical components observed in the aqueous methanol extracts of seagrasses were hexadecanoic acid methyl ester, n-hexdecanoic acid, 9, 12-octadecanoic acid (Z, Z) , 9-octadecanoic acid (Z)-methyl ester, phytol, 9, 12, 15-octadecatrienoic acid methyl ester (Z, Z, Z), octadecanoic acid, oleic acid, 9, 12, 15-octadecatrienoic acid and 13- octadecanol (Kannan et al., 2012). Anti inflammatory activity was found

in the hexane fraction and among these, higher activity was noticed in sub fraction five and its major constituents were hexadecanoic acid and its methyl ester, linoleic acid and its methyl ester, oleic acid, stearic acid and its methyl ester and testosterone (Hua et al., 2006).

The absorption peaks were between 270-290nm for phenolic acid derivatives whereas 317-340nm for flavonoid derivatives. An ethanolic extract showed higher absorptions at 270 and 275nm due to the presence of phenols and poly phenolic compounds at higher concentrations than methanol, hexane and chloroform (Vijayalakshmi and Ravindhran, 2012). The UV-VIS spectra results of seagrasses of Gulf of Mannnar implied the presence of phenolic acids (Kannan et al., 2013a). Simple phenolic compounds recorded an absorption maximum between 220-280nm and also closely related phenolic compounds exhibited variations in their molecular absorptions (Kannan et al., 2011b). The highest absorption found at 270 and 321nm was characteristic for flavonoids derivatives (Singh and Mendhulkar, 2015). Higher absorptions were at 270 and 275nm corresponds to phenolic compounds (Khan et al., 2015).

The leaf extracts of ethanol, methanol, isopropanol and acetone confirmed the presence of phenolic compounds and among these, more comparison was noticed towards standard gallic acid in ethanol extract (Khan et al., 2015). The major absorption bands observed in seagrasses of *C. serrulata* and H. *pinifolia* were 3415, 2954, 1649, 1408 and 1025cm^{-1} and this indicated the presence of functional groups alcohols, phenols, primary amines, aromatics, carboxylic acids, ethers and esters (Prabhakaran et al., 2012). *C. serrulata* exhibited absorption bands at 3431, 2070, 1580,

1482, 1402, 760 and 1123cm^{-1} and indicated the presence of bonded alcohols and phenols, N-H bond of primary and secondary amines, C-C stretch (in ring) aromatics, C-Cl stretch of alkyl halides and C-H bearing of alkanes (Chanthini et al., 2015). The major functional groups present in the aqueous methanol extracts of six seagrasses were compared with standards viz; phenolic acids of gallic acid, tannic acid, p-coumaric acid and vanillin, and the data confirmed the presence of phenolic compounds and also slight variations were observed in absorption bands (Kannan et al., 2011b). Among the solvents namely, methanol, ethanol, hexane, chloroform and water, phenolic compounds were absent in aqueous extract and more comparison with respect to gallic acid was observed in ethanol extract (Vijayalakshmi and Ravindhran, 2012).

Phytochemicals

Relatively higher phytochemicals were obtained in methanol extract than acetone. Alkaloids, saponins, phenolic compounds and reducing sugars were the chemicals found in both extracts (Mani et al., 2012a). Phytochemicals in seagrasses of Gulf of Mannar were present in benzene and petroleum ether extracts of *S. isoetifolium* whereas in chloroform extracts in the case of *C. serrulata* and *H. pinifolia* (Athiperumalsami et al., 2008). Antioxidant constituents of four seagrasses showed more numbers present in *H. pinifolia* followed by *T. hemprichii* and least constituents presented in *E. acoroides* and *S. isoetifolium* (Kannan et al., 2010c). Phytochemicals in seasgrasses of *C. rotundata* revealed that relatively higher numbers were found in acetone extract than both methanol and ethanol extracts (Mani et al., 2012b). Relatively more phytochemicals were investigated in seagrasses of *C. serrulata* than rest of the species (*H. pinifolia* and *H. stipulacea*) (Kannan et al.,

2010a). Among the seven seagrasses, saponins and alkaloids were present in all species whereas flavonoids were present only in *H. ovalis* and *T. hemprichii*. Anthroquinone was absent in all the samples (Lakshmanan and Dhanalakshmi, 1988). *C. serrulata* contained more number of phtochemicals in ethyl acetate extract and no differences were obtained between ethanol and aqueous (Sangeetha and Asokan, 2016). Phytochemicals present in the fresh leaves of *C. serrulata* were alkaloids, terpenoids, polyphenols and flavonoids (Hardoko et al., 2016). The methanol extract of *H. uninervis* revealed the presence of flavonoids, alkaloids and steroids (Baehaki et al., 2016). An aqueous methanolic extracts of six seagrasses of Gulf of Mannar were noticed the presence of 10 phytochemicals, and also alkaloids and glycosides were absent in all the seagrasses (Kannan et al., 2013b). Phytoconstituents present in different seagrasses include phenols, flavonoids, tannins, vitamin C and vitamin E (Kannan et al., 2013a), vitamins (A, B_3, C and E), β-carotene, tannin and phenol (Athiperumalsami et al., 2010). It is also noted that phenols and alkaloids were present only in the ethanol extract whereas sapanins found only in acetone (Berfad and Alnour, 2014). Phytoconstituents present in the hexane fractions were alkaloids, phenols, steroids and tannins whereas flavonoids were obtained only in the acetone fraction of *C. serrulata* (Ravikumar et al., 2011).

Antimicrobial activity

Majority of extracts from plant origin exhibited antibacterial activity to different orders. Extracts of *S. isoetifolium* exhibited antibacterial activity as well as antiinsecticidal activity and high antibacterial activity against acetone extracts than methanol (Mani et al., 2012a). The maximum zone of inhibition in *C. rotundata* extracts followed the order;

methanol>ethanol and acetone>butanol extracts. Moreover activity was not noticed in aqueous extract and *Vibrio chlorae* was inactive towards all the extracts (Mani et al., 2012b). Antimicrobial potential of seagrasses showed that extracts of *H. stipulacea* and *H. pinifolia* exhibit high activity against all pathogens in comparison with *C. serrulata* and in contrast to earlier observation, methanolic extract exhibited higher activity than chloroform and hexane (Arumugam et al., 2010). Crude aqueous ethanol extract of roots of *C. serrulata* and its sub fractions revealed that comparatively more activity against pathogens *Aeromonas hydrophila*, Serratia sp. and *Vibrio harveyii* in acetone fractions while hexane fraction against *Vibrio parahaemolyticus* and *Bacillus subtilis* (Ravikumar et al., 2011). Relatively more antifungal activity was exhibited by the extracts of *C. nodosa* while bacteria showed more inhibition towards *R. cirrhosa* (Abd El-Hady et al., 2007). *H. stipulacea* aqueous extract displayed activity against *Bacillus subtilis*, *Candida albicans* and *Aspergillus niger* (gram positive) whereas no activity towards gram negative bacteria of *Escherichia coli* and *Pseudomonas aeruginosa* (Abd El-Hady et al., 2012).

Comparison between *C. serrulata* and *S. isoetifolium* observed that *S. isoetifolium* was more active than *C. serrulata*. The zone of inhibition was obtained for *S. isoetifolium* and *C. serrulata* against three and two pathogens respectively while rest of them was resistant with no zone of inhibition (Kannan et al., 2012). Seagrasses of *Z. marina* crude methanol and its different partitioned solvents were treated against three human skin pathogens, and showed higher activity at lower concentrations of ethyl acetate (*Staphylococcus aureus*) and n-butanol (*Candida albicans*) extracts while at higher concentrations towards chloroform extracts (*C. albicans*)

and no inhibition obtained in the aqueous extract (Choi et al., 2009). *S. aureus* pathogen noticed maximum zone of inhibition in *H. pinifolia* followed by *E. acoroides* and *T. hemprichii* while rest of the pathogens varied with respect to species and relatively lower inhibition obtained in *S. isoetifolium*, *C. serrulata* and *C. rotundata* (Kannan et al., 2013b). Strongest antibacterial activity of *P. oceanica* was observed in the cyclohexane extract while no activity observed in the ethyl acetate extract, and maximum zone of inhibition was observed against the pathogen *P. aeruginosa* (Berfad and Alnour, 2014).

All the tested organisms displayed higher zone of inhibition against extracts of *H. pinifolia* except *S. paratyphi*-B and *Staphylococuss* sp., and higher activity observed in the ethanol extract than rest of the solvents (Umamaheshwari et al., 2009). Primary screening of methanol extracts of *Halodule* sp. and *H. ovalis* exhibited antifungal activity, and more activity found in *Halodule* sp. while on secondary screening, hexane fraction of *H. ovalis* and ethyl acetate fraction of *Halodule* sp. recorded antifungal activity (Devi et al., 2011). Higher activity was exhibited by methanol extracts of marine algae and *E. acoroides*, and most of micro organisms were gram positive bacteria whereas gram negative was *P. aeruginosa* and no activity towards *Klebsiella pneumoniac* (Alam et al., 1994). The degree of activity followed the order; *H. beaudettei*>*S. filiforme*>*T. testudinum* (Engel et al., 2006). An aqueous ethanolic extract of *S. isoetifolium* (root) showed higher larvicidal activity at lower concentrations followed by *S. isoetifolium* (leaf) and *C. serrulata* (root), and no activity was observed in *H. beccarii* and *C. serrulata* (leaf) (Ali et al., 2012). *E. acoroides* extracts inhibited different microbial types in the order; yeasts>filamentous>gram positive bacteria>gram negative bacteria (Supaphon et al., 2014). Methanol

extracts of *C. serrulata* and *S. isoetifolium* showed better antibacterial activity than other solvents of dichloromethane and acetone (Iyapparaj et al., 2014). Antifouling potential *C. rotundata* and brown algae showed inhibition against bacterial strains at different degrees (Bhosale et al., 2002). Anti inflammatory activity found in the hexane fraction and fraction has the highest capacity to inhibit proIL-1â expression as compared to other fractions in lipopolysaccharides (LPS)-stimulated J774A.1 murine macrophages (Hua et al., 2006). Among the three extracts of *C. serrulata*, higher antimicrobial activity exhibited in ethyl acetate followed by chloroform and ethanol and no activity towards hexane. Pathogens *P. aeruginosa*, *Enterococcus faecalis* and *S. aureus* did not show any activity in all the extracts (Sangeetha and Asokan, 2016). Antimicrobial activity of *H. uninervis* exhibited inhibition against both gram positive and negative bacteria, and highest inhibitory activity against gram negative bacteria (Supriadi et al., 2016).

1.12 Aim and Scope of the Study

Seagrases are marine flowering plants that grow abundantly in tidal and sub tidal coastal areas of the World except in the Polar Regions (Chanthini et al., 2015). Among the marine species of India, seagrasses are one of the specialized groups of marine flora which are poorly known compared to seaweeds and mangroves, especially for bio potential studies (Kannan et al., 2013 a,b&c; Mani et al., 2012 a&b). Total 15 species of seagrasses under seven genera are found in India and they are *Cymodocea rotundata*, *C. serrulata*, *Enhalus acoroides*, *Halodule pinifolia*, *H. uninervis*, *H. wrightii*, *Halophila beccarii*, *H. decipiens*, *H. ovalis*, *H. ovalis* var., *H. ovata*, *H. stipulacea*, *Syringodium isoetifolium*, *Thalassia hemprichii* and *Ruppia maritima*

(Jagtap et al., 2003; Thangaradjou et al., 2015). Chain of Islands stretching from India's Pamban Island to Srilanka's Mannar Island is called Adams Bridge (in English), Atham palam (in Tamil) and Rama sethu (in Malayalam) (Sriyanie, 2012), and seagrass density was reported to be higher at Palk Bay and Gulf of Mannar (Sulochanan, 2012).

The importance of the natural wealth in the Gulf of Mannar region gives through shelter to marine animals (fishes, sponges, dugongs, turtles etc), protecting the coastline (coral Reefs, sand dunes, mangroves and seagrass meadows against beating waves, storms and cyclones), regulating the local climate, producing food (through photosynthesis by tropical dry mixed evergreen forests, tropical scrublands, mangroves, coral Reefs, macro and microalgae and seagrass meadows), preventing floods (mud in mangroves), preventing erosion (seagrass meadows, mangroves and mud flats), trapping carbon dioxide (tropical dry mixed evergreen forests, tropical scrublands, mangroves and seagrasses as carbon sinks), maintaining soil productivity (seagrass meadows, mangroves and mud flats) and supporting traditional livelihoods (mangroves, coral Reefs and seagrass meadows support traditional fisheries) (Manikandan et al., 2011a; Maheswari et al., 2011; Sriyanie, 2012). Palk Bay is also rich in biodiversity and important groups associated includes are sponges and gorgonids (275 species out of 31 endemic form), corals (63 species out of 22 genera), stony corals (128 species out of 43 endemic form), fisheries (elasmobranches, squids, lobsters, crabs, cephalopods and pearl oyster culture contains 580 species), turtles (5 species), birds (61 species), crustacea (651 species out of 159 endemic form), mollusca (733 species out of 26 endemic form) mammals (11 species including dolphins and dugongs), algae and seagrasses (Kumaraguru et al., 2008; Manikandan et al., 2011b).

Secondary metabolites especially phenolic compounds are responsible for antioxidant, antibacterial and antifungal activities in seagrasses, and also are rich source of macro elements and trace metals which are essential for humans. Nowadays, in medicinal treatments many countries have incorporated traditional medicines. Antioxidant and antimicrobial activities exhibiting secondary metabolites are generated in seagrasses due to their interactions with micro and macro algae, epiphytes, etc. Studies showed that an interaction of *Caulerpa taxifolia* and *C. racemosa* with *P. oceanica* leaves exhibited an increase in phenolic content, due to their defensive mechanism against others (Dumay et al., 2004). Antioxidant and antimicrobial activities in seagrasses are decreased with an increase in the content of pollutants (Lei et al., 2012). In this context, antimicrobial and antioxidant studies are more important if it is obtained from an unpolluted environment. Many countries transplant seagrasses for their persistence in the universe and it provides ecological benefits to large varieties of organism as well as medicinal uses to humans (free from pollutants). The problem facing for transplantation process is cost of production and it mainly depends upon maintaining the nutrients as well as salinity level to attain suitable environmental conditions for the growth and productivity of seagrasses. Persistence of seagrasses at low salinity may also be more beneficial compared to higher salinity.

The investigations on seagrasses in India are scarcity and most of the studies are focused on seagrass biology, microbiology, utility of products from seagrasses, taxonomy and ecology and are given below (Table 1.3).

Table 1.3 List of Ph.D. works done in seagrasses in India

Sl No.	Name & year of completion of Ph.D. holder	Title	Nature of work
1	Aswathi Elizabeth Mani (2014)	Bioactive potentials of seagrasses of Gulf of Mannar Southeast coast of India.	Microbiology
2	Medo Merina R. (2014)	Seagrass ecology microbial association and bioactivity profiles with special reference to *Halodule pinifolia* miki hartog and *Syringodium isoetifolium* asch dandy occurring along the south Indian coast.	Microbiology
3	Gokulakrishnan R. (2013)	Production of biofuel ethanol from seagrass bio waste by using halotolerant microbes.	Utility of products from seagrasses
4	Dilipan E. (2012)	Taxonomy of Indian seagrasses: Molecular (RAPD, 18S and DNA) and Bioformatic (SNDI) tools for seagrass identification.	Taxonomy
5	Jeevitha M. (2012)	Studies on the nutritional value of seagrasses of Gulf of Mannar.	Nutrition
6	Pon Rathi T. (2012)	A comparative study on the effect of seaweed liquid fertilizer and seagrass liquid fertilizer on the growth and biochemicals of seedling.	Utility of products from seagrasses
7	Iyapparaj P. (2011)	Investigation on biofouling and identification of eco friendly antifouling compounds from selected seagrasses.	Microbiology
8	Kalaiarasi A. (2011).	Effect of eco friendly vermicompost from seagrass on the growth yield and biochemical properties of agricultural crop plants.	Utility of products from seagrasses
9	Jeba Ananthi K. (2011)	Studies on the effect of slf and sglf on water stressed plants.	Utility of products from seagrasses
10	Naganathan V. (2010)	Role of corals reefs mangroves and seagrasses in enhancing productivity of the Gulf of Mannar coastal ecosystem in the South eastern India and conservation measures.	Ecology
11	Nobi E. P. (2010)	Assessment and evaluation of the seagrass resources of Lakshadweep islands (India): A remote sensing and GIS approach.	Ecology
12	Athiperumalsami T. (2009)	Survey of seagrasses for phytochemical screening and pharmacognostic studies in the Gulf of Mannar region.	Biochemistry
13	Bindu Sulochanan (2009)	Sediment dynamics of seagrass beds off Mandapam and its influence on coastal erosion.	Ecology
14	Balaji V. (2008)	Assessment of seagrass beds associated fish assemblages and socioeconomics of northern Palk Bay southeast coast of India.	Ecology
15	Prabakaran M. P. (2008)	Ecological studies on the seagrass ecosystem of Minicoy lagoon Lakshadweep.	Ecology
16	Sobitha Bai R. (2008)	Comparative studies on the impact of seaweed *Laurencia papillosa* and seagrass *Cymodocea serrulata* extracts on germination growth senescence and biochemical changes in Zea mays.	Utility of products from seagrasses
17	Savithiri S. (2007)	Studies on the effect of native AM fungi and seagrass *Cymodocea serrulata* R Br Asch and Magnus on growth and productivity of sunflower *Helianthus annuus* L.	Microbiology
18	Dalia SusanVargis(2005)	Macrobenthos of Minicoy Island, Lakshadweep.	Ecology
19	Mary Elizabeth Gnanambal K. (2005)	Bioactivity of seagrasses of Tuticorin, southeast coast of India.	Microbiology
20	Sivakumar N. (2002)	Studies on the influence of VA_mycorrhizal fungi and seagrass on the growth nutrition and yield of *Saccharum officinarum* L.	Microbiology
21	Thangaradjou T. (2000)	Eco - biology, culture and experimental transplantation of seagrasses of the Gulf of Mannar Biosphere India, reserve.	Ecology
22	Kannan R. (1992)	Marine botanical – hydrographical and heavy metal studies in the Palk Bay, India.	Ecology
23	Ghevade K. S. (1983)	Ecophysiological studies in a seagrass, *Halophila beccarii* aschers.	Ecology

Seagrasses play a major role in biogeochemistry of the coastal environment, provides ecological, medicinal, commercial uses to humans and other related organisms. Although a variety of secondary metabolites are produced by seagrasses which have pharmacological significance, only very few studies were carried out to characterize these compounds. The present study is an attempt to evaluate the biochemical components as well as chemical characterizations of seagrasses collected from Gulf of Mannar and Palk Bay.

The objectives of the present study are,

1) Study the general ecological characteristics of seagrass meadows along Gulf of Mannar and Palk Bay.
2) Assessment of biochemical composition (total proteins, lipids and carbohydrates), elemental compositions, secondary metabolites, chlorophylls, macro elements and trace metals in the major species identified.
3) Evaluation of fatty acid, amino acid and carbohydrate profile of seagrasses.
4) Investigation on the bio potential of seagrasses grown at low salinities.

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Chapter 2

MATERIALS AND METHODS

Contents

2.1 Description of the Study Area

Seagrasses, water and sediments were collected from four stations [Thonithurai (area where seaweed farming is going on) and Chinnappalm (fishermen living area, boats and trawlers harbor) situated on the Gulf of Mannar] during November 2010 and [Munaikkadu (Farm pond) (geographically near to a lagoon and seaweeds are along with seagrasses) and Mathacovil (seaweeds and corals are growing along with seagrasses on the Palk Bay side] during June 2011. The latitude of stations ranged from 9° 16' to 9° 18 N' while longitude from 79° 08 to 79° 13' E and geographical location of the study area is given in Figure 2.1 & 2.2. Ocean currents due to southwest and northeast monsoons strongly influenced in trophic efficiency of plankton food webs (Anjusha et al., 2014), chlorophyll content (Jyothibabu et al., 2014), mesoplankton (Jagadeesan et al., 2013), picoplankton and nanoplankton (Jyothibabu et al., 2013) of Gulf of Mannar and Palk Bay. During southwest monsoon, high waves are noticed at Gulf of Mannar while Palk Bay showed high waves during northeast monsoon (Gowthaman et al.,

2013). A seasonal wise sampling was not done due to strong waves occurring at Gulf of Mannar and Palk Bay during 2010-2011 seasons.

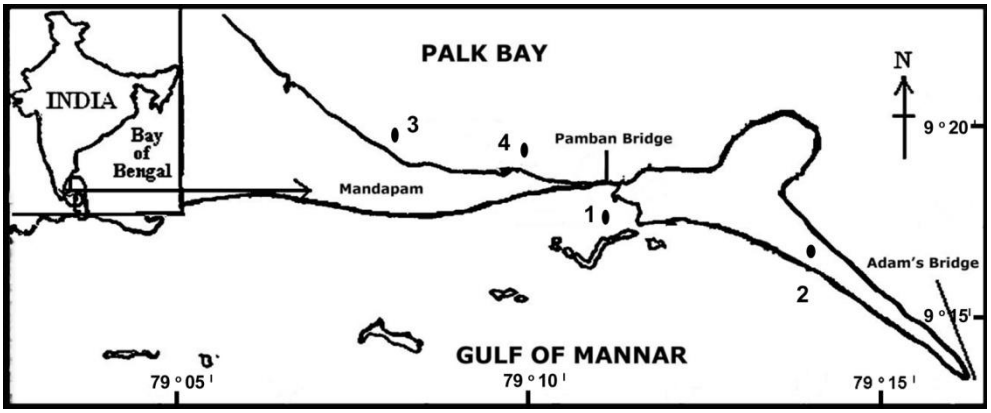


Figure 2.1 Geographical representation of the study area ((1) Thonithurai, (2) Chinnappalam, (3) Munaikkadu and (4) Mathacovil).

Figure 2.2 Geographical representation of the study area

2.2 Sampling and Analytical Methodology

Surface water samples were collected during low tide using a clean plastic bucket and sediment samples using a Van Veen grab (0.045 m^2). The water samples were stored in washed plastic bottles, previously rinsed with

the sample at the collection site. The sediment samples were collected in plastic bottles. The pH of the samples was measured using portable pH meter (Eutech, pH Tester 10) without delay. The samples were carried to laboratory in chilled condition and kept in deep freezer at –20^{o}C until analysis. All the analyses were carried out in triplicates and the average values were reported.

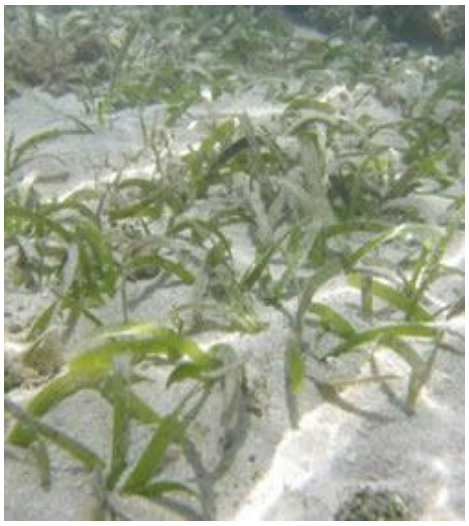
Four species of seagrasses (Figure 2.3) belonging to two different families were chosen for the present study (Table 2.1). The selection was made considering the abundance of seagrasses at the sampling sites and easiness for identification and collection. Sample collections were done during the period of low tides. Seagrasses were handpicked from their natural habitats, sorted out and washed in seawater. Samples were brought to the laboratory in plastic bags containing seawater to prevent evaporation and carried to the laboratory in chilled condition. Seagrasses were first washed in tap water to remove all sand particles, adhering particles and epiphytes followed by distilled water, and identified using standard references (Jagtap, 1983; Kaliaperumal et al., 1989; Dawes, 1998). Seagrasses were identified by seagrass taxonomists of Dr. M. P. Prabakaran (Assistant Professor, KUFOS, Panangad) and Dr. E. P. Nobi (Research Assistant (E), Wildlife Division, Ministry of Environment, Forest and Climate change), and voucher specimens (CST11/2010, SIT11/2010, CSC11/2010, EAC11/2010, CSF6/2011, THF6/2011, CSM6/2011 and SIM6/2011) were deposited in the Inter University Centre for Marine Biotechnology (IUCDMB), School of Marine Sciences, CUSAT. The scientific classification of seagrasses was given in Table 2.1. Fresh seagrasses were weighed and soaked in suitable solvents for the analysis of chlorophylls, amino acid and fatty acid profile while rest of

them were dried and powdered, and kept in a desiccator for further analysis. Sample blanks were carried out to nullify the color of the extracts during spectrophotometer analysis.

E. acoroides

C. serrulata

S. isoetifolium



T. hemprichii

Figure 2.3 Pictures of seagrasses

Table 2.1 Details of seagrasses from Gulf of Mannar and Palk Bay

SI No:	Location	Stations	Seagrsass species
1	Gulf of Mannar	Thonithurai	*Cymodocea serrulata* and *Syringodium isoetifolium*
2	Gulf of Mannar	Chinnappalam	*C. serrulata* and *Enhalus acoroides*
3	Palk Bay	Munaikkadu	*C. serrulata* and *Thalassia hemprichii*
4	Palk Bay	Mathacovil	*C. serrulata* and *S. isoetifolium*

Identification keys of seagrasses

1a. Leaves ligulate; male flowers without tepals; fruits indehiscent → ***Potamogetonaceae***

2a. Leaves terete, fleshy, grooved along adaxial side for a short distance; nerves absent → ***Syringodium isoetifolium***

2b. Leaves flat, not fleshy, without grooves; nerves present

3a. Rhizomes usually moniliferous, with scales; nerves 3, lamina 0.25 - 4 mm broad → ***Halodule***

4a. Leaf tips obtuse, serrulate; lateral teeth poorly developed or absent → ***H. pinifolia***

4b. Leaf tips not obtuse, not serrulate; lateral teeth well developed:

5a. Leaf tips tridentate; median tooth present; lamina 0.7 - 5 mm wide → ***H. uninervis***

5b. Leaf tips bidentate; median tooth absent; lamina 0.5 - 1 mm wide → ***H. wrightii***

3b. Rhizomes not moniliferous, without scales; nerves 7 - 22; lamina 4 - 10 mm broad → ***Cymodocea***

6a. Leaf scars forming closed rings; sheaths persistent; lamina 3 - 6 mm broad, rarely serrulate at apex; nerves 9 - 14 → ***C. rotundata***

6b. Leaf scars forming opened rings; sheaths not persistent; lamina 4 - 10 mm broad, serrulate at apex; nerves 12 - 22 → ***C. serrulata***

1b. Leaves eligulate; male flowers with 3 to 6 tepals; fruits dehiscent (except Halophila) → ***Hydrocharitaceae***

7a. Leaves differentiated into petioles and blades; lamina oblong, elliptic, linear, ovate, obovate or spathulate without tannin cells → ***Halophila***

8a. Leaves 6 - 12 at each node; cross-veins absent → ***H. beccarii***

8b. Leaves 2 at each node; cross-veins present:

9a. Lamina hairy, margins serrulate:

10a. Plants dioecious; leaves linear - oblong → ***H. stipulacea***

10b. Plants monoecious; leaves oblong - elliptic → ***H. decipiens***

9b. Lamina glabrous, margins entire: 11a. Seeds 6 - 12 → ***H. ovalis subsp. ramamurthiana***

11b. Seeds 20 or more:

12a. Lamina 20 - 30 mm long; cross-veins 13 - 22 pairs → ***H. ovalis subsp. ovalis***

12b. Lamina 4 - 15 mm long; cross-veins 3 – 9(-11) pairs → ***H. ovata***

7b. Leaves not differentiated into petioles and blades; lamina linear with tannin cells:

13a. Rhizomes 10 to 20 mm thick, without scales; roots stout; leaves ca 100 cm by 17 mm → ***Enhalus acoroides***

13b. Rhizomes 2 to 5 mm thick, with scales; roots thin; leaves ca 16 cm by 12 mm → ***Thalassia hemprichii***

2.2.1 General hydrography parameters

The general hydrographical parameters and nutrients of the water samples were analysed employing standard methods. Values of pH in the water column were measured using portable pH meter and temperature (sensitivity of 0.1°C) was recorded using a sensitive thermometer. Salinity of the water samples were estimated by Mohr-Knudsen method (Muller, 1999). Modified Winkler method was used for the estimation of dissolved oxygen (Hansen, 1999). Alkalinity of the water samples was estimated by the method of Koroleff (Anderson et al., 1999). Nutrients (nitrite, nitrate, ammonia, phosphate and silicate) were estimated using a spectrophotometer (Genesys 10UV Thermospectronic) (Hansen and Koroleff, 1999). Total hardness, Ca and Mg were measured by titration with EDTA solution (APHA, 1995). Turbidity was measured using a turbidity meter (Systronics Digital Nephelo-Turbidity Meter-132) according to APHA, 1995. Sulphates were estimated in the water spectrophotometrically (APHA, 1995). The total nitrogen and phosphorous content were determined after alkaline per sulphate oxidation (Hansen and Koroleff, 1999). Trace elements in the surface water were complexed with 1 %

ammonium pyrrolidine dithiocarbamate (APDC) and the metal complexes were extracted using chloroform, and reconstituted in nitric acid (HNO_3) (Kremling, 1999) and were estimated using ICP-OES (iCAP 600 Series, Thermo Scientific) while direct measurements were taken for macro elements, except Li (above mentioned extract), using flame photometer (BWB Technologies, UK Ltd).

2.2.2 General sedimentary parameters

Sediment containing inorganic carbonates was removed using 10% hydrochloric acid (HCl) and organic matter using H_2O_2, and grain size characteristics of the sediments (sand, silt, and clay) were determined using the method by Folk (1974). Sand fraction was estimated from wet sediment sieved through a 63μm sieve and the particles came through the sieve was divided into silt (63- 4μm) and clay (<4μm) fractions by timed gravimetric extraction of the dispersed sediments. Freeze-dried (Beetta Freeze drier, Chennai, India) sediment samples were finely powdered using agate mortar and kept for further analyses. Total carbon and nitrogen was determined using CHNS Analyser (Vario EL III). Total organic carbon (TOC) was analysed using TOC analyser (VARIO TOC SELECT- Elementar) (Chairi et al., 2010). Heavy metals in the surface sediments were estimated using ICP-OES (iCAP 600 Series, Thermo Scientific) after digestion using 1:5 $HClO_4$: HNO_3 (Loring and Rantala, 1992) except Hg, was quantitated using direct mercury analyser (Mile Stone). Macro elements of Na, K, Ca and Li were quantitated using flame photometer (BWB Technologies, UK Ltd) while Mg using ICP-OES after digestion using 1:5 perchloric acid ($HClO_4$): HNO_3, and total P was analyzed using the method Hansen and Koroleff, 1999.

2.2.3 Biochemical evaluation of seagrasses

Seagrasses were collected from the stations during low tides by the use of a 0.25m^2 quadrate (Lewis and Stoner, 1981). 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^oC for 12 hours (Khamsah et al., 2006). Seagrasses were powdered and kept in a desiccator for further analysis. Different body parts of seagrasses of *C. serrulata* and *E. acoroides* were taken for the study while seagrasses as a whole in the case of *T. hemprichii* and *S. isoetifolium*. spectrophotometric methods were employed for the determination of biochemical components in seagrasses. Analysis of total proteins were carried out following the procedure of Lowry et al. (1951), as modified by Rice (1982) with bovine serum albumin as the calibration standard. Total carbohydrates were analysed using the Dubois et al. (1956) method, using glucose as the standard. Total lipids were extracted according to Bligh and Dyer (1959), and estimated according to Barnes and Blackstock (1973) using cholesterol as the standard. All the analyses were carried out on triplicates and the average concentration is reported.

10g of the dried seagrasses were extracted for 24 hour 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 (Adomi, 2006) and made up to a known volume with methanol. Methanolic extracts were analysed as per Li et al. (2008) and Chang et al. (2002) respectively for total phenolic and flavonoids.

Acetone extracts of healthy seagrasses were measured at 647, 664 and 725nm for fresh chlorophylls estimation (Dennison, 1990). The extract was acidified with 0.1N HCl to estimate the amounts of pheophytins (Plante-Cuny, 1974). Tannin in seagrasses was extracted using 0.05M sodium hydroxide at 60^{o}C for 90 minutes and estimated spectrophotometrically by the sodium tungstate-phosphomolybdic acid method (Nair et al., 1989). Macro elements, total P and heavy metals of seagrasses carried out as described under 2.2.2 and total energy was calculated according to Pradheeba et al. (2011).

2.2.4 Biochemical profile of seagrasses

Total lipids in seagrasses were extracted using a cold mixture of chloroform: methanol (2:1) (Folch et al., 1957). The chloroform extract was filtered through sodium sulphate and concentrated using a rotary evaporator (Heidolph, Germany) and made up to a known volume with chloroform. The extracted lipid was saponified with potassium hydroxide-methanol and neutral lipid fraction was partitioned from the alkaline solution using n-hexane HPLC grade (HPLC) (AOAC, 1990), and remaining aqueous layer containing the fatty acid salts was acidified (pH 2) by adding 6M HCl. Fatty acids in this polar-lipid fraction were recovered separately into n-hexane (HPLC). The polar lipid fraction containing the fatty acids was evaporated to dryness using rotary evaporation. It is then converted to fatty acid methyl esters (FAMEs) by treating with 12% borontriflouride-Methanol (Sigma Aldrich, USA) (70^{o}C for 15 minutes) (Metcalfe et al., 1966).

The FAMEs were subsequently partitioned with n-hexane (HPLC) and was evaporated to dryness, and the extract was then made up to a known volume with n-hexane (HPLC) for gas chromatographic analysis. Analysis

of FAME was carried out by gas chromatography-mass spectrometry (GC-MS) (Perkin Elmer Clarus GC 620, with MS detector) equipped with a non-polar HP ultra-double-fused silica capillary column (30m, 0.32mm internal diameter, 0.25mm film thickness). Operating conditions were as follows: ion source of 200°C and electron voltage 70eV. Spectra were scanned from 50 to 600m/z with a scan time of 1.50seconds. A two-step temperature program was used: from 50°C to 200°C at 2°C per minute- then held for 5minutes. Then temperature again increased from 200°C to 280°C at 10°C per minute (held for 10minutes). The detector was operated at 290°C and helium was used as carrier gas. Full data acquisition was obtained with the use of MS (turbo mass version 5.3.2). Quantification was achieved by calibration of FAMEs standards (Sigma Aldrich, Supelco, 37 Component FAME Mix, 18919). Data were acquired and processed with the MS Turbomass version 5.4.2. Individual compounds were identified by comparison of mass spectra with literature and library data, comparison with authentic standards and interpretation of mass spectrometric fragmentation patterns. Mass spectral identification was confirmed by comparing the obtained mass spectra with those of authentic standards or mass spectra stored in the NIST MS Library (version: NIST MS Search 2.0) and then comparing mass fragmentation pattern with available literature. All the glasswares were cleaned by washing with tap water, chromic acid and distilled water followed by rinsing with methanol and n-hexane. All solvents were purchased from Merck (India/Germany).

For amino acid profiling, proteins were hydrolysed with 6N HCl (sample heated at 120°C for 24 hours in sealed test tubes in an air oven) and flash evaporated until acid free, and dissolved the amino acids in acidic

buffer (Ishida et al., 1981). The separation and quantification of amino acids were carried out with high performance liquid chromatography (Hitachi L 2130) with an ion exchange column Shodex CXPAK-no. P207074. Oven temperature was maintained at 55°C and flow rate was 0.4ml/min. The eluted amino acids were derivatised with ortho-phthalaldehyde (post column) and detected in a fluorescence detector (Hitachi L 2485) at 340nm excitation and 450nm (emission). Hydrolysis of polysaccharides was done using 2 M trifluoro acetic acid and autoclaved at 121°C for 1 hour done in screw capped vials. After cooling the contents of the vial were centrifuged for 5 minutes at 2000 g and trifluoro acetic acid was evaporated off in a rotary evaporator (Heidolph, Germany) (Stephen, 1988). The residue was dissolved in known volume of distilled water and analysed using HPLC method (Suhasini et al., 1997, Katayi-Chidewe, 2004 and Revanappa, 2009) [Shimadzu LC 2020 equipped with RID 10A detector and SUPELCOSIL LC-NH2 column (Sigma Aldrich)]. The separation was done at 30°C with the mobile phase as acetonitrile: water (8:2) at a flow rate of 0.8ml/min. The identification of individual compounds was done by comparing the retention time with those of standard compounds (Sigma Life Sciences).

2.2.5 Bio potential of seagrasses

C. *serrulata* and S. *isoetifolium* were the species taken for the bio potential studies. Crude extracts were prepared by soaking 100g of seagrass powder in 1.5L of methanol in amber colored conical flasks on a shaker overnight. The extraction was repeated twice more, and the pooled extract was filtered through Whatman No. 1 filter paper. The filtrate was concentrated nearly to dryness using a rotary flash drier and further freeze dried and stored in refrigerator until further analysis. All analysis was carried out by

standard methods. Antioxidant activities were evaluated by DPPH (Yen and Chen, 1995), total antioxidant activity (Prieto et al., 1999), reducing power assay (Oyaizu, 1986), hydrogen peroxide (Ruch et al., 1989 and Gulcin et al., 2003) and hydroxyl radical activity (Beara et al., 2009). Phytochemicals namely saponins, cardiac glycosides, terpenoids and reducing sugars were analysed according to Ayoola et al. (2008) while tannins, flavonoids, sterols and alkaloids by the method of Aiyegoro and Okoh (2010) and phenols and anthraquinone by Paul and Sheeba (2014). FTIR (Perkin Elmer Spectrum 100) spectrum of the powdered sample was carried out. UV-VIS spectra of methanolic extracts (10 times diluted) were obtained by scanning in the wavelength range from 200-1100nm using spectrophotometer (SPECORD 200 PLUS) at 1.0nm increments with a speed of 100nm/min using methanol as blank.

Active components in crude methanol extracts were analysed and evaluated by GC-MS using Perkin Elmer Clarus GC 620, with MS detector equipped with a non-polar HP ultra-double fused silica capillary column (30m, 0.32mm internal diameter, 0.25µm film thickness). Operating conditions were as follows: ion source of electron voltage 70eV kept at 200°C. Spectra were scanned from 50 to 600m/z with a scan time of 1.50s. The temperature ramping followed was 50 - 10°C /min - 220 °C (5 min)-1°C /min-290°C (10 min). The detector was held at 290°C and helium was used as carrier gas. Full data acquisition was obtained with the use of MS turbo mass version 5.3.2. Individual compounds were identified by comparison of mass spectra with literature and library data (NIST MS Library version: NIST MS Search 2.0), retention time of authentic standards and interpretation of mass spectrometric fragmentation patterns (Philp, 1985; Logan and Eglinton, 1994).

Antibacterial assay was carried out using the agar diffusion method (CLSI, 2012) with paper disc of 6mm diameter procured from Himedia, India. The antibacterial assay using *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212 and *Yersinia enterocolitica* ATCC 23715 were carried out using the Mueller Hilton Agar (MHA) (Himedia, India) and these strains for the study was obtained from Central Institute of Fisheries Technology, Cochin. The bacterial inocula were grown in Brain Heart Infusion (BHI) broth overnight and speared on MHA plate using sterile swab to form bacterial lawn. The 50µl extract was poured on sterile disc and left for drying at room temperature. Then discs were placed on the agar as per requirements. Plates were incubated for 18-24 hours at 37°C and zones of inhibition were measured (CLSI, 2012). Phenolics and flavonoids were estimated same as procedure mentioned in section (2.2.3).

2.3 Statistical Analysis

Statistical analysis was performed using Statistical Program for Social Sciences (SPSS version 13.0). Pearson correlation analysis was performed to identify inter-elemental relationship within seagrasses as well as its surrounding environments. All the data were normalised to create uniformity in the units of variables (Shaw, 2003). Principal component analysis (PCA) was employed to explore the origin and geochemical factors influencing the distribution of various parameters in seagrasses as well as its surrounding environments (Loska and Wiechula, 2003). The principal components, derived through varimax rotation, and significant factors within the data were established based on eigen value >0.50.

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Chapter 3

GENERAL ENVIRONMENTAL CHARACTERISTICS OF SEAGRASSES AND RELATED ECOSYSTEMS

Contents

3.1 Water Quality Parameters

3.1.1 Introduction

Distributions as well as growth of seagrasses are controlled by factors such as temperature, salinity, substratum characteristics, turbidity and sub marine irradiance of surrounding water system. Nutrients availability on the surroundings environments have influences on the morphology and seasonal cycling of seagrass communities (Sulochanan et al., 2011). Other ecological parameters related directly and indirectly on the physiology of seagrasses are light intensity and photoperiod (Thangaradjou and Kannan, 2007). The water quality is also influenced by natural phenomena's like rain fall, lagoon morphometry, etc. (Sridhar et al., 2008). Moreover the factors which strongly influence the sediment characteristics are wave action, tides, wind agitation and fresh water discharges (Thangaradjou and Kannan, 2005).

Seagrasses play a major role in maintaining the ecology by promoting secondary productivity in the surrounding region through stabilizing the sediments, producing particulate organic matter and transporting it to large varieties of other biological systems (Sridhar et al., 2008). The nutrient level in aquatic environment is generally increased through sewage and agricultural discharges, mainly nitrogen and phosphorous. Macrophytes have the ability to control the concentrations of nitrogen and phosphorous through accumulation from the aquatic system. Seagrasses by uptake through their roots and leaves controll the concentrations of these elements in the water column.

3.1.2 Results

General water quality parameters

pH of the water column exhibited no distinguishable variations and ranged from 7.70 (Mathacovil) to 8.12 (Chinnappalam) (Table 3.1.1). The salinity varied from 15*psu* (Gulf of Mannar) to 28*psu* (Palk Bay). Comparatively high turbidity was noticed at Gulf of Mannar while temperature was higher at Palk Bay, and differences were observed between Munaikkadu (35^{o}C) and Mathacovil (32.4^{o}C). The depths varied with respect to stations. The dissolved oxygen was almost same in the Palk Bay region while varied from 5.39 (Thonithurai) to 8.16mg/l (Chinnappalam) at Gulf of Mannar. The micronutrients nitrate, ammonia, phosphate, silicate, sulphate, total nitrogen and phosphorous recorded comparatively higher concentration in Gulf of Mannar whereas total hardness was higher at Palk Bay. The concentration of nitrate was same at Palk Bay while variations were noticed at Gulf of Mannar (2.73 to 5.18µmol/l). Total alkalinity varied with respect to stations. Concentrations of nitrite and total phosphorous did not exhibit any noticeable difference between stations of Gulf of Mannar

and Palk Bay. Remarkable differences in concentrations of micro nutrients were observed in the case of total nitrogen (both Gulf of Mannar and Palk Bay side), silicate (Gulf of Mannar) and ammonia (Palk Bay). Total hardness and sulphate content displayed significant variation among the stations with almost double in Palk Bay side.

Macro and trace elements

The levels of macro nutrients (sodium (Na), potassium (K), lithium (Li) and magnesium (Mg)) were relatively higher at Palk Bay than Gulf of Mannar except calcium (Ca) which showed a reverse trend. Variations were observed between stations in the case of Na and Mg. Li and K did not vary widely between the stations of Gulf of Mannar and Palk Bay. The Mg content varied from 310 to 463mg/l in Gulf of Mannar and 7750 to 8500mg/l in Palk Bay indicating a wide variation. The heavy metals studied were copper (Cu), chromium (Cr), manganese (Mn), nickel (Ni), iron (Fe), zinc (Zn), cadmium (Cd), lead (Pb), mercury (Hg), arsenic (As), selenium (Se) and cobalt (Co). Compared to Palk Bay, Cr, Zn, Se, Fe, Ni and Cd exhibited an increased value at Gulf of Mannar whereas a reverse trend was noticed in the case of Cu, Mn and Pb, and Co, Hg and As were not detected at all the stations. The contents of Cr, Ni and Zn were displayed spatial variation. Se was not detected at Palk Bay region whereas Cu only at Gulf of Mannar. Among the metals studied, Cu ranged from 0.117 to 1.027mg/l, Cr 0.265 to 2.039mg/l, Mn 0.163 to 0.330mg/l, Zn 2.06 to 11.61mg/l and Se 0.113 to 0.200µg/l. The Cd content varied from 9 to 65µg/l; while Pb ranged from 7 to 121µg/l and not detected at Chinnappalam (Table 3.1.2).Wide variations in concentrations were observed in the case of Pb compared to Cd.

Table 3.1.1 Hydrographic parameters

Parameters	Gulf of Mannar		Palk Bay	
	Thonithurai	Chinnappalam	Munaikkadu	Mathacovil
pH	7.93±0.15	8.12±0.18	8.02±0.16	7.70±0.21
Salinity (*psu*)	15.07±0.45	15.81±0.50	28.36±0.90	25.52±0.80
Turbidity (NTU)	63.6±3.50	27±2.50	2.6±0.40	3.1±0.60
Temperature (^oC)	26±0.12	26±0.15	35±0.25	32.4±0.18
Depth (m)	1.5-2.0	0.80-0.95	0.95-1.35	1.05-1.25
Dissolved oxygen (mg/l)	5.39±0.25	8.16±0.20	6.22±0.28	6.06±0.18
Nitrite (µmol/l)	0.15±0.03	0.11±0.02	0.06±0.01	0.018±0.02
Nitrate (µmol/l)	2.73±0.16	5.18±0.15	3.07±0.29	3.27±0.25
Ammonia (mg/l)	21.67±2.25	22.45±2.50	7.49±1.50	10.65±1.80
Phosphate (mg/l)	0.66±0.02	0.76±0.03	0.106±0.02	0.245±0.03
Sulphate (mg/l)	3654±0.28	3546±0.29	2363±0.21	2415±0.20
Silicate (mg/l)	22.39±2.24	10.89±2.18	1.72±1.25	1.07±1.50
Total Nitrogen (µmol/l)	109.91±3.50	126.90±4.50	15.23±1.25	22.54±1.50
Total Phosphorous (mg/l)	15.14±3.00	15.92±2.80	3.13±0.85	3.33±0.90
Total Hardness (mg/l)	5148±3.0	4924±3.5	10000±4.5	9500±3.5
Total Alkalinity (mg $CaCO_3$/l)	94±0.90	101±0.95	90±0.85	116±0.95

Table 3.1.2 Macro elements and trace metals in water (mg/l)

Elements	Gulf of Mannar		Palk Bay	
	Thonithurai	Chinnappalam	Munaikkadu	Mathacovil
Na	4450±0.85	4305±0.90	6600±1.50	7250±1.80
K	250±0.30	245±0.25	350±0.35	310±0.40
Ca	4686±3.00	4615±2.80	1750±1.50	1500±1.2
Li	0.8±0.11	0.9±0.12	2.40±0.22	2.20±0.24
Mg	463±1.00	310±1.50	7750±2.00	8500±2.00
Cu	0.117±0.04	ND	0.319±0.09	1.027±0.22
Cr	0.265±0.12	2.039±0.60	0.606±0.25	0.317±0.18
Mn	0.163±0.14	0.206±0.18	0.288±0.20	0.330±0.23
Ni	0.227±0.12	0.561±0.25	0.198±0.14	0.375±0.22
Fe	4.60±0.60	5.30±0.90	0.198±0.25	0.375±0.35
Zn	4.74±0.80	11.61±0.95	6.38±0.75	2.06±0.65
Se*	0.200±0.03	0.113±0.02	ND	ND
Co	ND	ND	ND	ND
Cd*	21±0.25	65±0.70	9±0.10	15±0.20
Pb*	7±0.10	ND	19±0.20	121±0.45
Hg	ND	ND	ND	ND
As	ND	ND	ND	ND

* µg/l

3.1.3 Discussion

Water quality parameters

Both at Gulf of Mannar and Palk Bay, a large numbers of rivers are connected to the sea and also drainages, which pass out their excess water into the sea. Comparatively higher rain fall during northeast monsoon noticed at Pamban and Mandapam regions than other areas while lower rain fall during southeast monsoon than other regions (Muthukrishnan et al., 2013). The currents patterns at Gulf of Mannar are swift and sea is rough from April to August, it goes to extreme levels during June to September (ENVIS, 2015). Both Gulf of Mannar and Palk Bay are joined at Pamban

and, the direction of current was clock wise during southwest monsoon while it was in anticlockwise direction during northeast monsoon (Sulochanan et al., 2011; Gowthaman et al., 2013; ENVIS, 2015).

The water was observed to be alkaline in all sites and was found to be higher than earlies reports while variations in surface temperature and salinity was in line with similar studies carried out earlier (Sulochanan and Muniyandi, 2005; Sridhar et al., 2006; Asha and Diwakar, 2007). Decrease in temperature was observed in Gulf of Mannar sample which could be related to the time of collection of sample (monsoon) and also could be attributed to the rainfall by the northeast monsoon during the period of this study. In general, turbidity was comparatively higher during monsoon (Sridhar et al., 2006, 2008) and it might be due to monsoon wave action, tides, wind agitation, fresh water discharges etc. stirring up the bottom portion. Dissolved oxygen (DO) also recorded only marginal differences again could be related to seasons stated above. The higher concentrations of nutrients viz., nitrate, nitrite, phosphate and silicate has already been reported during monsoon (Sridhar et al., 2006), and it compares well with the monsoon season in the Gulf of Mannar samples. An increase in salinity in Palk Bay might be due to an increase in evaporation rate in summer and variations in DO could be due to increase in temperature as well as salinity which strongly influence the dissolution of oxygen (Sampath Kumar and Kannan 1998).

The surface water temperature, salinity and dissolved oxygen were comparatively lower during northeast monsoon in the present study and were comparable with previous observations (Rajapandian et al., 1990; Thangardjou and Kannan, 2005; Kannapiran et al., 2008). It was obvious

that water quality parameters of Munaikkadu (Palk Bay) characterized by slightly lower pH and salinity; while DO, surface temperature and nutrients (nitrate, nitrite, phosphate and silicate) were relatively higher. Value corresponds to temperature, salinity, density and DO content of surface waters of Palk Bay were influenced by the influx through Palk Strait from the Bay of Bengal side. It has already been reported that DO content is relatively enriched in coral Reef environment than purely seagrass environment and is a common feature in coastal ecosystems (Sridhar et al., 2008). Higher DO levels indicated enhanced productivity during the period (Kannan and Kannan, 1996). Comparatively higher concentrations of nutrients were recorded in monsoon season was attributed to land runoff (Marsh, 1985; Kannan and Kannan, 1996; Sridhar et al., 2008) sewage discharges, ground water seepage (D'Elia et al., 1981), and low nutrients at summer due to the larger phytoplankton population. Generally, the non essential reactive silica was higher during monsoon compared to summer season and its importance changed to essential regarding to organisms like diatoms associated with the seagrass leaves (Kannapiran et al., 2008). No differences were noticed in the water quality parameters from Bardawil lagoon (Abd El-Hady and Khalifa, 2015) and the concentrations of pH and nitrite were higher whereas phosphate and nitrate lower than the present study. No wide variations were observed in the salinity, DO, temperature and pH of Southern Indian Ocean Island, Mauritius (Ramah et al., 2014).

Among the hydrographical parameters analysed pH, salinity and DO showed no significant variation. The high DO content was attributed due to the photosynthetic activity of zooxanthellae while observed lower levels could be due to decrease in photosynthesis and increased respiration of bottom communities. Phosphate reduction during the summer might be due to

an increased utilization by phytoplankton and zooxanthellae. The major controlling factors for the distribution of inorganic and organic nitrogen compounds are photosynthetic organisms and microbes formed by mineralization, oxidation and reduction of nitrogenous components. The nutrients as well as surface water temperature were higher in the purely seagrass area than seagrass along with coral Reef whereas salinity, pH and dissolved oxygen higher at coral Reef with seagrass areas (Kannapiran et al., 2008). Relatively high sea surface temperature, pH and salinity was noticed at Thonithurai and Farm pond (Munaikkadu) (Sulochanan et al., 2011) and these parameters were similar to the present study except salinity at Farm pond. Maximum ammonia, phosphate and nitrate observed at Gulf of Mannar in the present study were similar to earlier report (Sulochanan et al., 2011). The DO levels varied widely at most of the stations except at Thonithurai and it might be due to water mixing at Pamban (opposing currents) as well as cultivation of seaweeds. The nutrients were observed in relatively higher concentration at Shangumal except nitrate (Thonithurai) whereas phosphate and nitrite lower at Farm Pond and rest of them at Kundhukaal. The depleted levels of DO along with high nitrite content indicated the decomposition process triggered by algae and other microorganisms (Sulochanan et al., 2011).

Maximum surface water and meadow temperatures might be due to dry high radiation and clear sky (Ganesan 1992; Govindaswamy et al., 2000).The temperature had strong influence on the distribution of seagrasses and at the extreme cases it showed “heat burn” systems to species shift (Short and Neckles, 1999). The salinity at Palk Bay in this study ranged from 25.52 to 28.36*psu* and it was in line with appropriate salinity for the growth, abundance, species richness as well as seagrasses distribution

(ranged from 24 to 35*psu*) along Coromandal coast reported earlier (Rajeswari, 1991). Salinity varied with respect to differences in areas and time, and it ranged from 30 to 40*psu* (Arumugam et al., 2012), 32 to 34*psu* (Thangardjou and Kannan, 2005), 29 to 39*psu* (Thangardjou, 2000), 29.6 to 37.3*psu* (Asha and Diwakar, 2007) and 21.9 to 34.3*psu* (Sulochanan and Muniyandi, 2005), and it played an important role in the abundance and distribution of seagrasses as well as organisms living on seagrasses. The deviations in pH values could be related to removal of carbon dioxide by photosynthesis, dilution by land run off during rainy seasons leading to a decrease in salinity as well as temperature (low primary productivity) and decomposition of organic materials (Rajasekhar, 2003).The growth and reproductive capacity of seagrasses depend upon the availability of nutrients in the sediments and water column. High nutrient concentrations at Gulf of Mannar are more in line with similar studies reported earlier (Kannan and Kannan, 1996; Sridhar et al., 2006, 2008; Thangaradjou and Kannan, 2008).

Surface water temperature was relatively higher at Chinnapalam than Thonithurai whereas turbidity ranged from 10.4 to 14.7NTU. Relatively high turbidity was reported at coastal stations of Thonithurai, Tuticorin and Kathuvallimuni Reef and it might be due to the mixing up of silt and clay particles due to wave actions at coastal areas (Thangaradjou and Kannan, 2005). Along with wind agitation and freshwater discharges leading to resuspending of bottom settled particles as well as fine sand and mud particles in shallow coastal areas (Kannan, 1992). In the present study, wide variations in salinity, pH and nutrients compared well with earlier report (Thangaradjou and Kannan, 2005). Relatively low levels of sea surface temperature, salinity, phosphate, nitrate and nitrite were noticed at

Thonithurai and Chinnappalam whereas pH and turbidity was higher. The greater utilization by phyto benthic communities could be the reason for low phosphate content (Kannan and Kannan, 1996).

Remarkably higher nutrient content at Chinnappalam was due to discharge of domestic wastes and human excreta at coastal areas. The concentration of nitrate and nitrite at Chinnappalam were comparatively lower in the present study while no wide variations were observed in phosphate content compared with earlier report (Thangaradjou and Kannan, 2007). Relatively lower total nitrogen to phosphorous ratio (4.87 to 7.97) was observed in the present study related to earlier report (Williams et al., 2009). Accordingly higher total nitrogen content in water samples of subtropical seagrass estuary (Florida Bay) (0.29 to 1.14gN/m^2) and phosphorous (11.7 to 61.9mgP/m^2) and a high total nitrogen to total phosphorous ratio (33 to 111). Comparison of hydrographical parameters of seawaters of unpolluted (a clean area in Palk Bay) and polluted areas (polluted by effluents of fish processing units, aquaculture farms, disposal site of sewage etc) of Palk Bay and Gulf of Mannar showed that DO from unpolluted area was less than polluted area of Sethukari estuary of Gulf of Mannar, and also DO was comparatively higher at Gulf of Mannar. The nitrogen content in waters from Gulf of Mannar compared well with the study at Banten Bay, Indonesia (0.92 to 1.85mg/l) (Suwandana et al., 2011) while much lower concentrations were recorded at Palk Bay. Among the hydrographic parameters, pH of the present study was in line with similar other studies carried out while dissolved oxygen and silicate were slightly higher and salinity was lower (Table 3.1.3) (Sridhar et al., 2006, 2008; Kannapiran et al., 2008; Sulochanan et al., 2011).

Table 3.1.3 Comparison of water quality parameters.

Sl no.	Location	pH	Salinity(psu)	Temperature(°c)	Dissolved oxygen (mg/l)	Nitrate (µmol/l)	Nitrite (µmol/l)	Ammonia (mg/l)	Phosphate (mg/l)	Sulphate (mg/l)	Silicate (mg/l)	Total nitrogen(µmol/l)	Total phosphorous(mg/l)	References
1	Palk Bay	7.95-8.35	26 -34.5	28-32.5	4.15-7.18	2.15-3.28	0.12-0.62	--	1.28-2.15	--	5.15-12.52	--	--	Shridhar et al., 2006
2	Palk Bay	7.25-8.20	28-36	25-31.5	3.15-6.18	0.05-6.7	0.04-5.48	--	0.64-9.38	--	2.15-12.48	--	--	Shridhar et al., 2008
3	Gulf of Mannar	7.8-8.9	26.9-36.4	24.5-35.5	4-8.5	0.46-2	--	--	0.005-0.46	--	--	1.6-8.3	0.23-1.68	Kannanpiran et al., 2008
4	Gulf of Mannar & Palk Bay (Thonithuraii & Farm pond)	7.44-8.44	36.4-37.4	32.2-35	1.05-5.9	0.623-2.03	0.296-0.548	3.81-29.36	0-1.23	--	1.998-5.839	--	--	Sulochanan et al., 2011
5	Gulf of Mannar (Thonithuraii & Chinnappalam)	7.1-7.6	32-34	33.5-34	--	4.3-6.54	1.13-3.24	--	2.81-3.60	--	--	--	--	Thangaradjou and Kannan, 2005
6	Gulf of Mannar (Chinnappalam)	--	--	--	--	5.2-18.78	1.92-7.32	--	2.32-7.32	--	--	--	--	Thangaradjou and Kannan, 2007
7	Gulf of Mannar	--	30-40	--	--	--	--	--	--	--	--	--	--	Arumugam et al., 2012
8	Gulf of Mannar	--	29-39	--	--	--	--	--	--	--	--	--	--	Thangardjou, 2000
9	Tuticorin	--	29.6-37.3	--	--	--	--	--	--	--	--	--	--	Asha and Diwakar, 2007
10	Gulf of Mannar & Palk Bay	--	21.9-34.3	--	--	--	--	--	--	--	--	--	--	Sulochanan and Muniyandi, 2005
11	Bardawil Lagoon, Egypt	8-8.36	--	21-30	4.83-11.72	0.16-2.42	0-0.32	--	0.002-0.018	--	--	--	--	Abd El-Hady and Khalifa, 2015
12	Gulf of Mannar	7.93-8.12	15.07-15.81	26	5.39-8.16	2.73-5.18	0.11-0.15	21.67-22.45	0.66-0.76	3546-3654	10.89-22.39	109-126	15.14-15.92	Present study
	Palk Bay	7.7-8.02	25.52-28.36	32.4-35	6.06-6.22	3.07-3.27	0.018-0.06	7.69-10.65	0.106-0.245	2363-2415	1.07-1.72	15.23-22.54	3.13-3.33	Present study

Trace elements

Relatively lower levels of trace elements were noticed in this study compared to earlier reports (Table 3.1.4) (Lewis et al., 2007; Sulochanan et al., 2007; Govindaswamy et al., 2011; Suwandana et al., 2011; Lei and Xiaoping, 2012; Malltezi et al., 2012). Level of trace metal contamination in the areas from Palk Bay, Gulf of Mannar and Rameswaram were studied and areas were polluted due to effluents discharge from fish processing units, aquaculture farms and disposal site of sewage. Trace elements concentration followed the order Zn>Cu>Pb>Cd (Sulochanan et al., 2007). Spatial variations were observed in the levels of Cd and Pb from near to off shore irrespective of waters collected from bottom as well as surface (Palanichamy and Rajendran, 2000). Heavy metals were relatively higher concentrations in the Saranda Bay, Albania and followed the order Cu>Pb>Cd>Cr (Malltezi et al., 2012). Large variations were observed between stations in the distribution of metals from northwestern Mediterranean, where Co, Ni and Pb were higher while Cd and Cr showed lower concentrations than the present study (Lafabrie et al., 2007). Trace elements in the Banten Bay of Indonesia followed the order Fe>Zn>Cu>Pb=Cd (Suwandana et al., 2011).

Heavy metals distributions in the water column is strongly influenced by factors such as rainfall, ocean currents, winds, etc and its effect reflect immediately in the water column (Govindaswamy et al., 2011). Variations were noticed in the distribution of trace metals between stations in the present study and it was in line with the earlier report (Govindaswamy et al., 2011).

Table 3.1.4 Comparison of trace elements in water (mg/l).

Sl No.	Location	Cu	Cr	Mn	Ni	Fe	Zn	Se	Co	Cd	Pb	Hg	As	References
1	Gulf of Mannar and Palk Bay	5.3-8.3	--	--	--	--	30-120	--	--	0.18-0.35	2.3-4.5	--	--	Sulochanan et al., 2007
2	Sarandra Bay, Albania	9.3-72	0.70-1.70	--	--	--	--	--	--	.98-3.40	1.90-4.40	--	--	Maltazi et al., 2012
3	North western Mediterranean	--	0.152-0.616	--	0.197-1.380	--	--	--	--	0.006-0.016	0.038-0.075	--	--	Lafabrie et al., 2007
4	Banten Bay, Indonesia	0-34	--	--	--	0-37	0-34	--	--	N.D.	N.D.	--	--	Suwandana et al., 2011
5	Bay of Bengal (Palk strait)	0.05-0.70	--	0.02-0.89	--	0.12-0.30	0.03-60	--	--	--	--	--	--	Govindasamy et al., 2011
6	Gulf of Mannar	0.21-0.45	--	0.04-0.13	--	7.89-11.7	1.42-2.85	--	--	--	0.93-1.28	--	--	Yogesh Kumar and Geetha, 2012
7	Atlantic French coast	9	--	--	< 1.0	15	58.9	--	--	--	--	--	--	Wasserman and Wasserman, 2002
8	South China	2.43-3.89	--	--	--	--	13.66-31.69	--	--	0.06-0.56	1.47-8.04	--	--	Lei and Xiaping, 2012
9	Gulf of Mexico	13.9-61.1	< 0.8-25.4	--	< 0.9-20.6	--	< 0.3-66.4	--	--	--	< 0.6-16.7	N.D.	36.3-75.6	Lewis et al., 2007
10	Yellow river estuary, China	3.11	1.31	80.50	--	--	5.66	--	--	0.83	1.60	0.01	1.41	Lin et al., 2016
11	Gulf of Mannar Palk Bay	0-0.117 0.319-1.027	0.265-2.039 0.317-0.606	0.163-0.206 0.288-0.330	0.227-0.561 0.198-0.375	4.6-5.3 0.198-0.375	4.74-11.61 2.06-6.38	0.113-0.200 N.D.	N.D N.D.	0.021-0.065 0.009-0.015	0-0.007 0.019-0.121	N.D. N.D.	N.D. N.D.	Present study Present study

Among the metals studied Zn, Fe and Pb were higher in concentrations whereas Cu and Mn concentrations were very low. Comparatively high content of heavy metals related with environmental conditions mainly rain water runoff as observed during monsoon. The northward movement of sediments and currents along with continuous resuspension of bottom sediments tends to scavenge as well as an increase in metals presented in the water column (Yogesh Kumar and Geetha, 2012). It might be the reason for relatively high content of heavy metals noticed at Gulf Mannar in the present study. Distribution pattern of trace metals and their levels were varied with respect to sampling time and it was similar with earlier report (Lei and Xiaoping, 2012). Trace metals contents in surface waters of Gulf of Mexico followed the order As>Cu>Zn>Ni>Cr>Pb>Hg (Lewis et al., 2007). Heavy metals content in surface seawater followed the trend; Mn>Zn>Cu>As>Cr>Cd>Hg and comparatively higher levels were noticed in seagrasses area than nonseagrasses (Lin et al., 2016). All these metals were higher than the present study except Cr from Chinnappalam of Gulf of Mannar.

3.2 Sediment Quality Parameters

3.2.1 Introduction

Ecological importance of seagrasses includes stabilizing sediments, producing particulate organic matter and transporting these to various biological systems leading to an increase in secondary productivity in the related regions (Sridhar et al., 2008). Besides water quality parameters, sediments characteristics excert an important role in the morphology of seagrasses (Thangarajou and Kannan, 2005) because underground vegetative

portions of seagrasses contributes around 60-80% of the total biomass (Arumugam et al., 2012). This contributes to reduction in the sediment erosion and increase the water clarity by settling the suspended materials to the bottom (Mckenzie, 2008). Seagrass ecosystems act as a source of carbon sink, and the efficiency of these meadows are directly depend upon the species composition as well as surrounding environmental characteristics. Sediment size have an important role in the carbon storage and a fine size favours aggregation of organic particles, level of organic matter, oxygen levels, nutrient contents and microbial community composition (Dahl et al., 2016).

3.2.2 Results

Sedimentary parameters

Textural characterestics of the sediments followed the order, sand>silt>clay. Sand contents varied from 72.02 to 97.06%, silt 1.83 to 24.91% and clay 1.01 to 3.07% (Table 3.2.1). The sand content showed no remarkable variation between stations except from Chinnappalam. Clay and silt composition were comparatively higher at Chinnappalam followed by Thonithurai. Total organic carbon content varied from 0.24 to 0.58% and relatively higher concentrations were noticed at Gulf of Mannar than Palk Bay. The pH was alkaline at Gulf of Mannar (7.11-7.46) while it was acidic in the Palk Bay. The temperature was comparatively higher at Palk Bay (31-33.2) than Gulf of Mannar (24.1-24.4). Total phosphorous (TP) varied from 0.78 to 1.87% while total nitrogen was not detected in all the stations.

Macro and trace elements

The macro elemental levels were varied between the sampling locations and Na, Ca, Li and Mg contents were showed increased values at Chinnappalam while K showed higher values at Mathacovil site. The metals (Na, K, Ca, Mg and Li) concentrations in the sampling points followed the order: Chinnappalam>Munaikkadu>Thonithurai>Mathacovil. K content was higher at Mathacovil and lower at Munaikkadu and followed the trend: Mathacovil (0.75mg/g)>Thonithurai (0.60mg/g)>Chinnappalam (0.45mg/g)> Munaikkadu (0.36mg/g). An increased Zn and Co contents were noticed at Thonithurai (2.72mg/kg and 1.24mg/kg) and lower at Chinnappalam, and no major differences were observed at Munaikkadu and Mathacovil. The concentration of Cr, Mn and Fe revealed an increased values at Chinnappalam (6.40, 64.42 and 1195mg/kg) and lower at Mathacovil (4.82, 42 and 965mg/kg) (Table 3.2.2). Similar concentrations were noticed from Munaikkadu and Mathacovil in the case of Mn whereas Cr concentration was almost same except at Chinnappalam. Higher Ni and Cu concentrations were found at Thonithurai (2.02 and 2.16mg/kg) while lower Ni content at Munaikkadu (1.22mg/kg) and Cu at Mathacovil (1.55mg/kg). Among the toxic elements, Cd varied from 0.237 to 0.311mg/kg while Pb ranged from 0.540 to 0.811mg/kg. The maximum content of Cd was obtained at Chinnappalam and Pb at Munaikkadu. Spatial variation of Cd followed the order; Chinnappalam>Munaikkadu>Mathacovil>Thonithurai whereas Pb exhibited the trend: Munaikkadu>Thonithuraii>Mathacovil>Chinnappalam. As content was relatively higher at Gulf of Mannar than Palk Bay whereas variations were lesser in the levels of Hg between stations.

Table 3.2.1 Sedimentary parameters

Location	Sand (%)	Clay (%)	Silt (%)	TOC (%)	pH	Temperature (^{o}C)	Total P (%)
Thonithurai	93.60±0.29	1.96±0.30	4.44±0.28	0.42±0.18	7.11±0.09	24.4±0.08	1.44±0.42
Chinnappalam	72.02±0.38	3.07±0.25	24.91±0.33	0.58±0.16	7.46±0.07	24.1±0.07	0.78±0.30
Munaikkadu	96.60±0.23	1.01±0.17	2.39±0.28	0.24±0.12	6.60±0.12	33.2±0.11	1.83±0.48
Mathacovil	97.06±0.22	1.11±0.18	1.83±0.24	0.30±0.10	6.46±0.14	31.0±0.14	1.87±0.52

Table 3.2.2 Macro elements (mg/g) and trace metals in sediment (mg/kg)

Elements	Gulf of Mannar		Palk Bay	
	Thonithurai	Chinnappalam	Munaikkadu	Mathacovil
Na	2.58±0.18	5.83±0.25	4.15±0.24	2.49±0.20
K	0.60±0.14	0.45±0.12	0.36±0.16	0.75±.18
Ca	4.90±0.30	8.94±0.40	7.70±0.35	3.20±0.28
Li*	22±0.15	56±0.20	54±0.18	11±0.12
Mg	2.18±0.28	5.17±0.45	2.94±0.30	1.07±0.25
Cu	2.16±0.30	1.94±0.25	1.68±0.24	1.55±0.22
Cr	4.86±0.28	6.40±0.35	4.92±0.30	4.82±0.25
Mn	52.95±0.40	64.42±0.40	42.68±0.50	42±0.50
Ni	2.02±0.20	1.56±0.15	1.22±0.14	1.41±0.16
Fe	1095±1.00	1195±1.00	1040±0.60	965±0.60
Zn	2.72±0.25	1.34±0.20	2.24±0.20	2.01±0.22
Se	ND	ND	ND	ND
Co	1.24±0.30	0.81±0.25	0.91±0.20	0.84±0.18
Cd	0.237±0.03	0.311±0.03	0.276±0.02	0.238±0.02
Pb	0.679±0.24	0.540±0.21	0.811±0.38	0.618±0.25
Hg**	30±0.20	11±0.18	9±0.15	ND
As	7.66±0.45	8.62±0.55	ND	3.28±0.35

*mg/kg, **µg/kg

3.2.3 Discussion

Sediment quality parameters

pH of the sediments were slightly acidic in nature at Palk Bay during all seasons and it was comparable with previous studies (Table 3.2.3)

(Thangaradjou and Kannan, 2005; Arumugam et al., 2012). Acidity of the sediments was attributed to the decomposition of seagrasses and seaweeds (Kannan et al., 1998; Thangaradjou, 2000) as well as to the low carbonate contents in the seagrass dominated sediments (Vinith Kumar et al., 1999). The abundance of seagrass species and its distributions were favored by the sandy nature of sediment (Balakrishnan Nair et al., 1983; Thangaradjou and Kannan, 2005, 2007). The textural characteristics of the sediment followed the order; sand>silt>clay in the present study was compared to other studies carried out (Vinithkumar et al., 1999; Thangaradjou and Kannan, 2005, 2007; Arumugam et al., 2012). The suitable habitat for the growth and establishment of seagrasses were sand, mud or sand and mud with thin layers of sand (Rajeswari and Kamala 1987). Seagrasses were growing in 100% sand reported by Jagtap and Untawale (1984) at Lakshadeep while it was noted as 97% at Palk Bay and 72-94% at Gulf of Mannar in this study. Distribution and growth of seagrasses are directly influenced by sediment characteristics because nearly 60-80% of portions of seagrasses grow in the sediments (Arumugam et al., 2012). The total contribution of clay and silt was less than 7% (Arumugam et al., 2012), 12% (Thangaradjou and Kannan, 2005) and 18% (Thangaradjou and Kannan, 2007); while it was 6.4 to 27.98% at Gulf of Mannar and 2.84 to 3.5% at Palk Bay. Also a positive correlation was observed with silt and pH. Silt as well as clay compositions were comparatively high at coastal areas, and it could be due to the mixing up of silt and clay by wave action, tides, wind agitation and fresh water discharges (Kannan, 1992).

Table 3.2.3 Comparison of sediment parameters.

Sl No.	Location	Sand%	Clay%	Silt %	TOC%	pH	Temperature (°c)	C %	P %	N %	Na (mg/g)	K (mg/g)	Ca (mg/g)	Li (mg/g)	Mg (mg/g)	References
1	Gulf of Mannar	93-98.5	0.50-2.50	1-5	--	6.3-7.1	21-30	--	--	--	--	--	--	--	--	Arumugam et al., 2012
2	Gulf of Mannar	88.78-96.83	1.07-3.7	2.10-7.52	--	6.5-7	3.14-33.5	--	--	--	--	--	--	--	--	Thangaradjou & Kannan, 2005
3	Gulf of Mannar	81.93-93.66	2.11-5.65	4-12.42	--	--	--	--	--	--	--	--	--	--	--	Thangaradjou & Kannan, 2007
4	Gulf of Mannar	87.55	1.75	6.23	0.138-0.911	7.85-8.71	--	--	3.66-23.64µmol/g	19.5-266 µmol/g	--	--	--	--	--	Vinithkumar et al., 1999
5	South east coast of India	44.2-74.9	4.1-21	20.8-42.8	--	7.5-8.9	--	--	0.010-0.058	0.299-0.705	13.75-43.76	11.03-16.88	57-400	--	--	Sankaran et al., 2014
6	Bay of Bengal	--	--	--	0.05-6.14	--	--	--	0.10-0.83	1	24.3-64.4	3.6-25.6	26.2-73.2	--	0.4-2.3	Sundararajan & Natesan, 2010
7	Florida Bay	--	--	--	0.40-7.6	--	--	--	0.0004-0.0034	N.D.	--	--	--	--	--	Fourqurean et al., 2012
									0.0003-0.0045							
	Shark Bay				0.20-8.6					0.03-0.54						
8	Banten Bay	--	--	--	--	--	--	--	--	0.27-0.79	--	--	--	--	--	Suwandana et al., 2011
9	Gulf of Mannar	72.02-93.60	1.96-3.07	4.44-24.91	0.42-0.58	7.11-7.46	24.1-24.4	2.06-8.75	0.078-0.144	N.D.	2.58-5.83	0.45-0.60	4.9-8.94	0.022-0.056	2.18-5.17	Present study
	Palk Bay	96.60-97.06	1.01-1.11	1.83-2.39	0.24-0.30	6.46-6.60	31-33.2	1.66-2.86	0.183-0.187	N.D.	2.49-4.15	0.36-0.75	3.2-7.7	0.011-0.054	1.07-2.94	Present study

Nutrient concentrations and silt contents were directly linked with diversity as well as density of seagrasses and very low silt content was noticed at most of the stations except at Chinnappalam. Siltation occurred by the operation of fishing vessels and natural causes such as monsoon winds, and might be the reason for high silt at Chinnappalam. Also the rate of sedimentation was higher during the month of June, southwest monsoon season (Thangaradjou and Kannan, 2007). According to Palanichamy and Rajendran (2000), sand was the major component in five stations of South East coast of India and it contained very fine sand, fine, medium, coarse, very coarse and granule. The silt and clay concentrations decreased from near shore to off shore at most of the stations. Sedimentation of silt in the coral Reef environment of Palk Bay and it ranged from 1mg/cm^2/d to 42mg/cm^2/d (Wilson et al., 2005). Comparatively higher concentrations of total organic carbon (TOC) content were obtained at vegetated areas than adjacent non vegetated while sand content variations were less at most of the stations of vegetated and adjacent non vegetated areas, and relatively higher contents of silt noticed at non vegetated side. Also relatively higher clay content obtained at non vegetated area. TOC was relatively lower concentrations in the present study than earlier reports (Vinithkumar et al., 1999; Sundrarajan and Natesan, 2010; Arumugam et al., 2012; Fourquren et al., 2012). The texture composition revealed that sand was the major component followed by coarse silt and medium, fine slit and clay at the lowest. Sediment salinity varied from 9 to 42$^0/_{00}$ with maximum observed at seagrass beds and minimum at coral Reef. pH showed alkaline in nature and coral Reef areas showed higher pH than seagrass beds, and it might be due to the deposition of inorganic carbon as calcium carbonate. TOC content

followed the order: seagrass beds>adjacent area>coral Reef zone, and relatively high TOC at seagrass beds might be due to high benthic biodiversity and decayed seagrasses retained at the sediment, along with excrete products from migratory fishes, crustaceans and mollusks. Relatively high slit and clay as well as TOC at Gulf of Mannar were due to flow of water with high nutrients with influx of organic matter from Palk Bay to Gulf of Mannar through Pamban Pass (Vinithkumar et al., 1999).

Total Nitrogen (TN) showed more variations than TOC, and low nitrogen content seen at seagrass beds could be due to utilization of nitrogenous compounds as well as low nitrogen fixation. Nitrogen assimilated in sediments by benthic algal communities through nitrogen fixation process and an inverse relationship was observed in TN with salinity at seagrass beds. Total phosphorous (TP) concentrations followed the order coral Reef>seagrass beds>adjacent area. In seagrass beds an inverse order was found with TP and TOC, and low TP at seagrass beds due to the absorption of phosphorous by sediment for the growth of seagrasses along with release to the water column. TOC/TP ratio was highest at seagrass beds and lowest at coral Reef while the reverse order noticed in the case of N:P ratio (Vinithkumar et al., 1999). The alkaline nature of sediment was due to high water column temperature which tends to decrease the solubility of carbon dioxide. Relatively lower organic carbon could be due to low primary productivity and strong water currents, and nitrogen content was predominated over phosphorous (Sankaran et al., 2014). Variations in the concentration of TOC were attributed to the nature of sediment and rate of sedimentation (Sundararajan and Natesan, 2010). TOC in sediments varied from 111 to 321gcm^{-2} whereas TN 9.4 to 27.2gNm^{-2} and TP 212 to

1623mgPm^{-2}. TN:TP ratio ranged from 28 to 105. Total nitrogen in sediments of Banten Bay, Indonesia varied from 0.27 to 0.79% (Suwandana et al., 2011). The surface (cores) sediments of Florida Bay, nitrogen was not detected whereas phosphorous and organic carbon were varied while at Shark Bay exhibited a higher concentrations of nitrogen, phosphorous and of organic carbon respectively (Fourqurean et al., 2012).

Macro and trace elements

Among the macro elements, Ca is the element associated with carbonate minerals while Mg is the element associated with silicate. In the present study, macro elements followed the order Ca>Na>K>Mg and it was in line with earlier reports (Table 3.2.4) (Sundararajan and Natesan, 2010; Vinithkumar et al., 1999). K content in sediments in this study (0.036 to 0.075%) are lower than reported value in Thondi area (1.103 to 1.688%) (Sankaran et al., 2014). Relatively higher levels of K, Na and Ca were reported to be associated within seagrass beds than coral Reef, and K content in seagrass beds were directly proportional to the TOC content (Vinithkumar et al., 1999). Macro elements in the sediments of *Z. marina* followed the order: Na>Ca>Mg>K and comparatively more differences were observed in the case of Na and Ca (Lyngby and Brix, 1983). The trace elements individual levels were relatively lower in the present study compared to earlier studies carried out (Palanichamy and Rajendran, 2000; Lafabrie et al., 2007; Sulochanan et al., 2007; Suwandana et al., 2011; Malltezi et al., 2012; Yogesh Kumar and Geetha, 2012).

Table 3.2.4 Comparison of trace elements in sediment (mg/kg)

Sl. No	Location	Cu	Cr	Mn	Ni	Fe	Zn	Se	Co	Cd	Pb	Hg	As	Reference
1	Gulf of Mannar	10.60-25.41	--	--	--	--	8.75-21.10	--	--	1.59-1.66	24.80-55	--	--	Sulochanan et al., 2007
2	Gulf of Mannar and Palk Bay	--	--	--	--	--	--	--	--	0-4.3	0-40	--	--	Palanichamy & Rajendran, 2000
3	Sarandra Bay	13-44.3	31.4-75.6	--	--	--	--	--	--	0.36-1.34	2.12-49	--	--	Maltezi et al., 2012
4	North Western Mediterranean	--	9-1194	--	4-1325	--	--	--	2.5-55.33	.03-40	4.67-44.50	--	--	Lafabrie et al., 2007
5	Banten Bay	3.29-10.44	--	--	--	4100-105800	78.6-232.4	--	--	.030-3.13	< .06-17.34	--	--	Suwandana et al., 2011
6	Bay of Bengal	13- 45.50	--	29.40-61.50	--	16.50-74.50	21.40-49.50	--	--	--	--	--	--	Govindaswamy et al., 2011
7	Gulf of Mannar	--	--	529-642	--	3189-5484	64.3-88.9	--	--	.04-.33	16.5-22.8	--	--	Yogesh Kumar and Geetha, 2012
8	Atlantic French coast	3.13	--	52.2	--	3396	58.9	--	--	--	--	--	--	Wasserman and Wasserman, 2002
9	South China	1.01-7.98	--	--	--	--	6.61-63.87	--	--	.20-.62	4.13-13.71	--	--	Lei and Xiaoping, 2012
10	Mediterranean sea	23	--	--	--	--	79	--	--	83	134	--	--	Schlacher -Hoenlinger and Schlacher, 1998 a
11	Gulf of Mexico (Vegetated) (Non- vegetated)	0.6-70.8 < 0.08-0.44	1.1-6.2 < 0.2-2.3	-- --	0.30-17.3 < 0.12-0.19	-- --	3-10 2.2-3.5	< 0.5-0.62 < 0.5-0.87	-- --	< 0.2-0.35 < 0.2	0.74-28.8 0.21-0.70	< 0.004- 0.018 < 0.004- 0.057	0.83-17 0.16- < 1.5	Lewis et al., 2007
12	Yellow river estuary, China	16.69-21.38	52.30-56.77	499-556	--	--	50.41-63.08	--	--	0.014-0.015	17.3-21.09	0.027-0.041	9.92-11.37	Lin et al., 2016
13	Black Sea, Turkey	7	--	--	--	1300	21	--	--	0.006	4	0.015	16	Bat et al., 2016
14	Gulf of Mannar Palk Bay	1.94-2.16 1.55-1.68	4.86-6.4 4.82-4.92	52.95-64.42 42-42.68	1.56-2.02 1.22-1.41	1095-1195 965-1040	1.34-2.72 2.01-2.24	N.D N.D	0.81-1.24 .84-.91	0.237-0.311 0.238-0.276	0.540-0.679 0.618-0.811	0.011-0.030 N.D 0.009	7.66-8.62 3.28-3.62	Present study

Stations wise variations were exhibited in trace metals Cd, Cu, Pb and Zn from the Gulf of Mannar and Palk Bay and no remarkable differences were obtained in the case of Cd, and followed the order Pb>Cu>Zn>Cd (Sulochanan et al., 2007). Cd and Pb content declined from nearshore to offshore at most of the stations with Pb predominating over Cd, and the metal concentration followed the trend: Cr>Pb>Cu>Cd (Palanichamy and Rajendran, 2000).

The accumulation of heavy metals in sediments was affected by rain fall, ocean currents, wind and geographical conditions. Variations in the distribution of Mn, Fe, Cu and Zn were observed between stations (Govindaswamy et al., 2011). Trace metals displayed metals to metals variations in their concentrations at sediments in the present study and was similar to early investigation (Yogesh Kumar and Geetha, 2012). In marine environment, Fe and Mn form precipitate easily whereas Zn, Cd, Cu and Pb would take time for precipitation. Also metal minerals in the sediments had the capacity to absorb trace elements from the water column. The factors affecting the distribution of metals in the Gulf of Mannar include the presence of iron oxides and anthropogenic activities (Yogesh Kumar and Geetha, 2012). Similar findings were reported at South West coast of India as well as Cochin backwaters (Venugopal et al., 1982). It was observed that trace metals such as Cu, Cd, Pb and Zn in sediments showed lesser variations between stations except the stations near to wharf (Lei and Xiaoping, 2012). The trace metals analysed in sediments, followed the order Pb>Cd>Zn>Cu. An increased rate of sediment resuspension leads to high concentrations of metals in sediment during winter. Along with land run off, fluvial inputs and microbial degradation would produce an increased metal

level during winter (Schlacher-Hoenlinger and Schlacher, 1998). Relatively higher concentrations of Cr, Cu, Ni, Pb and Zn were found at vegetated areas than adjacent non vegetated while Cd was detected only at vegetated area. Se content was predominated at vegetated areas at most of the stations (Lewis et al., 2007). Trace metals content in sediments of Sinop coast of the Black Sea followed the sequence; Fe>Zn>As>Cu>Pb>Cd>Hg (Bat et al., 2016) and metals As, Pb, Fe, Zn and Cu were lower in the present study. Trace metals accumulation in sediments followed the order; Mn>Zn>Cr>Pb>Cu>As>Cd Hg and relatively higher concentrations of these metals were obtained from seagrasses area than non-seagrasses (Lin et al., 2016). All the metals except Cd were lower in this study.

3.3 Biochemical Evaluation of Seagrasses

3.3.1 Introduction

The primary production rates of seagrasses are comparable to the rates of associated fisheries. The reasons for the decline of seagrasses are linked with coastal development and associated reduction in water quality, anthropogenic activities and industrial wastes (Govindasamy et al., 2011). Seagrass meadows are playing an important role in the shore dynamics, nutrient cycling, remineralisation of minerals and sink for pollutants, and they are also significant for high primary productivity. Generally algae and seagrasses are used as pollution indicators (Thangaradjou et al., 2010). Seagrasses elaborate plenty of secondary metabolites (Kannan et al., 2010a; Ravi kumar et al., 2011; Mani et al., 2012) which have antibacterial, antiviral, antifungal, antialgal activities (Mani et al., 2012). Marine ecosystems are enriched with tannins and related phenolic compounds, and

are produced by submerged vascular plants, mergent salt marsh vegetation, mangroves and brown algae (Arnold and Targett, 2002).

Phenolics in seagrass act as chemical defences against herbivores mainly wound induced produtuion of condensed tannins, and reactive phenolics noticed in turtle grass *T. testudiunm*. Phenolics of seagrasses also showed resistance towards wasting disease, a disease characterized by rapid population declines and its resistant levels are related to level of phenolics in leaves as well as shoots (Arnold and Targett, 2002). Flavonoids present in the plants help them to interact with other organisms (Kannan et al., 2013a), and protect the poly unsaturated fatty acids in the phospholipids by donating the hydrogen atom thereby quenching of lipid peroxyradicals (Hamid et al., 2010). Tannins find uses in the treatment of burns (Athiperumalsami et al., 2008), and condensed tannins extracted from seagrasses are functioned as deterrents against herbivore feeding and also against fungal as well as bacterial invasion (McMillian, 1984).

Seagrasses accumulate heavy metals from sediments and interstitial water, though Cu, Mn, Fe, Zn, Mo and Cl are essential for the growth; they often become toxic when the threshold levels exceeds. These metals enter to human body through various trophic level food chain and they are concentrated through biomagnifications. Heavy metals in the coastal areas become one of the major health hazards in the World due to their toxicity, persistence, bioaccumulation and biomagnifications in the food chain (Thangarajou et al., 2013). Metals absorption in seagrasses and seaweeds takes place through surface reaction of metals by electrostatic attraction to negative sites, and these process influenced by temperature, light, pH, age of

plant and availability of metals in the surrounding water (Sudharsan et al., 2012). Health problems regarding heavy metals are renal failure, chronic toxicity, liver damage, physical distress, life threatening illness and damage to vital body system (Kannan et al., 2011; Sudharsan et al., 2012).

3.3.2 Results

3.3.2.1 Biochemical composition

The lipid content in seagrasses varied from 1.54 to 9.92%, protein 4.30 to 22.98% and carbohydrate 9.77 to 28.71% (Table 3.3.1&3.3.2). The low lipid and protein contents were observed in the rhizomes of *E. acoroides*. The protein and lipid contents in *T. hemprichii* were higher than *S. isoetifolium* and lower than that of *C. serrulata* from Palk Bay. The leaves of *C. serrulata* contained comparatively high content of lipid at Mathacovil (9.92%), protein at Munaikkadu (22.56%) and carbohydrate at Chinnappalam (18.21%). The roots and rhizomes of *C. serrulata* also showed high lipid of 5.31% (Mathacovil), protein 22.98% (Munaikkadu) and carbohydrate 26.44% (Chinnappalam). Among the seagrasses studied, comparatively higher content of biochemical constituents were found in the leaves and roots and rhizomes of *C. serrulata* except the carbohydrate content in the case of rhizomes (28.71%) in the *E. acoroides*. Slight differences were noticed in the biochemical composition of *S. isoetifolium* from Thonithurai and Mathacovil. The total energy contributed from biomolecules was higher in *C.serrulata* (2.49kcal/g) from Munaikkadu and low at Thonithurai *S. isoetifolium* (0.89kcal/g).

3.3.2.2 Secondary metabolites

The leaves, roots and rhizomes of *C. serrulata* from Mathacovil and Munaikkadu contained very high contents of phenolics and flavonoids compared with that from Chinnappalam and Thonithurai area. The leaves of *C. serrulata* contained phenolics in the range 41 to 298mg gallic acid equivalents (GAE)/100g and flavonoids 13 to 146mg quercetin equivalents (QE)/100g whereas in the roots and rhizomes phenolics varied from 29 to 292mg/100g GAE and flavonoids 9.1 to 75mg/100g (Table 3.3.3 &3.3.4). These results showed that leaves as well as roots and rhizomes of *C. serrulata* contained almost similar concentration except at Chinnappalam; while in the case of flavonoids *C. serrulata* leaves predominated over roots and rhizomes. *S. isoetifolium* of Mathacovil contained high phenolics and flavonoids compared to Thonithurai, and its concentrations were 21mgGAE /100g and 7mgQE /100g (Thonithurai) and 277mgGAE/100g and 58mgQE /100g (Mathacovil). The roots, rhizomes and leaves of *E. acoroides* contained relatively high contents of phenolics and flavonoids in leaves followed by roots and rhizomes. Comparatively higher concentration of phenolics and flavonoids were noticed in *T. hemprichii* than seagrasses from Gulf of Mannar, and also the flavonoid content of *S. isoetifolium* from Mathacovil. Phenolics in seagrasses ranged from 17 (*E. acoroides*) to 298mgGAE/100g (*C. serrulata*, Mathacovil) and flavonoid 5.20 (*E. acoroides*) to 146mg/100g (*C. serrulata*, Mathacovil). Observed tannin contents in *C. serrulata* was higher in leaves (1.21to 5.02mg/100g) than roots and rhizomes (1.11to 4.51mg/100g). Among the seagrasses, tannin varied from 0.20 to 5.02mg/100g. The leaves of *E. acoroides* predominated with tannins in roots and rhizomes, and also *T. hemprichii* exhibited a

concentration of 1.21mg/100g whereas *S. isoetifolium* from Mathacovil reported 0.78mg/100g.

3.3.2.3 Chlorophyll pigments

Chlorophylls (chls) and pheophytin denoted relatively high abundance in the leaves of seagrasses. Leaves of *C. serrulata* recorded high content of chl a in Chinnappalam, chl b in sample from Thonithurai whereas roots and rhizomes exhibited highest chl a and b at Thonithurai (Table 3.3.5&3.3.6). Pheophytin in roots and rhizomes were detected only at Thonithurai (7µg/g). Remarkable differences were observed in the chls content in *S. isoetifolium*, and relatively high concentrations of chls and pheophytin were noticed at Thonithurai.Variations were observed in chls content in the leaves of *C. serrulata*.

3.3.2.4 Elemental composition

Total nitrogen (N) content in seagrasses varied from 0.66 to 2.93%, carbon (C) 31.72 to 41.18% and phosphorous (P) 0.125 to 0.622%. Leaves of *C. serrulata* showed high N content at Chinnapalam (2.93%) and low at Mathacovil (1.49%) (Table 3.3.7&3.3.8). No wide variations were observed in the elemental composition of seagrasses between species as well as different body parts, and slightly more observed at Gulf of Mannar than Palk Bay. Elemental compositions in seagrasses were comparatively higher noticed in leaves than roots and rhizomes. N content in roots and rhizomes of *C. serrulata* varied from 0.77 (Munaikkadu) to 1.37% (Chinnappalam). In *S. isoetifolium* carbon content exhibited high quantity at Mathacovil while the rest of them were higher at Thonithurai. Seagrasses of *E. acoroides* and *T. hemprichii* showed relatively enhanced levels of elements.

3.3.2.5 Macro elements composition

Na content in seagrasses varied from 2.13 (*E. acoroides*) to 39.25 mg/g (*T. hemprichii*), K from 0.34 to12.41mg/g (*C. serrulata* from Thonithurai) while Ca from 1.30 (*E. acoroides*) to 18.23mg/g (*S. isoetifolium* of Thonithurai) and Mg from 1.37 (*E. acoroides*) to 8.90mg/g (*S. isoetifolium* of Thonithurai) (Table 3.3.9&10). Among the seagrasses under investigations, very low concentrations of macro elements were found in leaves of *E. acoroides*, and Li were not detected in any of the samples of seagrasses. The leaves of *C. serrulata* exhibited wide variations in the composition of macro elements with respect to stations, and variations were comparatively higher in the case of Na, Ca and K and lower for Mg. Na, Ca and K in the roots and rhizomes followed similar pattern as that of leaves, and more variations were noticed in K content. In comparison of *C. serrulata* leaves with roots and rhizomes, Na, Ca and Mg contents were comparatively higher in roots and rhizomes than leaves except at Thonithurai; while K contents were higher in roots and rhizomes than leaves. Na and K concentrations were relatively higher in *S. isoetifolium* of Mathacovil while Ca and Mg higher at Thonithurai. The results showed comparatively high contents of Na, K, Ca and Mg in *T. hemprichii* and the values were closer to the maximum value.

3.3.2.6 Heavy metals

Among the metals studied, most of them exhibited an increased value at Gulf of Mannar than Palk Bay side.The level of Co in seagrasses ranged from 0.12 to 3.39µg/g; Cr from 1.76 to 34.70µg/g; Cu from 4.78 to 156µg/g; Fe from 326 to 3745µg/g; Mn from 18.50 to 207µg/g; Ni from 1.39 to

13.50µg/g and Zn from 28 to 254µg/g (Table 3.3.11&12). A comparison of the seagrasses of *C. serrulata* leaves with roots and rhizomes indicated that Co and Fe contents were comparatively higher in roots and rhizomes than in leaves at Thonithurai and Chinnappalam, and a reverse pattern was observed in Munaikkadu. Cr and Mn concentrations were highest in the leaves of *C. serrulata* at all stations. Relatively high contents of Cu and Zn were noticed in the roots and rhizomes than in leaves of samples except from Thonithurai. Similarly Ni concentrations were highest in the leaves than roots and rhizomes except at Chinnappalam.

Among the heavy metals studied, Co, Cr, Mn, Ni, Cd and Pb showed an increased value in *S. isoetifolium* from Thonithurai whereas metals Cu, Fe and Zn dominated in *S. isoetifolium* from Mathacovil. The distribution of heavy metals in different body parts of *E. acoroides* showed an increased concentration of Fe, Mn, Ni and Cd at roots while Cu, Zn and Pb dominated in rhizomes and Co and Cr leaves, and *T. hemprichii* contained relatively enriched levels of heavy metals. Cd content in seagrasses varied from 0.15 (*C. serrulata* of Chinnappalam) to 1.73µg/g (*E. acoroides*) and Pb from 0.74 (*C. serrulata* of Mathacovil) to 8.22µg/g (*C. serrulata* at Chinnappalam). In the leaves of *C. serrulata*, Cd content varied from 0.19 to 0.91µg/g (Thonithurai) whereas roots and rhizomes varied from 0.15 (Chinnappalam) to 0.35 µg/g (Mathacovil). The highest values for Pb obtained in *C. serrulata* were 8.22µg/g and 7.42µg/g (Chinnappalam) in leaves and rhizomes respectively while lowest were 0.74µg/g (Mathacovil) and 1.21µg/g (Munaikkadu). Se was not detected in any of the samples of seagrasses while Hg found only in the roots and rhizomes of *C. serrulata* from Thonithurai (3.86µg/g) and As in the roots of *E. acoroides* (3.48µg/g).

Table 3.3.1 Biochemical composition of seagrasses from Gulf of Mannar (dry weight basis %)

Species	Station	Lipid	Protein	Carbohydrate	Energy (Kcal/g)
C. serrulata (leaf)	Thonithurai	2.28±0.08	10.86±0.58	13.66±0.11	1.39
C. serrulata (leaf)	Chinnappalam	3.61±0.12	8.35±0.62	18.21±0.12	1.56
C. serrulata (R&R)	Thonithurai	2.42±0.09	19.8±0.88	21.17±0.15	2.22
C. serrulata (R&R)	Chinnappalam	2.64±0.11	9.58±0.44	26.44±0.20	1.87
S. isoetifolium	Thonithurai	1.9±0.09	4.8±0.53	10.65±0.09	0.89
E. acoroides (leaf)	Chinnappalam	3.58±0.14	8.65±0.61	11.61±0.08	1.30
E. acoroides (Rh)	Chinnappalam	1.54±0.12	4.3±0.48	28.71±0.12	1.57
E. acoroides (Rt)	Chinnappalam	3.04±0.10	6.74±0.58	23.05±0.08	1.61

Table 3.3.2 Biochemical composition of seagrasses from Palk Bay (dry weight basis %)

Species	Station	Lipid	Protein	Carbohydrate	Energy (Kcal/g)
C. serrulata (leaf)	Munaikkadu	8.67±0.11	22.56±0.52	9.77±0.08	2.49
C. serrulata (leaf)	Mathacovil	9.92±0.14	14.03±0.48	12.87±0.12	2.26
C. serrulata (R&R)	Munaikkadu	3.43±0.10	22.98±0.62	9.79±0.09	2.02
C. serrulata (R&R)	Mathacovil	5.31±0.15	16.98±0.46	12.78±0.12	1.99
S. isoetifolium	Mathacovil	2.03±0.08	6.6±0.21	9.79±0.08	0.97
T. hemprichii	Munaikkadu	3.56±0.11	10.06±0.28	10.98±0.11	1.35

Table 3.3.3 Secondary metabolites in seagrasses from Gulf of Mannar (dry weight basis mg/100g)

Species	Station	Phenolics	Flavonoids	Tannin
C. serrulata (leaf)	Thonithurai	55±0.42	18±0.11	1.42±0.22
C. serrulata (leaf)	Chinnappalam	41±0.32	13±0.09	1.21±0.14
C. serrulata (R&R)	Thonithurai	50±0.38	16±0.11	1.34±0.18
C. serrulata (R&R)	Chinnappalam	29±0.25	9.1±0.08	1.11±0.22
S. isoetifolium	Thonithurai	21±0.17	7±0.06	0.20±0.08
E. acoroides (leaf)	Chinnappalam	108±0.56	34±0.21	1.82±0.28
E. acoroides (Rh)	Chinnappalam	27±0.24	9.3±0.06	1.32±0.21
E. acoroides (Rt)	Chinnappalam	17±0.20	5.2±0.07	1.14±0.25

Table 3.3.4 Secondary metabolites in seagrasses from Palk Bay (dry weight basis mg/100g)

Species	Station	Phenolics	Flavonoids	Tannin
C. serrulata (leaf)	Munaikkadu	281±0.70	104±0.44	4.26±0.18
C. serrulata (leaf)	Mathacovil	298±0.88	146±0.54	5.02±0.16
C. serrulata (R&R)	Munaikkadu	285±0.62	69±0.27	4.51±0.12
C. serrulata (R&R)	Mathacovil	292±0.81	75±0.30	3.70±0.10
S. isoetifolium	Mathacovil	277±0.66	58±0.17	0.78±0.09
T. hemprichii	Munaikkadu	227±0.51	85±0.22	1.21±0.10

Table 3.3.5 Chlorophylls in seagrasses of Gulf of Mannar (wet weight basis µg/g)

Species	Station	Chlorophyll a	Chlorophyll b	Pheophytin
C. serrulata (leaf)	Thonithurai	817±3.20	669±2.80	150±0.78
C. serrulata (leaf)	Chinnappalam	822±3.28	354±1.20	41±0.32
C. serrulata (R&R)	Thonithurai	22±0.12	18±0.11	7±0.08
C. serrulata (R&R)	Chinnappalam	11±0.08	8±0.08	0
S. isoetifolium	Thonithurai	850±3.32	1029±4.40	196±0.84
E. acoroides (leaf)	Chinnappalam	431±1.40	184±0.78	59±0.38
E. acoroides (Rh)	Chinnappalam	53±0.18	22±0.10	0
E. acoroides (Rt)	Chinnappalam	10±0.06	23±0.09	12±0.10

Table 3.3.6 Chlorophylls in seagrasses of Palk Bay (wet weight basis µg/g)

Species	Station	Chlorophyll a	Chlorophyll b	Pheophytin
C. serrulata (leaf)	Munaikkadu	127±0.66	29±0.25	204±1.10
C. serrulata (leaf)	Mathacovil	115±0.62	30±0.31	134±0.77
C. serrulata (R&R)	Munaikkadu	2.19±0.08	1.10±0.05	0
C. serrulata (R&R)	Mathacovil	2.19±0.06	1.10±0.06	0
S. isoetifolium	Mathacovil	211±0.88	11±0.08	23±0.18
T. hemprichii	Munaikkadu	136±0.69	69±0.51	109±0.96

Table 3.3.7 Elemental compositions of seagrasses of Gulf of Mannar (dry weight basis %)

Species	Station	N	C	P
C. serrulata (leaf)	Thonithurai	2.14±0.10	31.84±0.25	0.227±0.02
C. serrulata (leaf)	Chinnappalam	2.93±0.12	40.18±0.45	0.245±0.02
C. serrulata (R&R)	Thonithurai	0.89±0.08	31.72±0.28	0.187±0.02
C. serrulata (R&R)	Chinnappalam	1.37±0.09	39.87±0.41	0.213±.32
S. isoetifolium	Thonithurai	2.26±0.10	32.37±0.22	0.274±0.02
E. acoroides (leaf)	Chinnappalam	2.70±0.13	40.47±0.48	0.453±0.13
E. acoroides (Rh)	Chinnappalam	0.66±0.07	34.87±0.32	0.126±0.02
E. acoroides (Rt)	Chinnappalam	1.58±0.10	33.02±0.36	0.369±0.23

Table 3.3.8 Elemental compositions of seagrasses of Palk Bay (dry weight basis %)

Species	Station	N	C	P
C. serrulata (leaf)	Munaikkadu	1.58±0.09	41.18±0.51	0.466±0.03
C. serrulata (leaf)	Mathacovil	1.49±0.10	40.98±0.48	0.313±0.02
C. serrulata (R&R)	Munaikkadu	0.77±0.06	40.23±0.44	0.622±0.04
C. serrulata (R&R)	Mathacovil	0.85±0.07	38.78±0.38	0.407±0.03
S. isoetifolium	Mathacovil	1.19±0.08	35.21±0.28	0.125±0.02
T. hemprichii	Munaikkadu	1.19±0.10	35.21±0.29	0.166±0.02

Table 3.3.9 Macro elements in seagrasses of Gulf of Mannar (dry weight basis mg/g)

Species	Station	Na	K	Ca	Mg
C. serrulata (leaf)	Thonithurai	24.54±0.41	6.29±0.12	15.94±0.28	7.59±0.16
C. serrulata (leaf)	Chinnappalam	4.99±0.39	0.83±0.09	11.16±0.58	5.03±0.10
C. serrulata (R&R)	Thonithurai	19.92±0.28	12.41±0.36	9.40±0.79	6.26±0.64
C. serrulata (R&R)	Chinnappalam	7.99±0.12	1.29±0.08	11.86±0.18	6.22±0.22
S. isoetifolium	Thonithurai	16.20±0.24	1.01±0.09	18.23±0.36	8.90±0.28
E. acoroides (leaf)	Chinnappalam	2.13±0.10	0.34±0.06	1.30±0.09	1.37±0.11
E. acoroides (Rh)	Chinnappalam	10.32±0.16	4.82±0.09	6.57±0.13	3.23±0.12
E. acoroides (Rt)	Chinnappalam	6.63±0.22	2.72±0.16	16.85±0.55	6.85±0.47

Table 3.3.10 Macro elements in seagrasses of Palk Bay (dry weight basis mg/g)

Species	Station	Na	K	Ca	Mg
C. serrulata (leaf)	Munaikkadu	6.23±0.18	2.16±0.06	4.72±0.10	2.59±0.09
C. serrulata (leaf)	Mathacovil	4.71±0.11	3.44±0.08	5.66±0.14	2.36±0.06
C. serrulata (R&R)	Munaikkadu	8.99±0.16	3.79±0.10	6.99±0.18	3.39±0.08
C. serrulata (R&R)	Mathacovil	8.63±0.18	7.32±0.16	3.80±0.09	4.06±0.10
S. isoetifolium	Mathacovil	21.55±0.62	5.93±0.10	13.47±0.29	5.79±0.11
T. hemprichii	Munaikkadu	39.25±0.88	7.85±0.52	17.21±0.38	6.54±0.12

Table 3.3.11 Heavy metals in seagrasses of Gulf of Mannar (dry weight basis µg/g)

Species	Station	Co	Cr	Cu	Fe	Mn	Ni	Zn	Cd	Pb
C. serrulata (leaf)	Thonithurai	0.63±0.04	34.70±1.34	14.80±0.41	1157±3.06	139±0.88	13.50±0.66	46±0.38	0.91±0.14	3.51±0.19
C. serrulata (leaf)	Chinnappalam	0.35±0.09	11.10±0.45	98±0.98	1163±3.62	207±1.52	2.96±0.21	51.20±1.15	0.89±0.09	8.22±0.36
C. serrulata (R&R)	Thonithurai	1.55±0.34	9.18±0.64	6.83±0.26	1226±3.80	41.80±0.62	6.31±0.42	36.10±1.04	0.33±0.10	2.54±0.31
C. serrulata (R&R)	Chinnappalam	0.39±0.12	9.97±0.29	156±1.45	2074±4.04	47.30±0.98	3.72±0.34	82±0.44	0.15±0.04	7.42±0.80
S. isoetifolium	Thonithurai	0.25±0.11	8.09±0.41	9.20±0.40	612±1.58	152±1.02	5.18±0.46	28±0.28	0.65±0.12	4.09±0.28
E. acoroides (leaf)	Chinnappalam	3.39±0.24	7.89±0.54	47.90±0.78	736±1.89	40.70±0.64	2.42±0.28	29.90±0.25	0.60±0.16	7.45±0.38
E. acoroides (Rh)	Chinnappalam	0.15±0.08	5.94±0.32	130±1.08	548±1.62	18.50±0.48	1.39±0.12	42.50±0.48	0.47±0.08	8.08±0.31
E. acoroides (Rt)	Chinnappalam	3.01±0.19	1.80±0.24	10.80±0.42	3745±5.51	44.60±0.82	3.09±0.24	40.30±0.56	1.73±0.18	3.12±0.43

Table 3.3.12 Heavy metals in seagrasses of Palk Bay (dry weight basis µg/g).

Species	Station	Co	Cr	Cu	Fe	Mn	Ni	Zn	Cd	Pb
C. serrulata (leaf)	Munaikkadu	0.82±0.19	3.44±0.50	4.78±0.69	347±1.46	22.04±0.44	2.44±0.22	126±0.74	0.19±0.07	3.17±0.40
C. serrulata (leaf)	Mathacovil	0.80±0.16	4.12±0.48	7.55±0.85	416±1.78	48.40±0.62	2.70±0.28	151±0.62	0.19±0.09	0.74±0.20
C. serrulata (R&R)	Munaikkadu	0.44±0.14	1.91±0.51	5.05±0.58	326±2.04	20.88±0.42	1.70±0.24	230±1.08	0.23±0.12	1.21±0.30
C. serrulata (R&R)	Mathacovil	0.46±0.12	1.76±0.43	10.39±0.89	481±2.54	46.80±0.76	2.58±0.32	180±0.70	0.35±0.14	1.58±0.28
S. isoetifolium	Mathacovil	0.12±0.08	3.52±0.58	9.95±0.82	842±2.08	137±0.94	4.54±0.32	113±0.88	0.27±0.18	2.11±0.26
T. hemprichii	Munaikkadu	0.13±0.10	5.59±1.05	13.20±0.72	568±1.88	206±1.82	4.57±0.58	254±1.44	1.10±0.27	1.68±0.34

Hg - *C. serrulata* from Thonithurai (R&R) = 3.857 µg/g, **As** – *E. acoroides* (roots) = 3.478 µg/g and **Se** not detected. R&R- Roots &Rhizomes, Rh- Rhizomes and Rt- Roots.

3.3.3 Discussion

3.3.3.1 Biochemical composition

Variations in the composition of different elements were reported in seagrasses from different areas. Comparatively higher contents of lipid, protein and carbohydrate were observed in the present study than the earlier reports (Table 3.3.13) (Torbatinejad and Sabine, 2001; Abd El-Hady et al., 2007; Athiperumalsami et al., 2008; Kannan et al., 2010a; Pradheeba et al., 2011). Varying levels of protein content in leaves of *H. beccarii* (lower) and *E. acoroides* (higher) from Palk Bay, Bay of Bengal, and relatively lower concentrations in leaves and rhizomes were observed during monsoon and higher at summer. In seagrasses, protein was positively correlated with lipid, phenol and tannin (leaves and rhizomes) (Pradheeba et al., 2011). Species wise variations in biochemical compositions were noticed in this study and it compares well with the earlier study (Pradheeba et al., 2011). Lipids in seagrass leaves were lower than rhizomes and comparable results were seen in *C. serrulata* from Gulf of Mannar. Most of the seagrasses showed variations in lipid content in the leaves and rhizomes of seagrasses throughout the season. Carbohydrate in leaves of seagrasses displayed lower content in comparison with rhizomes, and variations were noticed with respect to season. Moreover carbohydrate content showed a positive correlation with protein in leaves and lipid in rhizomes (Pradheeba et al., 2011). Relatively higher carbohydrate was stored at rhizomes as food, and moreover seagrasses having thick rhizomes were predominated over lean. Seasonal variations in the biochemical composition of seagrasses depend on nutrient availability as well as environmental conditions.

Table 3.3.13 Comparison of biochemical composition of seagrasses.

Sl No.	Species	Location	Protein %	Carbohydrate %	Lipid %	Calorific value kcal/g	Reference
1	Seagrasses leaves	Palk Bay	0.01-0.59	0.2-0.87	0.1-3.2	24.08-68.82	Pradheeba et al., 2011
	Seagrasses rhizomes (*E. acoroides, H. beccarii, H. ovalis, C. rotundata, C. serrulata, H. uninervis, H. pinifolia* and *S. isoetifolium*)	Palk Bay	0.06-7.2	0.3-0.91	0.03-4.1	28.23-77.84	Pradheeba et al., 2011
2	Seagrasses (*C. serrulata, H. ovalis, H. pinifolia* and *S. isoetifolium*)	Gulf of Mannar	0.263-1.575	0.014-0.318	0.002-0.003	1.987-2.926	Athiperumalsami et al., 2008
3	Seagrasses (*C. serrulata, H. pinifolia* and *H. stipulacea*)	Mandapam coast	6.79-7.89	6.01-6.32	0.92-1.02	---	Kannan et al., 2010 a
4	Seagrasses leaf blade	Thailand	---	---	0.28-0.38	---	Yamamuro and Chirapart, 2005
	Leaf sheath	Thailand	---	---	0.53-0.71	---	Yamamuro and Chirapart, 2005
	Roots/rhizomes (*E. acoroides, H. ovalis, C. rotundata* and *T. hemprichii*)	Thailand	---	---	0.17-0.35	---	Yamamuro and Chirapart, 2005
5	Seaweeds	South Australia	4.4-7.3	---	1.1-1.7	---	Torbatinejad and Sabine, 2001
	P. australis	South Australia	4.8-6.8	24.4-28.8	5.5		Torbatinejad and Sabine, 2001
6	*C. nodosa* and *R. cirrhosa*	Egypt	13-18.6	6.2-13	1.5-3.65	---	Abd El - Hady et al ., 2007
7	Macro algae	Baja California	11.25-19.18	---	0.68-0.88	---	Serviere -Zaragoza et al., 2002
	P. torreyi	Baja California	12.53	---	0.45	---	Serviere -Zaragoza et al., 2002
8	*H. engelmannii*	Florida	4.2-11.5	5.2-34.7	0.1-1.5	1.6-3.3	Dawes et al., 1987
9	*E.acoroides*	Philippines	0.8-8.8	72.4-87.6	0.1-0.2	3.27-3.55	Montano et al., 1999
10	*C. nodosa* and *P. oceanica*	Egypt	51-60.8	2.90-4.722	4.05-10.08	3.93-4.03	El Din and El Sherif, 2013
11	*P. australis*	Australia	4.81-6.11	20-30	---	---	Torbatinejad et al., 2007
12	Seagrasses (*E.acoroides, C. serrulata* and *S. isoetifolium*)	Gulf of Mannar	4.3-19.8	10.65-26.44	1.54-3.61	0.89-2.22	Present study
13	Seagrasses (*C. serrulata, T. hemprichii* and *S. isoetifolium*)	Palk Bay	6.6-22.98	9.77-12.87	2.03-9.92	0.97-2.49	Present study

Rhizomes and shoots are the body parts that store soluble proteins in seagrasses, and lipid content varied with respect to age, stage of growth and ecology (Pradheeba et al., 2011; El Din and El-Sherif, 2013). Lipid also followed peak levels during summer and minimum at monsoon seasons.

Species wise and location wise variations in biochemical constituents were observed in this study and it might be due to changes in environmental conditions of seagrass meadows as well as stage of growth and ecology. Remarkable differences were noticed in the energy contributed by seagrasses of Gulf of Mannar as well as Palk Bay. In comparison with seaweeds, seagrasses contained lower levels of carbohydrates and lipids while protein content predominated over seaweeds (Athiperumalsami et al., 2008). Biochemical composition of seagrasses from the Mandapam coast revealed absence of variations in the case of protein, carbohydrate and lipid content (Kannan et al., 2010a). Seagrasses of slowest growth rate showed relatively lower variation in lipid content in leaf blade followed by roots, rhizomes and leaf sheath (Yamamuro and Chirapart, 2005) while this study observed *E. acoroides* with large leaf had high lipid content followed by *C. serrulata* and *S. isoetifolium* of spherical shaped leaf, and lipid content in leaves predominated over roots and rhizomes except *C. serrulata* from Thonithurai. *E. acoroides* from Gulf of Mannar showed higher carbohydrate and lower protein content while vice versa was observed in *E. acoroides* from Palk Bay (Kannan and Kannan, 2002). Rhizomes of seagrasses normaly act as photosynthate storage tissue and stored carbons were used for respiratory demands and plant growth (Lee and Dunton, 1996). Variations were observed in the biochemical constituents and it was more in protein content. Wide variations in protein content could be due to strong

allelopathic effect of seagrasses (Abd El-Hady and Khalifa, 2015). Protein content in *T. testudinum* ranged from 0.30 to 4.12% while carbohydrate varied from 6.7 to 26.78% (Hernández et al., 2016), and concentration of carbohydrate was comparable and protein content was higher in the present study. Variations in the concentrations of proteins and carbohydrates in seagrasses were associated with its surrounding environment, and it depends upon nutrients level, salinity and temperature which directly related to photosynthetic rate (Hernández et al., 2016).

Crude protein content displayed species wise variations in marine plants and it was lesser in lipid content (Torbatinejad and Sabine, 2001; Serviere-Zaragoza et al., 2002). Similar results were seen in seagrasses, and *R. cirrhosa* contained higher protein content whereas *C. nodosa* exhibit higher carbohydrate and lipid content (Abd El-Hady et al., 2007). Seagrass of *P. torreyi* recorded lower protein and lipid than macroalgae and wide variations were observed in the biochemical composition of seagrass with macroalgae (Serviere-Zaragoza et al., 2002). Total non starch polysaccharides in *P. australis* followed the order fresh >green>dry washed > dry unwashed while crude protein followed the order green>dry unwashed> fresh>dry washed (Torbatinejad et al., 2007). Proximate composition of *H. engelmannii* showed relatively higher concentrations of protein content in most of the body parts except at roots, and almost similar concentrations in seagrasses (as a whole). Relatively higher concentrations of protein and lipid were noticed in leaves than roots and rhizomes, and variations were observed with respect to species as well as region in this study, and it was in line with the earlier report (Dawes et al., 1987). Biochemical compositions of the seagrasses of *C. nodosa* and *P. oceanica* followed the order protein>lipid>carbohydrate.

Total lipids and carbohydrates were higher in *C. nodosa* and protein higher in *P. oceanica*. Wide variation between total carbohydrate and protein was attributed to the factors of depth, light intensity, turbidity and nutrient availability in the water column, its uptake and translocation to different body parts of the plant (El Din and El-Sherif, 2013). Relatively higher protein and lipid contents were noticed in the present study than *Z. marina* from Swan Lake, China (Song et al., 2016).

Relatively lower calorific value was observed in the present study than earlier findings (Dawes et al., 1987; Montano et al., 1999; Athiperumalsami et al., 2008; Pradheeeba et al., 2011; El Din and El-Sherif, 2013). Calorific value in leaves ranged from 24.08 (*H. ovalis*) to 68.82 Kcal/g (*E. acoroides*) while in rhizomes 28.23 (*H. pinifolia*) to 77.84 K cal/g (*E. acoroides*) (Pradheeeba et al., 2011), and it was lower in the present study. Relatively higher energy contributed by *C. serrulata* followed by *E. acoroides* and *T. hemprichii* and lowest with *S. isoetifolium* in this study. Calorific value ranged from 8.39 (*H. ovalis*) to 12.25 KJ (*S. isoetifolium*) and followed the order *S. isoetifolium>C. serrulata>H. pinifolia>H. ovalis* (Athiperumalsami et al., 2008). No noticeable variation in calorific value was reported in seagrasses of Egypt (El Din and El-Sherif, 2013). Calorific values were highest at Homosassa River than Indian Bluff Island, and no wide variations were observed between the month of December and September at Indian Bluff Island (Dawes et al., 1987). The energy corresponds to seeds flour and starch of *E. acoroides* was 327cal/100g and 355cal/100g respectively (Montano et al., 1999).

3.3.3.2 Secondary metabolites in seagrasses

Total phenolics

Comparatively higher phenolics were noticed in the present study than similar other studies carried out (Table 3.3.14) (Athiperumalsami et al., 2008; Kannan et al., 2010b, c; Abd El-Hady et al., 2012; Rengasamy et al., 2012; Girija et al., 2013). Relatively higher concentrations of phenolics were reported in the ethyl acetate fraction and lower in water, and no major differences were observed between the rest of the solvent fractions and crude methanol extract (Choi et al., 2009). Comparatively higher total phenol content was noticed in summer than monsoon season at the rhizomes than leaves and it was similar to the earlier report (Rotini et al., 2013). Higher concentrations at summer season was attributed to disturbing events affecting the meadow such as exposure of environmental pressures of turbidity, metal contamination, pollution, ocean acidification, competition with invasive seaweed, infection by *Labyrinthula*, etc., and phenols are toxic to nematodes and can be used as a bioindicator (Rotini et al., 2013). No remarkable differences were noticed between leaves, roots and rhizomes of seagrasses of Palk Bay in this study, and it might be due to an increased plant growth seemed in summer and directly related to synthesis of structural phenols, because they are the building blocks for the new cells (Migliore et al., 2007). A comparison between leaves and rhizomes indicated that rhizomes had long life time and less affected by common changes in the physiological process (Migliore et al., 2007; Pradheeba et al., 2011). In the present study, total phenolic content in seagrasses of Gulf of Mannar was found to be minimum in *S. isoetifolium* and maximum in *E. acoroides* whereas in Palk Bay lower concentrations were observed in

T. hemprichii and higher in *C. serrulata*. Phenol content in the leaves as well as rhizomes of *H. beccarii* from Palk Bay were relatively lower and higher at *E. acoroides*, and a positive correlation was obtained between phenol and tannin contents (Pradheeba et al., 2011). Relatively higher phenol contents were observed in the leaves than roots and rhizomes of seagrasses of Gulf of Mannar in the present study. Higher phenol contents in leaves might be due to the defensive mechanisms of seagrass leaves against epiphytes because they are exposed more than rhizomes (Pradheeba et al., 2011).

Seagrasses of Tuticorin were reported to have comparatively higher concentrations of phenolics in methanol extract of *H. pinifolia* and lower in *H. ovalis* hexane extract, and in *S. isoetifolium* no variations were observed in phenolic contents between solvents, and seagrasses followed the order; *S. isoetifolium*>*H. ovalis*>*H. pinifolia* (Girija et al., 2013). Total phenolic content in *H. stipulacea* contained 0.611mg/g (Abd El-Hady et al., 2012) whereas in this study a wide variation was observed in phenolics from 0.41 to 2.98mg/g (leaves) and 0.17 to 2.92 mg/g (roots and rhizomes). Roots and rhizomes of *P. perfoliatus* and *R. maritima* showed a decrease in the concentration of total reactive phenolics and an increase in carbon dioxide content (Arnold et al., 2012). Seagrasses from Indonesia water display comparatively higher phenolic contents in the methanol extract followed by ethyl acetate and n-hexane except *S. isoetifolium*, with predominance in solvent ethyl acetate followed by methanol and n-hexane (Santoso et al., 2012). Total phenolic content in *H. uninervis* was 20.17µg/g (Supriadi et al., 2016) and it was much lower than the present findings. Polyphenolics in the hydrated ethanol extract of *T. testudinum* was ranged from 7.19 to 58.81mg/g (Hernández et al., 2016) while concentrations were lower in this study. The

concentration of polyphenolics in different body parts are related with changes in temperature and salinity, and these two are related to the variations in the intensity of sunlight and was due to self protection of seagrasses against UV radiation. A direct linear relationship was showed between level of phenolic compounds and UV radiation exposure. Both in summer and winter season, total phenolic content in seagrasses predominated over seaweeds of Southern Indian Ocean Island, Maritus (Ramah et al., 2014). Total phenolics in seagrasses of Palk Bay were ranged from 3.77 to 15.38mgGAE/g in leaves while 2.08 to 16.26mgGAE/g in rhizomes (Jeyapragash et al., 2016) and it was comparatively higher than the present study.

Species wise variations in phenolic contents were noticed in the present study and it was similar to the earlier reports (Spencer and Ksander, 1994; Athiperumalsami et al., 2008; Kannan et al., 2010c; Pradheeba et al., 2011; Rengasamy et al., 2012; Santoso et al., 2012; Girija et al., 2013; Kannan et al., 2013a). Station wise variation was observed in this study which comprised well with the study carried out by Grignon-Dubois et al. (2012). Total phenolic acid content in *Z. noltii* fresh leaves were higher in concentration and wide variations were observed between locations as well as time of samplings. The predominated phenolic acids were rosmaric acid (RA) followed by Zosteric acid (ZA) and Caffeic acid (CAF) at most of the stations, and the variations in phenolic acids depend on the abiotic and biotic factors including depth, environmental parameters, herbivory, species competition and invasions (Grignon-Dubois et al., 2012). Phenol content in the seagrasses of Gulf of Mannar followed the order *C. serrulata*>*S. isoetifolium*>*H. pinifolia*>*H. ovalis* and seagrasses contained lower levels of phenolics (Athiperumalsami et al., 2008).

Table 3.3.14 Comparison of phenolic content in seagrasses.

Seagrass Species	Solvent used for extraction	Phenol content in mg/g GAE	Location	References
Z. marina	Methanol	204.63	korea	Choi et al., 2009
Z. marina	Ethyl acetate fraction	968.50	Korea	Choi et al., 2009
P. oceanica	-----	22.31-27.62 (FW)	Italy	Rotini et al., 2013
Seagrass leaves * (*E. acoroides* , *H. beccarii*, *H. ovalis*, *C. rotundata*, *C. serrulata*, *H. uninervis*, *H. pinifolia* and *S. isoetifolium*)	-----	2.61 – 7.52	Palk Bay	Pradheeba et al., 2011
Seagrass rhizome *	----	0.74 -7.03	Palk Bay	Pradheeba et al., 2011
H. pinifolia	Methanol	0.64	Tuticorin	Girija et al., 2013
S. isoetifolium	Methanol	0.58	Tuticorin	Girija et al., 2013
H. ovalis	Methanol	0.34	Tuticorin	Girija et al., 2013
H. stipulacea *	Methanol	0.611	Egypt	Abd El-Hady et al., 2012
C. nodosa (leaves) *	-----	2.21 – 5.45 WM	Italy	Arnold et al., 2012
Potamogeton perfoliatus * (roots/rhizomes)	---	0.91 – 2.49 WM	Maryland,USA	Arnold et al., 2012
Ruppia maritima * (roots/rhizomes)	----	1.95 – 4.01 WM	Maryland,USA	Arnold et al., 2012
T. hemprichii	Methanol	0.94 – 10.22	Indonesia	Santoso et al., 2012
S .isoetifolium	Ethyl acetate	0.36 – 7.32	Indonesia	Santoso et al., 2012
C. rotundata and E. accordies	n-hexane	0.026 -0.10	Indonesia	Santoso et al., 2012
Potamogeton species *		32.3-53.1 µM/g DW	California	Spencer and Ksander,1994
Hydrilla species *		6.9-204 µM/g DW	California	Spencer and Ksander,1994
Z. noltii **	Methanol	12049 µg/g DW	Bay of cadiz	Gringnon-Dubois et al., 2012
Z. noltii **	Methanol	21322 µg/g DW	Sa Nitja Bay,Menora	Gringnon-Dubois et al., 2012
Z. noltii **	Methanol	933 µg/g DW	Alfacs Bay	Gringnon-Dubois et al., 2012
Z. noltii **	Methanol	7334 µg/g DW	Arcachon lagoon	Gringnon-Dubois et al., 2012
Z. noltii **	Methanol	6206 µg/g DW	Arcachon lagoon	Gringnon-Dubois et al., 2012
Seagrasses (*C. serrulata*, *H. ovalis*, *H. pinifolia* and *S. isoetifolium*)		0.070-0.226	Gulf of Mannar	Athiperumalsami et al., 2008

P. oceanica ***		64.6-296.9 µg/g DM	France	Dumay et al., 2004
Interaction with *C. taxifolia* ***		99.8-356.9 µg/g DM	France	Dumay et al., 2004
P. oceanica ***		56-358 µg/g DM	France	Dumay et al., 2004
Interaction with *C. racemosa* ***		72.4-382.9 µg/g DM	France	Dumay et al., 2004
Seagrass (*E. acoroides* , *C. rotundata*, *C. serrulata*, *T. hemprichii* , *H. pinifolia* and *S. isoetifolium*)	Aqueous methanol	1.62-21.64	Chinnappalm	Kannan et al., 2013a
Seagrasses * (*E. acoroides* , *H. pinifolia*, *S. isoetifolium* and *T. hemprichii*)	Aqueous methanol	0.337 – 1.263	Mandapam	Kannan et al., 2010c
E. acoroides leaf *	Ethanol	0.378	Chinnappalam	Kannan et al., 2010b
E. acoroides root *	Ethanol	0.301	Chinnappalam	Kannan et al., 2010b
E. acoroides rhizome *	Ethanol	0.121	Chinnappalam	Kannan et al., 2010b
Seagrasses (*E. acoroides* , *H. ovata*, *H. ovalis*, *H. stipulacea*, *C. serrulata*, *T. hemprichii* , *H. pinifolia* and *S. isoetifolium*)	Ethanol	0.4078-1.3987	Mandapam	Rangasamy et al., 2012
Seagrass leaves (*E. acoroides*, *H. ovata*, *C. rotundata*, *H. uninervis*, *T. hemprichii* and *S. isoetifolium*) Seagrass rhizomes	Methanol	3.77 - 15.38 2.08 - 16.26	Palk Bay	Jayapragash et al., 2016
Seagrasses leaves (*E. acoroides*, *C. serrulata* and *S. isoetifolium*) Roots and rhizomes	Methanol	0.41 – 1.08 0.17 – 0.50	Gulf of Mannar	Present Study
Seagrasses leaves (*C. serrulata*, *T. hemprichii* and *S. isoetifolium*) Roots and rhizomes	Methanol	2.27 – 2.98 2.85 – 2.92	Palk Bay	Present Study

GAE= Gallic acid equivalents.

* $A^{1\%}$ gallic acid = 970.8, $A^{1\%}$ tannic acid = 830, $A^{1\%}$ Catechin = 1188.7 and $A^{1\%}$ pyrogallol = 1551.8. Blainski A., Lopes G. C. and de Mello J. C. P. (2013). Application and analysis of the Folin Ciocalteu method for the determination of the total phenolic content from Limonium Brasilience L. Molecules, 18, 6852 -6865.

** ∑ Rosmaric acid + Zosteric acid +Caffeic acid.

*** ∑ 4 – Hydroxybenzoic acid, 4 – Coumaric acid, t – Cinnamic acid, Ferulic acid and Caffeic acid.

Seasonal variations were not noticed in the adult leaves of *P. oceanica* while with the interactions of *Caulerpa taxifolia* and *C. racemosa*, adult *P. oceanica* leaves exhibited increased phenolic content (Dumay et al., 2004). A similar trend was noticed in *C. serrulata* from Gulf of Mannar, where comparatively higher concentration of phenolics noticed at Thonithurai (seaweed culturing area) than Chinnappalam while a slight increase was observed at Mathacovil (place with seaweeds as well as corals) than Munaikkadu of Palk Bay. Total phenolic content was higher in the intermediate leaves than adult at all the stations while sheaths contained lower concentrations than adult leaves. *E. acoroides* of Gulf of Mannar of this study displayed relatively higher concentrations of phenol in leaves followed by rhizomes and roots while the order was leaves> roots> rhizomes (Kannan et al., 2010b). 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 (Dumay et al., 2004). Phenolic content in seagrasses of Gulf of Mannar followed the order *H. pinifolia*>*C. serrulata*>*C. rotundata*>*S. isoetifolium*>*T. hemprichii*> *E. acoroides* (Kannan et al., 2013a). Total phenolic content in seagrasses showed higher concentrations at *H. pinifolia* and lower content in *E. acoroides* (Kannan et al., 2010c). Phenolic compounds act as potential antioxidants and free radical scavengers (Kannan et al., 2010a). *Halophila* genera of *H. ovalis* and *H. ovata* with *H. stipulacea* exhibited a wide variation in concentration (Rengasamy et al., 2012).

Total flavonoids

The concentration of flavonoids in seagrasses of the present study showed that *C. serrulata* predominated over *S. isoetifolium*, *E. acoroides*

and *T. hemprichii*. Flavonoids present in plants help them to interact with other organisms (Kannan et al., 2013b). Comparatively higher concentrations of flavonoids were reported in *C. serrulata* and lower at *H. pinifolia*, and wide variations were observed between species (Kannan et al., 2013a). Flavonoid level in seagrasses of Gulf of Mannar in this study followed the order *E. acoroides*>*C. serrulata*>*S. isoetifolium* while the trend was *C. serrulata*>*C. rotundata*>*T. hemprichii*>*S. isoetifolium*>*E. acoroides*> *H. pinifolia* (Kannan et al., 2013a). 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, and these flavonoids act as sun green pigments or compatible solutes (Gavin and Duracko, 2012). The geographical variations in flavonoids content in aqueous crude extract of *Z. noltii* contained Apigenin 7-sulfate (71.3-82.7%) and Diosmetin 7-sulfate (85.1-92.9%) as dominated flavonoids while Zosteric acid, Caffeic acid, Luteolin 7- sulfate, Apigenin 7-glucoside, Apigenin, Luteolin and Diosmetin showed below 5% of total flavonoids (Grignon-Dubois and Rezzonico, 2012). 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 subtidal while relatively lower concentrations in *H. decipiens* (Gavin and Durako, 2011).

Table 3.3.15 Comparison of flavonoid content in seagrasses.

Species name	Solvent	Flavonoid concentration	Location	References
Zostera noltii (leaves)	Aqueous	3378- 4355µg/g DW	Bay of Cadiz	Grignon- Dubois and Rezzonico,2012
Z. noltii (leaves)	Aqueous	6623- 9895 µg/g DW	Bay of Arachon	Grignon- Dubois and Rezzonico,2012
Halopila johnsonii		3.5- 3.8 nmol QE /mm^2 leaf	Munyon Island	Gavin and Durako,2011
H. decipiens		1.4 nmol QE/mm^2 leaf	Munyon Island	Gavin and Durako,2011
H. johnsonii		1.5 - 3.5 nmol QE/mm^2 leaf	Florida	Gavin and Durako,2012
Seagrass (*E. acoroides*, *C. rotundata*, *C. serrulata*, *T. hemprichii*, *H. pinifolia* and *S. isoetifolium*)	Aqueous methanol	0.22 to 5.12 mg QE/g	Chinnappalam	Kannan et al., 2013a
Seagrasses leaves (*E. acoroides*, *C. serrulata* and *S. isoetifolium*) Roots and rhizomes	Methanol	0.13- 0.34 mg QE/g 0.05- 0.16 mg QE/g	Gulf of Mannar	Present study
Seagrasses leaves (*C. serrulata*, *T. hemprichii* and *S. isoetifolium*) Roots and rhizomes	Methanol	1.04- 1.46 mg QE/g 0.69- 0.75 mg QE/g	Palk Bay	Present study

QE = Quercetin equivalents

Flavonoids in the hydrated ethanol extract of *T. testudinum* were ranged from 9.47 to 51.30mg/g (Hernández et al., 2016). Species wise variations in total flavonoid content in seagrasses were noticed. Biosynthesis of flavonoids is affected by light intensity and temperature, and also due to the level of maturation of different body parts (Ramah et al., 2014). Relatively lower concentration of flavonoids were noticed in the present study than other similar studies (Table 3.3.15) (Grignon-Dubois and Rezzonico, 2012 Kannan et al., 2013a).

Tannin

Comparatively lower concentrations of tannin were noticed in the present study compared to other similar works carried out (Table 3.3.16) (Torbatinejad et al., 2007; Athiperumalsami et al., 2008; Pradheeba et al., 2011; Kannan et al., 2013a). Seagrasses are used as feed for animals at coastal areas (de la Torre-Castro and Ronnback, 2004) and high intake of tannins more than 4mg/g tannin in feeds may cause protein precipitation followed by a decrease in platability which further leads to lower food intake as well as digestibility to animals (Torbatinejad et al., 2007). Relatively higher concentrations of tannin were noticed at leaves than roots and rhizomes, and also comparatively high content of tannin found at *C. serrulata* and lower at *S. isoetifolium* in this study. Comparatively higher concentrations of tannin were observed in leaves and rhizomes of *E. acoroides* while lower concentrations in *H. uninervis* and *H. pinifolia* (Pradheeba et al., 2011). Variations in tannin content based on species as well as stations were observed both at Gulf of Mannar and Palk Bay in this study.

Table 3.3.16 Comparison of tannin content in seagrasses.

Species name	Tannin content in mg/g	Location	References
Seagrass leaves (*E. acoroides* , *H. beccarii*, *H. ovalis*, *C. rotundata*, *C. serrulata*, *H. uninervis*, *H. pinifolia* and *S. isoetifolium*)	0.56-2.4	Palk Bay	Pradheeba et al., 2011
Seagrass rizhomes	0.56-1.9	Palk Bay	Pradheeba et al., 2011
Posidonia australis	17.4-18.5	South Australia	Torbatinejad et al., 2007
P. australis	17.4-18.5	South Australia	Torbatinejad and Sabine, 2001
Seagrass (*C. serrulata*, *H. ovalis*, *H. pinifolia* and *S. isoetifolium*)	2.94 - 9.80	Gulf of Mannar	Athiperumalsami et al., 2008
Seagrass (*E. acoroides* , *C. rotundata*, *C. serrulata*, *T. hemprichii*, *H. pinifolia* and *S. isoetifolium*)	1.13-17.12	Gulf of Mannar	Kannan et al., 2013a
Seagrasses (*E. acoroides* , *C. serrulata* and *S. isoetifolium*)	0.002-0.018	Gulf of Mannar	Present study
Seagrasses (*C. serrulata*, *T. hemprichii* and *S. isoetifolium*)	0.008-0.050	Palk Bay	Present study

Tannin content showed variations from species to species and seasonality, and the class of compounds in leaves usually play an active role in the defensive mechanism against epiphytes (Pradheeba et al., 2011). It was reported that cell wall constituents in different forms of *P. australis* recorded lower content of tannin in green and fresh leaves and high at dry washed leaves (Torbatinejad and Sabine, 2001; Torbatinejad et al., 2007). Tannin content in seagrasses of Gulf of Mannar in this study followed the trend; *E. acoroides*>*C. serrulata*>*S. isoetifolium* while the order was *H. ovalis* >*H. pinifolia*>*C. serrulata*>*S. isoetifolium* (Athiperumalsami et al., 2008), and *H. pinifolia*>*C. serrulata*>*C. rotundata*>*T. hemprichii*> *S. isoetifolium*>*E. acoroides* (Kannan et al., 2013a). Tannins are used for the treatment of burns as well as against chronic diseases (Athiperumalsami et al., 2008). It has been established that condensed tannins extracted from seagrasses functioned as deterrents against herbivore feeding, and antifungal as well as antibacterial (McMillian, 1984). Tannin content in *H. uninervis* was 1.22mg/g (Supriadi et al., 2016) and it was comparable to seagrasses of Gulf of Mannar while lower than at Palk Bay in this study.

3.3.3.3 Chlorophyll pigments

Chlorophyll content in seagrasses of Gulf of Mannar and Palk Bay in this study was comparable to similar studies carried out (Table 3.3.17) (Kannan et al., 2010a; Pradheeba et al., 2011). In previous studies, wide variations were observed between control and salt-stressed plants in the case of total pigments (Marin-Guirao et al., 2013). Both species as well as station wise variations in chl a and b were observed in the present study.

Table 3.3.17 Comparison of chlorophylls content in seagrasses.

Sl no.	Species	Location	Chl a in mg/g	Chl b in mg/g	Total Chl	Reference
1	*P. oceanica*	South Eastern Spain	282- 334 mg/m^2			Marin- Guirao et al., 2013
2	Seagrasses (*E. acoroides, T. hemprichii* and *T. ciliatum*)	Tanzania	0.3 - 0.4			Duadi et al., 2012
3	*T. testudinum*	Texas, USA			6.3- 8.3mg/g	Lee and Dunton, 1996
4	*C. nodosa* and *C. cirrhosa*	Bardawil Lake, Egypt	0.828- 1.187			Abd El- Hady et al., 2007
5	*C. nodosa*		1.9- 5.2	0.6- 2.1		Zavodnik et al., 1998
6	*P. oceanica*	Italy			2- 4 mg/g	Rotini et al., 2013
7	Seagrasses (*E. acoroides*, *H. beccarii*, *H. ovalis*, *C. rotundata*, *C. serrulata*, *H. uninervis*, *H. pinifolia* and *S. isoetifolium*)	Palk bay	0.02- 0.19	0.03- 0.149	0.05- 0.29mg/g	Pradheeba et al., 2011
8	Seagrasses (*C. serrulata*, *H. pinifolia* and *H. stipulacea*)	Mandapam coast	0.454 - 0.885	0.247 - 0.541	0.701- 1.424 mg/g	Kannan et al., 2010a
9	Seagrasses (*C. serrulata*, *H. ovalis*, *H. pinifolia* and *S. isoetifolium*)	Gulf of Mannar	0.385- 1.403	0.161- 0.719	0.545 - 2.003 mg/g	Athipermalsami et al., 2008
10	Seagrasses (*E. accordies*, *C. serrulata* and *S. isoetifolium*)	Gulf of Mannar	0.010 - 0.850	0.008- 1.029	0.018 – 1.879	Present study
11	Seagrasses (*C. serrulata*, *T. hemprichii* and *S. isoetifolium*)	Palk bay	0.002 - 0.136	0.001- 0.069	0.003 – 0.205	Present study

Chl concentration in seagrasses biofilm in *E. acoroides*, *T. hemprichii* and *T. ciliatium* showed no significant differences; both species and locations wise (Daudi et al., 2012). Total chl content in the leaves of *T. testudinum* varied with respect to seasons and chl a/b ratios varied from 2.9 (September) to 3.2 (December), and relatively higher concentrations were found during spring and lower in winter (Lee and Dunton, 1996). Chl content in seagrass varied with changes in temperature and light intensity. 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 due to turbidity (Dennison and Albarte, 1985; Abal et al., 1994; Lee and Dunton, 1997). The light reductions on the seagrass meadows responded by an increase in chl content and thereby a decrease in the ratio of chl a:b (Lee and Dunton, 1996). Species and location wise variations were noticed in three seagrasses of Malaysia (Wan Hazma et al., 2015).

Chl content was more obtained in *C. nodosa* than *R. cirrhosa* and comparatively higher concentrations during summer might be due to the presence of thick layer of epiphytic algae on the seagrasses (Abd El-Hady et al., 2007). In this study much lower concentrations of chl a and b investigated at rhizomes in comparison with leaves. Chl a predominated was over chl b in leaves in seagrass of Palk Bay (Pradheeba et al., 2011). Chl a content in the leaves of *C. nodosa* showed seasonal variations and highest concentrations of chl a and b was obtained during the month of September, and the maximum concentration was169mgdw/m^2 (Zavodnik et al., 1998). Total chl content in the leaves of *P. oceanica* increased from summer to winter, and chl a/ b ratio as well as total chl/soluble proteins were higher

during winter. The low chl content during summer might be due to its degradation and related to the surrounding environmental factors such as temperature, light, stress, etc. (Rotini et al., 2013). Plants generally exhibit an increase in chl a/ chl b ratio during summer to winter season. 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 (Lichtenthaler and Babani, 2004). Chl a and b in *T. testudinum* was 0.26-1.52µg/gDW and 0.43-1.56µg/gDW respectively (Hernández et al., 2016) and the level of chls were much higher in this study. A positive correlation of chls with protein was observed. Major factors to be affected in the concentration of chls in marine plants are light, temperature and photosynthetic rate, and these three are inter connected and showed a decrease in chls at a temperature of 30-35 ^{o}C (Hernández et al., 2016). This could be the reason for comparatively higher chls found in Gulf of Mannar than Palk Bay. Ecophysiological parameters of pH and temperature were strongly affected in the pigment content of *Z. noltii* from Portugal. Total chl content was not strongly influenced by pH and temperature while chl a and b concentrations were decreased with increase in pH and temperature. The level of pheophytins increased with increase in pH and temperature (Repolho et al., 2017).

Chl content in seagrasses were affected by the availability of light and morphology of the seagrass leaves. The species to species variations in chl content depends on the depth of seagrass habitat and surface areas of the leaf (Pradheeba et al., 2011). In this study, seagrasses with cylindrical leaf of *S. isoetifolium* showed higher concentrations than *C. serrulata* and

E. acoroides at Gulf of Mannar while the order was *S. isoetifolium* followed by *T. hemprichii* and *C. serrulata* at Palk Bay. Seagrass with broad leaf showed more chl content than cylindrical as well as linear leaf (Pradheeba et al., 2011). It is also seen that the accumulation of trace metals of Zn, Cd and Cu in the tissue of *T. hemprichii* had an effect on the photosynthetic pigments, and consequent decrease in the concentrations of chl a and b (Lei et al., 2012). Enhanced levels of chlorophyll pigments in seagrasses correspond to high photosynthetic activity and productivity (Kannan et al., 2010a). Chl a content in seagrasses of Gulf of Mannar followed the trend; *H. pinifolia*>H. *ovalis*>*C. serrulata*>*S. isoetifolium* while chl b followed the order; *H. pinifolia*>*H. ovalis*>*C. serrulata*>*S. isoetifolium* (Athipermalsami et al., 2008).

3.3.3.4 Elemental composition

Comparatively higher elemental compositions were noticed in the present study compared to earlier reports in C contents (Table 3.3.18) (Mateo et al., 2003; Yamamuro and Chirapart, 2005; Athiperumalsami et al., 2008; Campbell et al., 2012; El Din and El-Sherif, 2013), N contents (Table 3.3.18) (Spencer and Ksander, 1994; Athiperumalsami et al., 2008; Campbell et al., 2012; El Din and El-Sherif, 2013; Wan Hazma et al., 2015) and P contents (Table 3.3.18) (Mateo et al., 2003; Campbell et al., 2012; El Din and El-Sherif, 2013; Wan Hazma et al., 2015). Species wise variations in elemental compositions were noticed and comparatively higher concentrations were noticed in the leaves than roots and rhizomes except *C. serrulata* from Palk Bay.

Table 3.3.18 Comparison of elemental composition of seagrasses.

Sl.No.	Species	Location	C %	N %	P %	References
1	*T. testudinum*	America	34.6-37.9	2.14-3.02	0.09-0.70	Campbell et al., 2012
2	*H. uninervis* and *S. isoetifolium* (leaves) *H. uninervis* and *S. isoetifolium* (rhizomes)	Australia	--- ---	1.60-3.0 0.50-1.60	0.26-0.30 ---	Udy et al., 1999 Udy et al., 1999
3	Seagrasses (*H. uninervis*, *T. ciliatum*, *C. rotundata* and *S. isoetifolium*	Kenya	36.6-42.1	2-3.1	---	Mariani and Alcoverro, 1999
4	*P. oceanica*	Spain	28-39.1	0.70-1.48	0.021-1.24	Mateo et al., 2003
5	Seagrasses (27 species average)		33.6	1.92	0.23	Duarte, 1990
6	*Potamogeton nodosus* and *Hydrilla verticillate*	California	40.1-43	1.3-3.1	---	Spencer and Ksander, 1994
7	Seagrasses leaf blades leaf sheath roots and rhizomes *(E. acoroides , H. ovalis, C. rotundata and T. hemprichii)*	Thailand Thailand Thailand	31.4-38.9 29.6-34.3 17.7-36	1.90-3.02 1.38-1.72 0.41-0.86	0.236-0.264 0.217-0.23 0.041-0.18	Yamamuro and Chirapart, 2005 Yamamuro and Chirapart, 2005 Yamamuro and Chirapart, 2005
8	Seagrasses *(C. serrulata, H. ovalis, H. pinifolia and S. isoetifolium)*	Gulf of Mannar	1.76-3.35 (OC)	0.56-2.22	---	Athiperumalsami et al ., 2008
9	*C. nodosa* and *P. oceanica*	Egypt	12.4-13.23	8.45-10.6	1.21-2.13	El Din and El Sherif, 2013
10	*Halophila ovalis, H. spinulosa* and *Halodule uninervis*	Johore, Malaysia	---	0.81-1.73	0.15-0.31	Wan Hazma et al., 2015
11	Seagrasses *(E. acoroides, C. serrulata and S. isoetifolium)*	Gulf of Mannar	31.72-40.47	0.66-2.93	0.126-0.453	Present study
12	Seagrasses *(C. serrulata, T. hermprichii and S. isoetifolium)*	Palk Bay	35.21-41.18	0.77-1.58	0.125-0.622	Present study

Variations in N, C and P contents were reported in seagrass species (Udy et al., 1999; Campbell et al., 2012; Wan Hazma et al., 2015), and comparatively higher concentarions of N and P were observed in leaves followed by roots and rhizomes in *H. uninervis* whereas in *S. isoetifolium*, N content predominated in leaves followed by rhizomes (Udy et al., 1999). Total organic carbon content in *T. testudinum* varied from 7.2 -93.3gcm^{-2} while total nitrogen 0.44 - 6.54gNm^{-2} and total phosphorous 2.7- 276mgPm^{-2} (Williams et al., 2009). Lower concenrtrations of C and N content has already been noted in seagrasses *S. isoetifolium* and *H. Uninervis* while higher at *T. ciliatum* and *C. rotundata* (Mariani and Alcoverro, 1999). Seagrass species *S. isoetifolium* and *C. rotundata* are preferred by fishes due to their relatively higher contents of carbon as well as low carbon fibre whereas sea urchins prefer long lived seagrasses of *T. hemprichii* and *T. testudinum* (Valentine and Heck, 1991; Klump et al., 1993). The elemental composition of seagrass *P. oceanica* leaves were reported to be abundant in P content whereas in leaf litter (banquette) C and N content predominated (Mateo et al., 2003).

Station wise variation in elemental compositions was observed in the present study. It is seen that seagrasses are a good source of major elements and the distribution pattern for C, N and P were 36%, 1.5% and 0.2% respectively, in line with surrounding environmental conditions (Duarte, 1990). Further in this study relatively higher species to species variations in C and N content were observed particularly from Gulf of Mannar and Palk Bay, and P content diplayed more variations at Palk Bay (0.13 to 0.62%). The nutrient availability in seagrasses increased to meet the needs and was enriched in nitrogen and phosphorous with

respect to carbon content, and thereby a decrease in the C:N and C:P ratios (Duarte, 1990). A large variation in C/N ratios in seagrasses were reported (from 18.3 (*T. testudinum*) to 22.4 (*P. oceanica*)) and C/P ratios (from 256.6 (*E. acoroides*) to 732.5 (*P. oceanica*)) (Cebrian and Duarte, 1998), while in this study C/N ratio showed much more variation from 13.81 to 48.06 and a marginally lower C/P ratio ranged from 66 to 271. Comparatively higher values in *P. oceanica* related to high carbon content (Cebrian and Duarte, 1998). Total C content in rooted aquatic plants revealed that relatively higher C and N contents were found in these species and variations among species were more in N content than carbon (Spencer and Ksander, 1994). Seagrasses of *Z. japonica* was taken as a tool for the determination of changes in elemental composition against changes adopted in the sediment nutrient as well as organic matter by Han et al. (2017). Based on this nutrients loadings in sediment showed an increase in N content in leaves as well as roots and rhizomes while a decrease in P and N content was observed with respect to organic matter. The effect of changes in the environmental parameters of salinity, temperature, pH and sediment characteristics with respect to season showed that differences were obtained in the leaves of *Z. noltii* meadows of Portugal in C and N biomass by Sousa et al. (2017) while relatively lower changes noticed in the roots and rhizomes. In the present study, no wide variations were observed in the leaves of seagrasses of Gulf of Mannar and Palk Bay.

The distribution pattern of C, N and P showed higher levels in the leaf blades followed by leaf sheath and roots and rhizomes, and comparatively higher differences noticed at roots and rhizomes than other body parts in the

case of P and N content; and relatively higher concentrations were observed in seagrasses with slow growing rate (Yamamuro and Chirapart, 2005). Athiperumalsami et al. (2008) reported that N content in seagrasses of Gulf of Mannar were comparable to other seagrasses and organic carbon content varied from 1.76 (*H. ovalis*) to 3.35% (*C. serrulata*). Elemental components in a sulphated polysaccharide isolated from *H. pinifolia* contained 18.25% of C and 1.77% N with a C/N ratio of 2.04 (Kannan et al., 2013b). The decay kinetics of C, P and N content in scales and rhizomes of *P. oceanic*a showed that C loss with time was very lower in rhizomes and scales, and a sharp decrease was observed in P and N content in rhizomes (Romeo et al., 1995). Comparatively higher P content was noticed in *P. oceanic*a than *C. nodosa* whereas vice versa in organic carbon and N content (El Din and El-Sherif, 2013). Relatively higher concentrations of P found in the seeds of flour than starch of *E. acoroides* of Philippines (Montano et al., 1999).

3.3.3.5 Macro elements composition

Distribution pattern of macro elements in the present study showed predominance of Na followed by Ca, K and Mg and was similar to the earlier report (Malea, 1998). The differences in concentrations of these elements of same species from the same locations or different locations might be related to the differences in the age of plants, their time of collection and environmental factors (pH, salinity, temperature, etc.) which influence metal uptake (Malea, 1998). In this study, relatively higher concentrations of K, Ca and Mg noticed in seagrasses at Gulf of Mannar while at Palk Bay, higher Na was noticed. Species as well as location wise variations in the level of macro elements were observed in the present study and it was more pronounced at Gulf of Mannar rather

than Palk Bay. Leaves of *P. australis* from the West coast of Australia followed the order Na>K>Ca>Mg and Ca content was relatively lower in comparison with other seagrasses of the same genera (Hocking et al., 1980). *P. oceanica* leaves of Tunisia showed distribution of macro elements in the order Mg>Ca>K and K content was comparatively very low (Saidane et al., 1979). Distribution of macro elements at different body parts in *E. acoroides* showed higher contents of Na and K in rhizomes followed by roots and leaves whereas Ca and Mg followed the order roots>rhizomes>leaves in this study. The Mg concentration in leaf of selected seagrass species from Gulf of Aqaba (Jordan) followed the order of *H. stipulacea>H. ovalis>H. uninervis* and the same in different body parts followed the order leaves>roots>rhizomes at both *H. stipulacea* and *H. ovalis* (Wahbeh, 1984). In the same way, species to species variation in different body parts were reported (Jagtap, 1983; Kannan et al., 2011) and among the different body parts, comparatively higher concentrations of minerals in most of the seagrasses were noticed at the rhizomes whereas lesser at roots (Jeevitha et al., 2013).

Among these elements in seagrasses of Gulf of Mannar analysed in this study, relatively higher concentrations of Na and K noticed at *C. serrulata* and Ca and Mg in *S. isoetifolium*. These elements in seagrasses followed the order Mg>Na>K>Ca except in *Cymodocea* genera. Relatively more variations in seagrasses between seasons were observed in the case of Na and it was lesser in K, Ca and Mg (Jeevitha et al., 2013). Relatively higher concentrations of K and Na observed at monsoon and lower at premonsoon whereas higher concentrations of Mg in premonsoon and low at post monsoon (Jeevitha et al., 2013). The effect of hypersaline stress on the

minerals of seagrass of *P. oceanica* leaf has been estimated and Na levels increased with respect to control, and comparatively more differences with the control were exhibited after three months period (Marin-Guirao et al., 2013).

Further, comparatively higher Mg, K and Ca contents were noticed in the roots and rhizomes of *Z. marina* and variations observed were in higher in Na while the leaves showed higher concentrations of K and Ca levels (Lyngby and Brix, 1983). Relatively higher concentration of Ca was found in the seeds of flour than starch of *E. acoroides* of Philippines (Montano et al., 1999). Major elements in the leaves of *C. nodosa* and *P. oceanica* revealed that Na and Ca were predominated in *P. oceanica* while K in *C. nodosa*. The differences in minerals were related to metabolic reactions, environmental conditions, seasonal variations and differences in the requirement of plants. Moreover concentration of minerals in the interstitial as well as overlying waters, because roots uptake minerals from these areas and translocated to different body parts (El Din and El-Sherif, 2013). Most of the macro elements studied were in lower concentrations than similar earlier reports (Table 3.3.19) (Hocking et al., 1980; Malea, 1998; Jeevitha et al., 2013; El Din and El Sherif, 2013;Wan Hazma et al., 2015).

Table 3.3.19 Comparison of macro elements in seagrasses (µg/g).

Sl no.	Species	Location	Na	K	Ca	Mg	References
1	Seagrasses (*H . stipulacea*, *P. oceanica* and *C. nodosa*)	Greece	20557-62596	13023-24320	13285-25533	6791-11788	Malea,1998
2	*P. australis*	Australia	40956	26532	88013	5406	Hocking et al.,1980
3	*P. oceanica*	Tunisia	---	350	14200	18000	Saidane et al.,1979
4	Seagrasses leaves rhizomes roots (*H. stipulacea*, *H. ovalis* and *H. uninervis*)	Jordan Jordan Jordan	--- --- ---	--- --- ---	--- --- ---	12574-15760 9508-11003 14086-14408	Wahbeh, 1984
5	*H. beccarii*	India	2650-7750	3150-6500	275-4475	7225-9800	Jagtap, 1983
6	Seagrasses (*E. acoroides, H. pinifolia*, *C. rotundata*, *C. serrulata*, *T. herprichii*, and *S. isoetifolium*)	Gulf of Mannar	30-690	10-300	19-220	91-912	Kannan et al., 2011
7	Seagrasses (*C. serrulata*, *H. ovalis*, *H. pinifolia* and *S. isoetifolium*)	Tuticorin	18300-60600	17300-37400	11700-28100	17800-39800	Jeevitha et al., 2013
8	*E. acoroides*	Philippines	---	---	320-923	---	Montano et al.,1999
9	*C. nodosa* and *P. oceanica*	Egypt	10444-27650	4816-6754	24700-38900	---	El Din and El Sherif, 2013
10	Seagrasses *(H. decipiens, C. rotundata, C. serrulata, H. uninervis, T. hemprichii , H. pinifolia* and *S. isoetifolium)*	Lakshadweep	---	---	---	5123-10368	Thangaradjou et al., 2013
11	*Halophila ovalis, H. spinulosa* and *Halodule uninervis*	Johore, Malaysia	---	4126-10942	4705-10576	3244- 3534	Wan Hazma et al., 2015
12	Seagrasses (*E. acoroides*, *C. serrulata* and *S. isoetifolium*)	Gulf of Mannar	2130-24540	340-12410	1300-18230	1370-8900	Present study
13	Seagrasses (*C. serrulata*, *T. hemprichii* and *S. isoetifolium*)	Palk Bay	4710-39250	2160-7850	3800-17210	2360-6540	Present study

3.3.3.6 Heavy metals

Seagrasses are important contributors to primary production and had the ability to uptake the trace metals from water through leaves and sediment as well as interstitial water through roots. Seagrass consumers (green turtle, sea cow, sea urchins, fishes and other organisms) feed the leaf, root, rhizomes and detrital materials of seagrasses and leads to higher concentrations of heavy metals in predators. The major sources for the metals in seagrasses is marine ecosystem which in turn is due to land run off due to rain, sewage, boating operation, boat maintenance activities, paint industries, fertilizers, herbicides, etc. (Thangaradjou et al., 2010). Heavy metal concentrations were also affected by northwest and southeast monsoon along with bidirectional asymmetric diurnal tidal flows (Suwandana et al., 2011). Fe content in seagrass species was relatively higher from Thonithurai and Chinnappalam and this goes with earlier study in seagrasses (*E. acoroides*, *S. isoetifolium*, *H. pinifolia* and *C. rotundata*) (Kannan et al., 2011). Heavy metals (Fe, Mn, Zn, Ni, Co, Cu, Cr, Cd and Pb) in seagrasses showed species to species and location wise variations in their concentration. Species to species variations were pronounced more in Gulf of Mannar than Palk Bay in the present study and species wise variations were similar to ealier findings (Malea, 1998; Schlacher-Hoelinger and Schlacher, 1998a; Khristoforva and Chernova, 2005; Lewis et al., 2007; Kannan et al., 2011; Thangaradjou et al., 2010, 2013; Sudharsan et al., 2012). The trace metals content particularly Pb, Cr and Cd were within limit prescribed by WHO for foods (WHO 1991) (Kannan et al., 2011). Location and species wise variations were observed in the concentration of trace metals in seaweeds while only species wise variations in seagrasses could be due to the adsorption, intracellular

compartmentalization of metals, chelation and secretion of extracellular exudates mechanism chosen by the macrophytes for metal tolerance or resistance (Sudharsan et al., 2012). In the present study, Mn and Pb from Gulf of Mannar noticed higher concentrations in *S. isoetifolium* than *C. serrulata* and Ni, Mn, Cd and Pb from Palk Bay side. Species to species variations were noticed in Mn content and most of the trace metals were relatively higher in *S. isoetifolium* than *C. serrulata* except Cd. Trace metals like Cd, Ni and Pb were lower concentrations in seagrasses than seaweeds whereas Cu, Mn and Zn were higher in seagrasses (Sudharsan et al., 2012). Marine algae, seagrasses and other aquatic biota can be used for the absorption metals to find out the level of pollution (Thangaradjou et al., 2010; Sudharsan et al., 2012). Presence of heavy metals also affected in the biological process of cellular metabolism, inhibition of photosynthesis, etc. Cd content in these organisms causes disturbances in concentrations of protein, carbohydrate and pigment (Sudharsan et al., 2012).

Heavy metals (Mn, Fe, Cu and Zn) in seagrasses demonstrated that they have the ability to accumulate from the surrounding environment. Mechanisms that contribute to magnify the contaminants in the environment include rainfall, ocean currents, wind and geographical condition, and trace metal concentration in seagrass tissues gives an outline on the pollution in the water column (Govindasamy et al., 2011). Variations in the concentrations as well as distribution of trace metals in the leaves and roots and rhizomes of *C. serrulata* showed higher levels at Gulf of Mannar than Palk Bay except Fe while trace metals of Co, Cr, Ni, Zn, Cd and Pb predominated in *S. isoetifolium* of Gulf of Mannar and Fe, Cu and Mn at Palk Bay. The leaf, root and rhizome of *E. acoroides* in this study showed

that Fe, Mn, Ni and Cd predominated in roots while Cu, Zn and Pb rhizomes and Co and Cr in leaves. It was also reported that Mn, Fe and Cu were higher in leaves of *C. serrulata* and Zn in *S. isoetifolium*, while Fe and Mn predominated in *C. serrulata* and Cu and Zn in *S. isoetifolium* (Govindasamy et al., 2011). Differences in the rate of accumulation of trace metals in soft tissues varied with respect to the mechanisms of metal binding and regulation. The availability of trace metals were influenced by the cation exchange capacity, pH of sediment as well as water, redox potential, water temperature, salinity, organic concentration and concentration of other metals in the ecosystem (Ward, 1989; Govindasamy et al., 2011). Wide variations in the concentration of heavy metals were noticed between species to species and depends on the factors of sediment resuspension, run off from the land, fluvial inputs, microbial degradation, etc. (Schlacher-Hoelinger and Schlacher, 1998b; Thangaradjou et al., 2013). An inter and intra specific variations in the heavy metal concentrations in seagrasses were also affected by environmental and metabolic levels of different phonological stages (Eide et al., 1980).

Interestingly, comparatively lower concentrations of Cd, Cu, Fe and Pb were noticed at *C. rotundata* while lower contents of Co, Cr, Zn, Mn and Ni were repoted in *S. isoetifolium* and *C. serrulata* (Thangaradjou et al., 2013). The rate of productivity as well as growth had important role in the variations of trace metal concentrations, rapid growth leads to dilution of metals within the plant tissue (Lyngby and Brix, 1982) and this is in support of the findings in *C. serrulata* between Gulf of Mannar and Palk Bay in the present study. Mn, Zn and Fe concentrations in seagrass species with small leaves of *C. serrulata* observed higher metal concentrations than large

leaves of *E. acoroides* in this study and seagrasses of larger leaves might be utilizing these metals for their physiological activities (Thangaradjou et al., 2013). Most of the metal concentrations in the seagrasses of Andaman Islands were higher than the present study and rest of them comparable (Thangaradjou et al., 2010). Out of ninety heavy metals, fifty three are naturally occurring and among these Cu, Mn, Fe and Zn are essential micronutrient for plant growth (Thangaradjou et al., 2010). Utilisation of Mn in plants includes maintaining osmotic balance, ion regulation and enzyme catalysis and it occur at higher concentrations in plants (Clarkson and Hanson, 1980).

Seasonal variations in trace metals concentration showed that the accumulation of Cu, Pb and Zn were higher in the month of January while Cd in July. Among the seagrasses, comparatively high content of Cd and Zn obtained in *T. hemprichii* whereas Cu and Pb in *E. acoroides* (Lei and Xiaoping, 2012). Higher concentrations of Cu, Zn and Pb in January might be due to the growth dilutions per unit mass of seagrasses. Young blades become numerous during January while adult predominated in July (Malea and Haritonidis, 1995). Also Cd concentrations increased with age of seagrasses (Ancora et al., 2004). Higher concentrations of Cd in seagrasses and other macrophytes lead to the synthesis of phytochelatin and metallothionein (metal biomolecule proteins) which have the ability to reduce the oxidative stress formed by metals (Alvarez-Legorreta et al., 2008). A linear absorption pattern was observed in Cd and Zn from ambient seawater by seagrass shoots and blades, and then transported basipetally (Warnau et al., 1996).

Comparatively higher concentrations of Cu, Ni and Pb were noticed in the leaves of *H. uninervis* than rhizomes while almost similar results were observed in Fe (Al-Bader et al., 2014). Relatively lower concentrations of Cd, Cu and Zn noticed in *S. isoetifolium* whereas higher Pb found at *E. acoroides* from Indonesia region (Nienhuis, 1986). A comparison between seagrass *P. oceanica* and mussel *M. galloprovinciallis* were used as pollution indicators revealed comparatively higher bio sediment accumulation factor for *P. oceanica* for Cd, Co and Ni (Lafabrie et al., 2007). Lower concentration of pollutants in seagrasses and higher in sediments of same location might be due to the availability of large amounts of calcium carbonate grains in the sediment which decreased the absorption of trace metals by plants. This might be the reason for the absence or less accumulation of trace metals even though higher concentrations were noticed in sediments (Sanchiz et al., 2001; Balestri et al., 2004). Also aquatic plants have the ability to remove Pb from the sorrunding water (Axtell et al., 2003). There are reported variations in aquatic plants in accumulating heavy metals. Comparatively high contents of Cu and Mn were found in seagrasses while Fe, Ni and Cd were higher in brown algae, and species wise variations were lesser in trace metals of Cu, Ni and Cd in seagrasses as well as brown algae. Iron, one of the major metal used in metabolic reactions in plant showed wide variations, with higher concentrations in *Z. marina* while Cd predominated in *P. iwatensis* (Khristoforva and Chernova, 2005). The ability of algae to form more stable insoluble organic complexes might be the reason for the relatively high Cd content in algae than seagrasses (Khristoforva and Chernova, 2005).

Species wise variations in the trace metal concentration were noticed in *T. testudinum* and *H. wrightii* (Lewis et al., 2007), and in *P. oceanica*, *C. nodosa* and *H. stipulacea* (Malea, 1998). Metal absorption pattern in marine phanerogams were happened through their roots as well as leaves but the rate of absorption vary with respect to each metal (Nicolaidou and Nottu, 1998; Schlacher-Hoelinger and Schlacher, 1998a; Wasserman and Wasserman, 2002). Important factors contributing to the absorption of metals are salinity and photosynthesis (Wasserman and Wasserman, 2002) and also the nature of plant organ whether live or dead leaves, roots, rhizomes, blade, sheath, etc. as well as living environmental conditions (Schlacher-Hoelinger and Schlacher, 1998a; Gosselin et al., 2006). The rate of metal decrease in seagrasses followed the order Pb>Cd>Zn, and fishes also followed the same order while no clear picture was noticed in crustaceans and no comparisons in the case of ascidian and molluscs (Ward et al., 1986). Stations with high human activities were showed high metal concentrations. High content of Cd, Pb and Zn were reported in lagoon mainly attributed to entry of metals to lagoon via rain water (Sanchiz et al., 2000). The concentrations of Fe, Cr and Ni were lower in lagoons with limited tidal and unrenewal of water than seagrass beds while Cu and Pb concentrations were similar or higher (De Casabianca et al., 2004). Trace metals content in *Z. noltii* from Sinop coast of the Black Sea followed the trend; Fe>Zn>Cu>As=Pb>Hg (Bat et al., 2016) and these metals concentrations were lower in the present study. Heavy metals content in the shoots and roots of *Z. japonica* followed the order; Mn>Zn>Pb>As>Cu>Cr>Cd>Hg (Lin et al., 2016) and the levels of Hg, Cr and Zn were lower and As, Cu, Pb and Mn were higher than this study.

Table 3.3.20 Comparison of heavy metals in seagrasses (µg/g).

Sl. No.	Species name	Location	Co	Se	Cr	Cu	Fe	Mn	Ni	Zn	Cd	Pb	Hg	As	References
1	Seagrasses (*E. acoroides*, *H. pinifolia*, *C. rotundata*, *C. serrulata*, *T. hemprichii* and *S. isoetifolium*)	Chinnappalam	0.283-0.431		0.983 - 3.956	2.946-7.800	67.586 - 156.56	7.173-23.42	0.413-1.513	4.563- 17.59	0.121-0.356	0.813-2.040			Kannan et al., 2011
2	Seaweeds (8 species)	Kanyakumari,	- - - -		- - - - -	0.3-6.55	- - - - -	0.62-11.38	0.16-.803	0.45-1.24	0-0.300	0.2-0.52			Sudharsan et al., 2012
	S. isoetifolium & *C. serrulata*	Ervadi Thondi	- - - -		- - - - -	3.84-5.09	- - - - -	4.21-14.38	0.10- 0.16	1.02-1.75	0-0.012	0.26-0.3			
3	*S. isoetifolium* & *C. serrulata* (Above ground level)	Palk Strait	- - - -		- - - - -	0.05-0.53	0.22-1.33	0.24-0.90	- - - - -	0.05-0.59	- - - - -	- - - - -			Govindasamy et al., 2011
	Below ground level	Palk Strait	- - - -		- - - - -	0.40- 1.48	0.64-2.85	0.67-7.93	- - - - -	0.50-3.88	- - - - -	- - - - -			
4	Seagrasses (*H. decipiens*, *C. rotundata*, *C. serrulata*, *H. uninervis*, *T. hemprichii* , *H. pinifolia* and *S. isoetifolium*)	Lakshadweep Islands	0.07-11.2		2.94- 19.8	4.87-21.5	124.3-10368	19.97-1255	1.91- 19.4	27.36-69.17	0.97-2.15	5.18-23.1			Thangaradjou et al., 2013
5	Seagrasses (*E. acoroides*, *H. pinifolia*, *H. ovalis*, *H. ovata*, *H. uninervis*, *C. rotundata*, *T. hemprichii*, and *S. isoetifolium*)	Andaman Islands	1.3.28		58.28-49.44	30.52-109.52	525- 1920	508- 2224	1.76-10.04	28.88-85.52	2.24- 6.92	4.16-17.72			Thangaradjou et al., 2010
6	*C. rotundata* (leaves)	Indonesia (Baten Bay)	- - - -		- - - - -	5.43- 9.08	700- 4000	- - - - -	- - - - -	51.85-195.5	0-1.05	N.D.			Suwandana et al., 2011
	C. rotundata (corns)	Indonesia (Baten Bay)	- - - -		- - - -	3.97- 10.18	2200- 4600	- - - - -	- - - - -	57.50-103.4	N.D.	N.D.			

7	*T. hemprichii*, *E. acoroides* and *C. rotundata*	South China (Hainan Island)	- - - -		- - - - -	9.0– 22.3	- - - - -	- - - - -	- - - - -	2.89– 63.6	1.2– 3.2	9.8– 18.2		Lei and Xiaoping, 2012
8	*H. uninervis* (leaves) *H. uninervis* (rhizomes)	Kuwait Kuwait	- - - - - - - - - -		- - - - - - - - - -	50.6–89.8 52.3–86.7	5– 8.4 6.1– 7	- - - - - - - - - -	55.6–87.2 55.7–72.1	- - - - - - - - - -	- - - - - - - - -	20.5–32.6 17.4–29.9		Al Bader et al., 2014
9	Seagrasses (*E. acoroides*, *H. pinifolia*, *C. rotundata*, *C. serrulata*, *T. hemprichii*, *H. ovalis*, *T. ciliatum*, *H. uninervis* and *S. isoetifolium*)	Flores Sea, Indonesia	- - - - -		- - - - -	3.9– 17.3	- - - - -	- - - - -	- - - - -	15– 63	0.16– 1.54	1.7– 3.9		Nienhuis, 1986
10	*P. oceanica* *M. galloprovincialis* (Mussel)	North western Mediterranean	1.7– 12.070.0 6–1.43		0.20– 1.270 43– 3.00	- - - - -	- - - - -	- - - - -	27.4– 60.301.10 – 3.67	- - - - -	2.10–5.38 1.13–1.82	1.40– 1.80 1.07– 1.43	0.05– 0.13 0.09- 0.12	Lafabrie et al., 2007
11	*Z. marina* and *Phyllospadix iwantensis* *S. pallidium* and *S. meyabei* (Brown algae)	Peter the Great bay Peter the Great bay	- - - - - - - - - -		- - - - - - - - - -	20.9– 2.39 1.69– 1.84	42.62– 111.1 100.7– 251.9	47.38– 239.7 8.95– 26.1	2.09– 2.13 4.22– 5.30	- - - - - - - - - -	1.61– 2.16 3.05– 3.41	- - - - - - - - - -		Khiristoforova and Chernova, 2005
12	*T. testudinum* and *H. wrightii* (Blades) roots & rhizomes	Florida Florida	- - - - - - - - - -	<1.0– 2.5 <1.0– 1.95	<0.1– 3.5 0.2 – 2.9	<5– 20.5 <5– 15.3	- - - - - - - - - -	- - - - - - - - - -	<1– 3.5 0.6– 2.7	3.4– 7.3 2.50– 52	<0.1– 1.2 <0.1– 1.3	0.3– 4.8 0.3– 4.1	0.0 – 2.38 0.0 – 0.19	<2.0– 8.0 <2.0 – 3.7	Lewis et al., 2007
13	Seagrasses (*H. stipulacea*, *P. oceanica* and *C. nodosa*)	Greece	- - - - -		- - - - -	16.1– 18.0	372– 851	- - - - -	- - - - -	25.4– 43.4	11.9– 20.8	37.8– 50.9			Malea, 1998
14	*P. oceanica* *M. galloprovincialis* (Mussel)	Saranda Bay, Albania Saranda Bay, Albania	- - - - - - - - - -		1.29– 37.1 0.56– 2.52	6.29– 16.53 15.20– 88.40	- - - - - - - - - -	- - - - - - - - - -	- - - - - - - - - -	- - - - - - - - - -	0.22– 1.86 1.97– 4.10	2.21– 19.1 1.15– 3.88			Malltazi et al., 2012
15	*Z. noltii*	Atlantic French coast	- - - - -		- - - - -	6.09– 7.75	2411– 8135	128.5– 599	- - - - -	50.4– 77.5	- - - - -	- - - - -			Wasseman and Wasseman, 2002

16	*P. oceanica*	North western Mediterranean Sea	- - - -	52 - 80	22 - 123	9 - 16	- - - - -	- - - - -	9 - 111	14 - 31	0.4- 1.2	6 - 25		14 - 21	Gosselin et al., 2006
17	*P. oceanica* (leaves) (rhizomes) (roots)	Mediterranean	- - - - - - - - -		- - - - - - - - -	14 16 27	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	167 60 70	1 0.6 1.2	2 10 2			Schlacher-Hoenlinger and Schlacher, 1998
18	*P. oceanica* (leaves) Algaes (2 species) Mullusks (2 species)	Linosa Island, Sicily Linosa Island, Sicily Linosa Island, Sicily	- - - - - - - - -		0.11- 0.24 0.32- 0.68 0.42- 0.64	4.97- 13.25 4.91- 13.25 5.87- 20.18	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	31.7- 103.4 26.2- 39.3 43.2- 55.4	1.85- 3.51 0.86- 1.07 1.09- 3.57	1.32- 4.29 2.94- 4.78 1.01- 1.17			Conti et al., 2010
19	*Z. marina* (roots) leaves	Thau lagoon, France Thau lagoon, France	- - - - - -		2 0.3	19 10	921 186	- - - - - -	1 0.6	44 83	- - - - - -	2 1			De Casabianca et al., 2004 De Casabianca et al., 2004
20	*Halophila ovalis, H. spinulosa* and *Halodule uninervis*	Johore, Malaysia				13- 17	3737- 5954	160- 173		146- 212					Wan Hazma et al., 2015
21	*Z. japonica* (shoots and roots)	Yellow river estuary, China			4.31- 5.84	10.47- 18		928- 2544		33.16- 43.36	1.74- 4.52	4.32- 37.3	0.01- 0.02	5.19- 33.76	Lin et al., 2016
22	*Z. noltii*	Black Sea,Turkey				2	500			14	0.18	1	0.04	1	Bat et al., 2016
23	Seagrasses (*E. acoroides, C. serrulata and S. isoetifolium*)	Gulf of Mannar	0.25- 3.391		1.8- 34.7	9.2- 47.9	374- 2074	18.5- 207	1.39- 13.5	29- 82	0.14- 1.733	3.116- 8.22			Present study
24	Seagrasses (*C. serrulata, T. hermprichii* and *S. isoetifolium*)	Palk Bay	0.12- 0.815		3.52- 5.59	4.78- 13.2	326- 842	22- 206	1.70- 4.57	113- 254	0.191- 1.095	0.738- 3.172			Present study

No remarkable variations were observed in the concentrations of phytochelatins between different body parts as well as stations except *E. acoroides* from Thuy Trieu. Relatively higher Pb contents were obtained in the roots than leaves and rhizomes (Nguyen et al., 2017). Among the micro nutrients (Fe, Zn, Mn and Cu) of seagrasses of Malaysia, only Fe was relatively higher concentrations (Wan Hazma et al., 2015) than the present study. Heavy metals namely Cu, Mn, Fe, Ni and Zn showed comparatively higher concentrations than similar other studies carried out (Table 3.3.20) (Malea, 1998; De Casabianca et al., 2004; Govindasamy et al., 2011; Sudharsan et al., 2012; Bat et al., 2016).

Statistical analysis

General characteristics of seagrasses

Pearson correlation matrix

Pearson correlation matrix was performed to identify inter-elemental relationship existing between the general characteristics of seagrasses (Table 3.3.21). Phenolics in seagrasses of Gulf of Mannar and Palk Bay showed strong positive correlations with lipid and protein and negative correlations with carbohydrate while flavonoids were positively correlated, and a high correlation of tannin was noticed with lipid, protein and phenolics. Pheophytin exhibited strong positive correlations with chl a and chl b and negatively with carbohydrate. N was positevly correlated with chl a and chl b whereas C with lipid and tannin.

Reactive oxygen species scavenging flavonoids reduces lipid peroxidation which directly protect anti peroxidative enzymes from oxidative damage, there by a reduction in membrane damage and inhibition

of the oxidation process in different intracellular cells (Asha et al., 2012) might be the reason for a positive correlation between lipid and phenolic compounds. Also phenolic compounds have the ability to protect lipids from decay through scavenging the radicals involved in initiation as well as propagation reactions of lipid peroxidation (Hamid et al., 2010; Hernández et al., 2016). Generally phenolic compounds and bio active proteins (example lectins) from marine origin are used for intracellular communication (signalling), and among these only bio active proteins can bind with carbohydrates (Mohd Rosni et al., 2015) and this can be the reason for positive correlation of phenolic compounds with protein, and negative correlation with carbohydrate. Marine plants with high protein leads to high growth, and phenolic compounds and tannins are formed from amino acid phenylalanine, a common precursor. If dehydroshikimic acid leads to hydrolysable tannins (gallic acid) and phenyl propanoids leads to condensed tannins; and hydrolysable tannins are metabolically more effective in defence mechanism than condensed tannins (Zhang et al., 2009) and hence address the positive correlation of protein with phenolics and tannins. Photosynthetic pigment chlorophyll is nitrogen containing macro molecule with Mg metal in the porphyrin ring and degradation of chlorophyll leads to form pheophytin (free of Mg), it might be the explanation for a positive correlation between chlorophyll and pheophytin. It was confirmed on the basis of nitrogen and carbon isotopic ratios in marine phytoplankton by Sachs et al. (1999). The protective effects of gallic acid in streptomycin induced rat lipid and its peroxidation products showed that phenolic compounds are antilipid peroxidative (Punithavathi et al., 2011). It may be

also due to phosphoglycerolipids presents in plants involved in the signalling mechanisms against in environmental conditions (Ruellanda et al., 2015).

Table 3.3.21 Pearson correlation matrix for general characteristics of seagrasses

General Characteristics of Seagrasses												
	Lipid	**Pro**	**Carb**	**Phe**	**Flav**	**Tan**	**Chla**	**Chlb**	**Pheo**	**N**	**C**	**H**
Lipid	1.00											
Pro	0.52	1.00										
Carb	-0.37	-0.34	1.00									
Phe	** 0.67	** 0.70	* -0.56	1.00								
Flav	* 0.55	0.37	-0.43	0.35	1.00							
Tan	** 0.82	** 0.76	-0.37	* 0.87	0.54	1.00						
Chla	* 0.55	0.13	-0.38	0.06	0.43	0.22	1.00					
Chlb	0.11	-0.21	-0.33	-0.28	0.34	-0.18	0.81	1.00				
Pheo	0.43	0.04	* -0.54	0.04	0.33	0.12	** 0.67	** 0.69	1.00			
N	0.00	-0.37	-0.20	-0.45	0.37	-0.29	* 0.63	** 0.79	0.43	1.00		
C	* 0.64	0.37	-0.22	* 0.54	0.46	** 0.66	0.43	0.06	-0.04	0.12	1.00	
H	0.06	0.29	-0.45	0.40	0.43	0.33	0.10	0.05	-0.09	-0.08	0.33	1.00

N=14, ** Correlation is significant at the 0.01level (2- tailed).
* Correlation is significant at the 0.01level (2- tailed).

Principal Component Analysis

PCA of general characteristics of seagraases of Gulf of Mannar and Palk Bay revealed four components accounting for a total variance of 87.81%. First component accounts for about 37.04% of the total variance, characterised by high positive positive loadings on chla, chlb and N whereas component two exhibited strong loadings on lipid, protein and tannin and it account for 31.19% of the total variance. Component three accounts for 10.86% of the total variance characterised by positive loadings on phenolics and hydrogen, and a strong negative loadings on carbohydrate. Only one positive variable was included in component four and accounts for 8.70% of the total variance (Table 3.3.22&3.3.23).

Table 3.3.22 Total variance explained (Extraction sums)

	Total	% of Variance	Cumulative %
1	4.815911	37.04547	37.04547
2	4.055805	31.1985	68.24398
3	1.412299	10.86384	79.10781
4	1.131707	8.70544	87.81325
5	0.728233	5.601792	93.41504
6	0.40527	3.117459	96.5325
7	0.162567	1.250518	97.78302
8	0.119051	0.915775	98.69879
9	0.087018	0.669372	99.36817
10	0.058036	0.446434	99.8146
11	0.014922	0.114781	99.92938
13	3.38E-05	0.00026	100

Table 3.3.23 Factor loadings of PCA analysis of general characteristics of seagrasses

Parameters	Component			
	1	2	3	4
Lipid	0.243	0.887	0.023	0.252
Protein	-0.308	0.874	0.175	-0.142
Carbohydrate	-0.272	-0.320	-0.735	0.101
Phenolics	-0.259	0.667	0.590	0.200
Flavonoids	0.286	0.670	0.418	-0.017
Tannin	-0.197	0.871	0.181	0.313
Chla	0.974	0.036	0.096	0.020
Chlb	0.943	-0.165	0.075	0.007
Pheophytin	0.847	0.128	0.183	-0.392
N	0.881	-0.041	-0.130	-0.109
C	0.155	0.550	0.117	0.765
H	-0.040	0.093	0.908	0.151
% Variance (Rotation sums)	30.03	28.22	15.64	13.92

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.

Macro and Micro elements of Seagrass

Pearson correlation matrix

Na was shows positive correlations with K, Ca and Mg whereas Mn and Ni and negatively correlated with P. Mg exhibited strong positive correlations with Ca whereas Mn with Ca and Mg, and Ni with Mg and Cr. Cd shows strong positive correlation with Ca and Fe while Pb shows a high positive correlation with Cu and a negative correlation with Zn (Table 3.3.24).

The level of Na in plants are directly related with salinity of the surrounding ecosystem and an excess level of Na increases the level of K via uptake of Na by K. Plants grow under nitrogen stress (due to high salinity), they make a protective effect against the stress by calcium. The level of Mg in plants are related with chlorophyll content which directly related to plant growth, and this can be the reason for the correlation of Na with Ca, K and Mg (Rande-Malvi, 2011; Sivakumar and Arunkumar, 2009). The rate of productivity as well as growth have important role in the variation of trace metal concentrations; rapid growth leads to dilution of metals within the plant tissue (Lyngby and Brix, 1982) may be the reason for a negative relationship of Zn with Pb. Generally, toxic metals disturbs the biosynthesis of chlorophylls and Mg is the central metal in chlorophylls, and Cd can replace Mg and the scarcity of metal in the porphyrin is replaced by Mn in damaged chlorophyll structure due to toxicity of Cd can be the reason for positive correlation between Mg and Mn (Gomes et al., 2013). A positive correlation between Cd and Ca may be due to competition of Cd metal for Ca channel in plants (Gomes et al., 2013).

Table 3.3.24 Pearson correlation matrix for macro and micro elements of seagrass.

	Na	K	Ca	Mg	P	Co	Cr	Cu	Fe	Mn	Ni	Zn	Cd	Pb
Na	1													
K	* 0.62	1												
Ca	* 0.63	0.086	1											
Mg	* 0.59	0.219	** 0.93	1										
P	* -0.54	-0.354	-0.493	-0.488	1									
Co	-0.387	-0.175	-0.222	-0.233	0.361	1								
Cr	0.307	0.099	0.327	0.397	-0.305	-0.083	1							
Cu	-0.291	-0.360	-0.095	-0.092	-0.359	-0.165	0.101	1						
Fe	-0.235	-0.163	0.389	0.384	-0.077	0.519	0.063	0.192	1					
Mn	* 0.59	0.010	* 0.66	* 0.57	-0.482	-0.381	0.350	-0.055	-0.133	1				
Ni	* 0.56	0.363	0.524	* 0.61	-0.362	-0.095	** 0.89	-0.233	0.098	0.404	1			
Zn	0.309	0.241	-0.119	-0.231	0.306	-0.412	-0.388	-0.333	-0.490	0.051	-0.263	1		
Cd	0.231	-0.066	* 0.59	0.447	-0.111	0.407	0.177	-0.126	* 0.57	0.403	0.226	-0.212	1	
Pb	-0.362	-0.508	-0.106	-0.095	-0.249	0.127	0.217	** 0.85	0.202	0.047	-0.145	* -0.63	0.073	1

N=14, ** Correlation is significant at the 0.01level (2- tailed).
* Correlation is significant at the 0.01level (2- tailed).

The positive correlations between Fe-Cd and Cu-Pb may indicate synergistic interactions for the binding sites of the plant. Seasonal variations in the level of trace metals in *P. oceanica* are associated with growth dynamics while an increase in biomass leads to a decrease in toxic metals content due to dilution effect (Malea et al., 1994) and increased with the age of the tissue (Lyngby and Brix, 1989).

Principal Component Analysis

PCA of macro elements of Gulf of Mannar and Palk Bay investigated that four components accounting for a total variance of 81.39%. First component account for about 34.80% of the total variance with strong positive loading on Ca, Mg, Zn and Na whereas component two displayed strong positive loading on Cu and Pb and a high negative loading on K, accounting for 26.56% of the total variance. Variables included in component three accounts for 12.32% of total variance and showed positive loadings on Cr, Ni and Na while negative loadings on phosphorous, and component four account for 7.70% of total variance, with high positive loading on Co and Fe and a high negative loading on Zn (Table 3.3.25&3.3.26).

Table 3.3.25 Total variance explained (Extraction sums)

	Total	% of Variance	Cumulative %
1	5.220221	34.80148	34.80148
2	3.984587	26.56391	61.36539
3	1.848452	12.32301	73.6884
4	1.156118	7.707451	81.39585
5	0.996626	6.644173	88.04003
6	0.722921	4.819477	92.8595
7	0.35432	2.362133	95.22163
8	0.311218	2.074784	97.29642
9	0.166009	1.106728	98.40315
10	0.12948	0.863203	99.26635
11	0.061784	0.411892	99.67824
12	0.031953	0.21302	99.89126
13	0.016311	0.108738	100

Table 3.3.26 Factor loadings of PCA analysis of macro and micro elements of seagrass.

Metals	Component			
	1	2	3	4
Na	0.514	-0.383	0.599	-0.387
K	0.010	-0.662	0.481	-0.280
Ca	0.863	-0.089	0.302	-0.141
Mg	0.845	-0.107	0.396	-0.014
P	-0.315	-0.280	-0.673	0.337
Co	-0.184	-0.099	-0.266	0.874
Cr	0.148	0.386	0.741	0.217
Cu	-0.028	0.933	0.113	-0.052
Fe	0.161	0.299	0.109	0.718
Mn	0.742	0.081	0.262	-0.162
Ni	0.472	-0.249	0.725	0.257
Zn	-0.155	-0.483	-0.276	-0.714
Cd	0.723	0.054	-0.165	0.340
Pb	0.036	0.915	0.125	0.259
% Variance (Rotation sums)	25.31	20.83	17.77	17.49

3.4 Conclusions

The water was noticed as alkaline and variations were slightly higher in this study whereas surface temperature and salinity changes were in line with other studies carried out. Relatively higher turbidity at Gulf of Mannar attributed to strong waves and wind agitations during rainy seasons. Variations in the concentrations of nitrate, nitrite, silicate, phosphate and DO were observed along the Gulf of Mannar and Palk Bay, and relatively higher nutrients contents were also noticed at Gulf of Mannar than Palk Bay. Heavy metals content in sea waters of Gulf of Mannar were slightly higher than Palk Bay except Pb, and the toxic pollutants Hg and As were not detected in this study.

The pH of the sediment was acidic in nature at Palk Bay in comparison to previous studies, and slightly higher values were observed at Gulf of Mannar. The sediment texture revealed that sand predominated over silt followed by clay. TOC content in the sediments were lower than similar studies carried out in World wide while the concentration of P was comparatively higher. Among the macro elements, Na, K and Ca were much lower in concentration whereas Mg content was comparable to the studies carried out at Palk Bay and higher at Gulf of Mannar. Most of the heavy metals in the sediments of Gulf of Mannar showed higher concentrations than Palk Bay and metals of Cu, Cr, Ni, Fe, Zn, Co and Pb were at lower concentrations compared to the values reported from different regions of the World. Most of the trace metals of vegetated areas were higher than the adjacent non vegetated areas. The Hg, As and Cd levels were comparable and slightly higher than other studies carried out. The results indicated that these stations are less contaminated.

Species as well as location wise variations in biochemical composition were observed in seagrasses of Gulf of Mannar and Palk Bay, and it may be due to changes in environmental conditions of seagrass meadows and differences were noticed in the energy contribution of seagrasses from both sides. Comparatively higher carbohydrate content was found in roots and rhizomes than leaves. Protein was positively related with lipid, phenol and tannin of leaves and rhizomes of seagrasses. Lipid content in seagrasses is varied with respect to age, stage of growth and ecology. Seagrasses of larger leaf of *E. acoroides* with high lipid content followed by *C. serrulata* and lowest with spherical shaped *S. isoetifolium*, and also lipid content in leaves predominated over roots and rhizomes except *C. serrulata* from Thonithurai.

Secondary metabolites in seagrasses revealed that they are a good source of phenols, flavonoids and tannins. Phenolic content was comparatively higher at leaves than roots and rhizomes in seagrasses of Gulf of Mannar and Palk Bay, and the content varied with reference to environmental pressures such as turbidity, metal contamination, pollution, ocean acidification, competition with invasive seaweed, infection by *Labyrinthula*, etc. Phenol content was positively related to tannin content. Phenols are used as a bio indicator due to its toxicity against nematodes. Relatively higher phenolics found at *C. serrulata* and lower at *S. isoetifolium*. Higher phenol contents in leaves may be due to its defensive mechanisms against epiphytes because they are exposed more than rhizomes. Phenolics in seagrasses of Gulf of Mannar and Palk Bay were comparable and lower than similar studies carried out at Gulf of Mannar, Palk Bay, Tuticorin, etc. Flavonoids are concentrated at the leaf surfaces and extracellular cuticular layer in the epidermal cells. Flavonoids present in

plants help to interact with other organisms. Species to species variations in the concentrations of tannin, flavonoids and phenolics contents were noticed both at Gulf of Mannar and Palk Bay, and relatively higher concentrations of tannin were found in leaves than roots and rhizomes. Condensed tannins extracted from seagrasses are functioned as deterrents against herbivore feeding and also against fungal as well as bacterial invasion.

Chlorophyll content in seagrasses of Gulf of Mannar and Palk Bay showed species as well as station wise variations and comparatively higher contents of chl a and b obtained from Gulf of Mannar, and it may be due to reductions in light intensity due to high turbidity. 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. Among the different body parts, relatively higher concentrations of N, C and P were noticed in leaves rather than roots and rhizomes except P content. Species to species variations were noticed in C and N content of seagrasses from Gulf of Mannar while P content at Palk Bay. Macro elements in seagrasses followed the order Na>Ca>K>Mg and comparatively more station wise variations in concentration of these elements were noticed at Gulf of Mannar rather than Palk Bay. Among these elements, K, Ca and Mg were abundant in Palk Bay while Na in Gulf of Mannar. Most of the seagrasses contained higher contents of macro elements at roots and rhizomes rather than leaves, relatively higher concentrations of Ca and Mg noticed in *S. isoetifolium* while K predominated in *C. serrulata* and Na in *T. hemprichii*. Macro element concentrations in seagrasses of Gulf of Mannar were comparatively higher than similar studies

carried out at this area and lower than Tuticorin. The results showed that seagrasses of Gulf of Mannar and Palk Bay are a good source of macro elements.

In this study, species to species as well as location wise variations in the concentrations of heavy metals were observed and species to species variations were more observed in Gulf of Mannar than Palk Bay. Among the different body parts, concentrations of metals in leaves, roots and rhizomes varied with respect to stations. *C. serrulata* and *S. isoetifolium* were common seagrasses noticed at Gulf of Mannar and Palk Bay and among the metals studied; location wise variations were noticed in *C. serrulata* than *S. isoetifolium*. The concentrations of Cu, Mn, Fe, Ni and Zn were comparatively higher in seagrasses of Gulf of Mannar than Palk Bay, and similar studies carried out at southwest coast of India, and Andaman and Lakhshadweep Islands demonstrated much higher concentration than the present study as well as global studies. The distribution and variation pattern of Co and Cr in seagrasses were similar to metals of micro essentials. The natural processes of bioaccumulation and biomagnification in the food chain of seagrasses with consumers of mussels and mullusks showed less variation in concentrations in the case of Co and Cr. Cd content in seagrasses were comparable to most of the studies carried out except seagrasses of Andaman and Lakhshadweep Islands. The concentrations of Hg was slightly higher in this study while As was comparable to seagrasses of Florida and lower than Northwestern Mediterranean.

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Chapter 4

BIOCHEMICAL PROFILE OF SEAGRASSES

Contents

4.1 Fatty Acid Profile

4.1.1 Introduction

Major sources of energy in food webs are contributed by photosynthetic organisms. Both aquatic and near shore marine environments, primary production takes place through macrophytes (macro algae and seagrasses), diatoms, dinoflagellates and terrestrial plants (Galloway et al., 2012) and among these, seagrass meadows act as one of the most productive ecosystems. Organic compounds released by seagrasses and its detritus have influence on benthic microbial abundance and activity (Gillan et al., 1984). Seagrasses are growing in tidal and sub tidal areas of sea except Polar regions. Seagrasses in India find uses as medicinal which include heart diseases, sea sickness, low blood pressure, etc., besides other usage as food, fertilizer and live stock feed (Kannan et al., 2012). Marine macrophytes are characterized by high content of polyunsaturated fatty acids (PUFAs) which act as precursors in the biosynthesis of oxylipins of prostaglandins and other

eicosanoids. The distribution of individual fatty acids (FAs) in structural complex lipids such as glycolipids, phospholipids and neutral lipids are used to understand variations of FAs with respect to habitats, biosynthetic pathways, localization of individual FAs and functional role of lipids (Khotimchenko, 2003). The functional importance of n-6 PUFAs, increased by their presence of plasma membrane than photosynthetic membrane, are also more as potent mediators participating in the signal transduction (Sanina et al., 2008). Phenolic compounds showed the ability to protect lipids from decay through scavenging the radicals involved in initiation as well as propagation reactions of lipid peroxidation (Hernández et al., 2016).

4.1.2 Results

Saturated fatty acids (SFAs) were predominated in seagrasses followed by polyunsaturated and monounsaturated fatty acids (MUFAs) in most of the species studied (Table 4.1.1 and 4.1.2). A total of 18 fatty acids were quantitated in seagrasses. The major SFAs were Palmitic acid (16:0), Stearic acid (18:0), Lignic acid (24:0) and Myristeric acid (14:0), while PUFAs were Linoleic acid (C18:2n-6) and α-Linolenic acid (C18:3n-3). The MUFAs were characterized by Oleic acid (18:1n-9c), Palmitoleic acid (C16:1n-9), Elaidic acid (18:1n-9t) and Erucidic acid (C22:1n-9). In seagrasses, total SFAs varied from 35.65 to 90.07%, PUFAs 6.43 to 51.97% and MUFAs 3.26 to 13.76% (Figure 4.1.1 & 2). The content of predominant SFAs were C16:0 (19.51 to 54.80%), C18:0 (2.53 to 24.75 %) and C24:0 (0.50 to 20.09%). C18:2n-6 (3.38 to 47.31%) and C18:3n-3 (0.68 to 32.75%) were the PUFAs while the MUFAs include C18:1n-9c (0.68 to 10.19%), C16:1n-9 (0.11 to 4.17%) and C22:1n-9 (0.07 to 3.14%). A comparison of PUFAs between leaves and rhizomes of *E. acoroides* did not

show any noticeable changes and relatively lower PUFAs (18.81 %) were estimated in roots of *E. acoroides*. In *C. serrulata* comparatively higher concentrations of PUFAs were displayed at the roots and rhizomes than leaves except from Chinnappalam.

The total SFAs of the leaves at *C. serrulata* varied from 61.82 to 87.08%, PUFAs from 9.66 to 31.92% and MUFAs from 3.26 to 9.86% (Table 4.1.1). The major SFAs (leaves of *C. serrulata*) of palmitic acid exhibited maximum in samples from Chinnappalam (54.80%) and minimum at Thonithurai (41.10%) and stearic acid showed highest content at Thonithurai (10.06%) and minimum at Munaikkadu (7.47%). PUFAs such as linoleic acid and α-linolenic acids were maximum at Thonithurai (16.47% and 10.49%) and lowest at Chinnappalam (3.38% and 1.82%), and MUFAs of oleic acid was highest at Mathacovil (3.81%) and lowest at Chinnappalam (1.46%). PUFAs were the dominant fatty acids in the roots and rhizomes of *C. serrulata* (Munaikkadu) followed by SFAs and MUFAs, in which PUFAs varied from 6.43 to 51.97%, SFAs 35.65 to 90.07% and MUFAs 3.51 to 12.39%. The PUFAs and MUFAs were lower at Chinnappalam. Palmitic acids were almost comparable and highest at Thonithurai and Mathacovil while stearic acid was higher at Chinnappalam (11.82%) and lowest at Munaikkadu (2.53%). The major PUFAs of linoleic acid in the roots and rhizomes were highest at Munaikadu whereas α-linoleic acid showed no wide variation among stations except at Chinnappalam. Among the seagrasses, roots and rhizomes of Munaikkadu found more than 50% of PUFAs and the rest of the stations exhibited more than 40% except at Chinnappalam.

Table 4.1.1 Fatty acid composition (% of total fatty acids) of seagrasses of Gulf of Mannar.

Fatty acids	*C. serrulata* (leaf)		*C. serrulata* (roots & rhizomes)		*S. isoetifolium*	Leaf	Rhizomes	Roots
	Thonithurai	Chinnappalam	Thonithurai	Chinnappalam	Thonithurai	*E. acoroides* (Chinnappalam)		
12	ND	6.13±0.39	ND	1.36±0.15	0.61±0.08	0.21±0.04	0.16±0.04	ND
14	4.50±0.30	2.66±0.23	0.12±0.04	1.45±0.19	0.73±0.08	0.57±0.08	0.25±0.06	0.04±0.02
15	ND	0.56±0.08	0.38±0.06	ND	0.07±0.02	0.20±0.04	0.29±0.06	0.22±0.06
16: 1n-9	0.67±0.10	0.62±0.07	1.05±0.16	0.11±0.04	1.25±0.24	0.59±0.09	0.87±0.12	0.93±0.12
16	41.10±0.78	54.80±0.89	28.52±0.69	49.10±0.94	40.45±0.82	29.69±0.64	24.18±0.58	53.17±0.96
18: 4n-3	4.96±0.41	4.46±0.38	0.57±0.07	ND	3.15±0.29	11.42±0.56	6.36±0.40	3.17±0.28
18: 2n-6	16.47±0.56	3.38±0.36	36.59±0.75	5.75±0.44	15.91±0.58	11.35±0.50	11.67±0.60	9.57±0.68
18: 3n-3	10.49±0.52	1.82±0.24	4.57±0.36	0.68±0.12	1.02±0.19	28.51±0.68	32.75±0.73	6.07±0.62
18: 1n-9c	2.96±0.24	1.46±0.19	6.95±0.44	2.72±0.28	7.05±0.52	3.17±0.33	6.08±0.42	0.68±0.10
18: 1n-9t	1.02±0.19	0.28±0.08	1.19±0.20	0.13±0.05	0.98±0.16	0.89±0.14	0.71±0.16	1.84±0.28
18	10.06±0.41	9.19±0.44	4.85±0.28	11.82±0.42	20.80±0.58	7.78±0.38	5.47±0.31	17.10±0.49
20: 1n-9	0.80±0.09	ND	ND	ND	0.06±0.02	0.32±0.04	0.47±0.06	0.22±0.04
20	0.33±0.06	0.11±0.02	0.08±0.02	0.09±0.02	0.92±0.21	0.84±0.16	1.22±0.24	0.33±0.04
21	0.04±0.02	0.17±0.06	0.32±0.09	0.13±0.05	0.03±0.02	0.35±0.04	0.07±0.02	0.10±0.02
22: 1n-9	0.62±0.08	0.90±0.14	0.59±0.10	0.55±0.11	0.07±0.02	0.52±0.08	0.18±0.03	0.86±0.10
22	1.88±0.19	1.86±0.18	1.17±0.16	2.83±0.24	1.12±0.20	1.34±0.22	0.49±0.14	1.05±0.19
23	0.46±0.09	1.14±0.14	0.89±0.11	3.20±0.38	1.37±0.26	0.70±0.15	1.01±0.18	0.58±0.12
24	3.43±0.32	10.48±0.48	12.15±0.59	20.09±0.70	4.39±0.34	1.07±0.21	7.44±0.58	3.52±0.33

In the case of *S. isoetifolim*, PUFAs and MUFAs showed comparatively higher concentration at Mathacovil whereas SFAs at Thonithurai (Table 4.1.2). Oleic acid was similar at both stations while large differences were noticed for palmitic acid (40.45 to 53.84%) and stearic acid (6.98 to 20.80%). Among the PUFAs, slight variations were observed in α-linolenic acid viz., 1.02% (Thonithurai) and 4.47% (Mathacovil). Palmitoleic acid also displayed marked differences between stations. Leaves as well as rhizomes contained more than 50% as PUFAs in *E. acoroides* and comparatively less PUFAs were exhibited in *T. hemprichii* compared to other seagrasses.

Table 4.1.2 Fatty acid composition (% of total fatty acids) of seagrasses of Palk Bay

Fatty acids	*C. serrulata* (leaf)		*C. serrulata* (roots & rhizomes)		*S. isoetifolium* (Mathacovil)	*T. hemprichii* (Munaikkadu)
	Munaikkadu	Mathacovil	Munaikkadu	Mathacovil		
12	0.98±0.12	1.44±0.19	ND	0.60±0.09	0.15±0.04	0.34±0.06
14	0.99±0.14	1.17±0.18	0.04± 0.01	0.12±0.02	0.55±0.08	0.61±0.09
15	0.23±0.06	0.21±0.06	ND	0.38±0.05	0.12±0.04	0.17±0.04
16: 1n-9	0.90±0.16	0.57±0.18	0.23±0.06	1.04±0.21	4.17±0.33	0.67±0.22
16	50.13±0.91	53.28±0.98	19.51±0.48	28.35±0.59	53.84±1.12	39.70±0.72
18: 4n-3	1.56±0.26	0.76±0.16	0.30±0.08	0.56±0.12	3.29±0.40	1.73±0.28
18: 2n-6	15.56±0.62	8.97±0.34	47.31±0.72	36.37±0.60	14.29±0.46	8.04±0.30
18: 3n-3	5.43±0.44	2.51±0.30	4.36±0.38	4.54±0.41	4.47±0.46	1.41±0.28
18: 1n-9c	3.74±0.38	3.81±0.32	10.19±0.54	6.91±0.48	7.74±0.42	8.00±0.52
18: 1n-9t	0.78±0.20	0.66±0.18	1.43±0.26	1.19±0.20	0.27±0.10	0.74±0.14
18	7.47±0.52	8.20±0.48	2.53±0.34	4.82±0.38	6.98±0.43	24.75±0.76
20: 1n-9	1.31±0.22	1.46±0.22	ND	ND	0.70±0.16	0.57±0.12
20	0.86±0.18	1.22±0.16	0.06±0.02	0.08±0.02	0.13±0.04	0.86±0.20
21	0.10±0.04	0.18±0.06	0.13±0.04	0.32±0.08	0.18±0.04	0.25±0.08
22: 1n-9	3.14±0.36	3.14±0.33	0.53±0.14	0.58±0.12	0.87±0.28	0.79±0.25
22	1.39±0.18	3.06±0.24	0.81±0.12	1.17±0.18	0.24±0.06	2.97±0.28
23	0.92±0.15	1.60±0.21	0.96±0.14	0.88±0.15	1.50±0.18	1.46±0.18
24	4.51±0.34	7.76±0.46	11.60±0.60	12.08±0.54	0.50±0.16	6.95±0.48

4.1.3 Discussion

The fatty acids (FAs) in the leaves as well as roots and rhizomes of *C. serrulata* followed the trend: SFAs>PUFAs>MUFAs except *C. serrulata* from Munaikkadu which followed the order PUFAs>SFAs>MUFAs. Seagrass of *E. acoroides* (leaves and rhizomes) followed the trend: PUFAs>SFAs>MUFAs while in roots SFAs predominated over PUFAs. SFAs were the dominating FAs in *S. isoetifolium* and *T. hemprichii* followed by PUFAs and MUFAs. Species to species variations were observed in the present study similar to earlier reports. The FA composition of seagrasses of *C. serrulata*, *S. isoetifolium*, *H. ovalis* and *H. uninervis* from Tuticorin Bay showed that FAs varied with respect to species and concentration, and PUFAs were the predominated fatty acid followed by SFAs and MUFAs (Jeevitha et al., 2013). Major PUFAs were 18:2n-6 and 18:3n-3 whereas SFAs were C16:0 and C18:0, and C18:1n-9 was the major MUFA, and these FAs were in line with similar studies carried out (Gillan et al., 1984; Dembitsky et al., 1991; Milkova et al., 1995; Gonocharova et al., 2000; Khotimchenko, 2003; Sanina et al., 2004, 2008; Sousa et al., 2017). Two non common fatty acids, C17:0 (SFA) and 18:4n-3(PUFA) were detected at relatively lower concentrations in *S. isoetifolium* and *H. ovalis* respectively (Jeevitha et al., 2013). In general among the seagrasses, total PUFAs followed the order: *S. isoetifolium>H. ovalis>H. pinifolia>C. serrulata* (Jeevitha et al., 2013). In this study *C. serrulata* predominated over *S. isoetifolium* (as a whole basis) except *C. serrulata* from Chinnappalam. Flavonoids protect phospholipids PUFA by donating hydrogen atom to quench lipid peroxyl radicals generated as a result of hydroxyl radical attack (Hamid et al., 2010; Hernández et al., 2016).

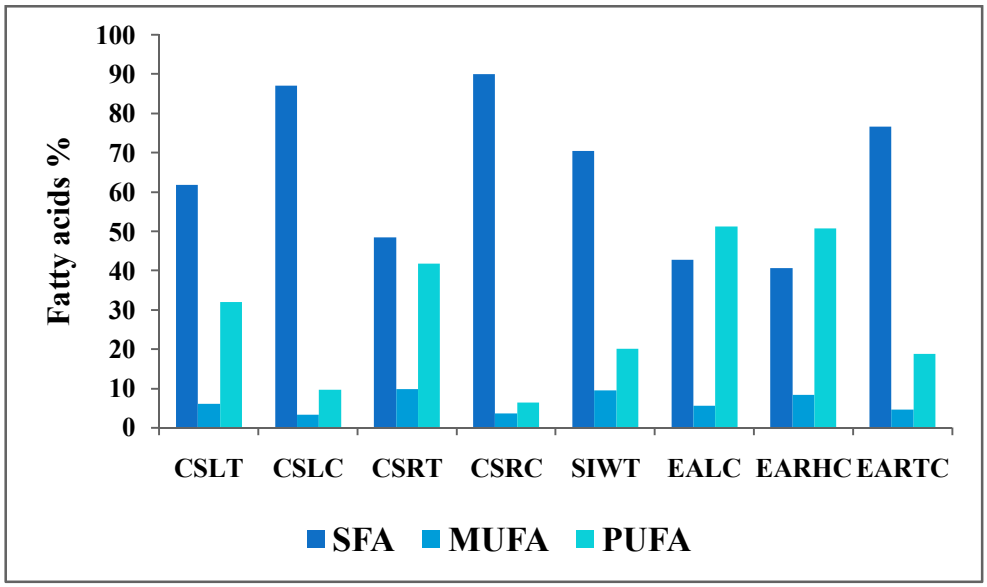

Figure 4.1.1 Fatty acid composition of seagrasses of Gulf of Mannar

The lipid classes of monogalactosyl diacylyglycerol (MGDG), digalactosyl diacylglycerol (DGDG), phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE) were major lipid classes noticed in seagrasses (Table 4.1.3) (Milkova et al., 1995; Vaskovsky et al., 1996; Gonocharova et al., 2000; Khotimchenko, 2003; Sanina et al., 2004, 2008) and FA compositions followed the order PUFA>SFA>MUFA (Milkova et al., 1995; Vaskovsky et al., 1996; Gonocharova et al., 2000; Khotimchenko, 2003; Sanina et al., 2004, 2008; Sousa et al., 2017). The PUFAs present in seagrasses were C18:3n-3, C18:2n-6 and C18:4n-3, and among these C18:2n-6 predominated except *E. acoroides* (leaves and rhizomes) in this study. The major PUFAs from *Z. marina* were C18:3n-3, C18:2n-6 and C16:3n-3, and among these C18:3n-3 was predominated (Gonocharova et al., 2000). FAs of C18:3n-3, C18:2n-6, C18:0, C16:0, C18:1n-9c and

C18:1n-9t in this study accounted for almost 80% of total fatty acids. Investigations on the tropical seagrasses from North Queensland, Australia also have reported similar observation (Gillan et al., 1984). Location wise variations in the FA composition of seagrasses were noticed in this study, and *S. isoetifolium* from Gulf of Mannar and Palk Bay showed less variations in PUFAs, SFAs and MUFAs whereas *C. serrulata* showed wide variations in FA profile from the same locations. The FA compositions of *T. hemprichii* from two different locations were almost similar whereas *H. spinulosa* contained comparatively higher concentrations of SFAs C20:0, C21:0, C22:0, C23:0, C24:0 and C26:0 (Gillan et al.,1984).

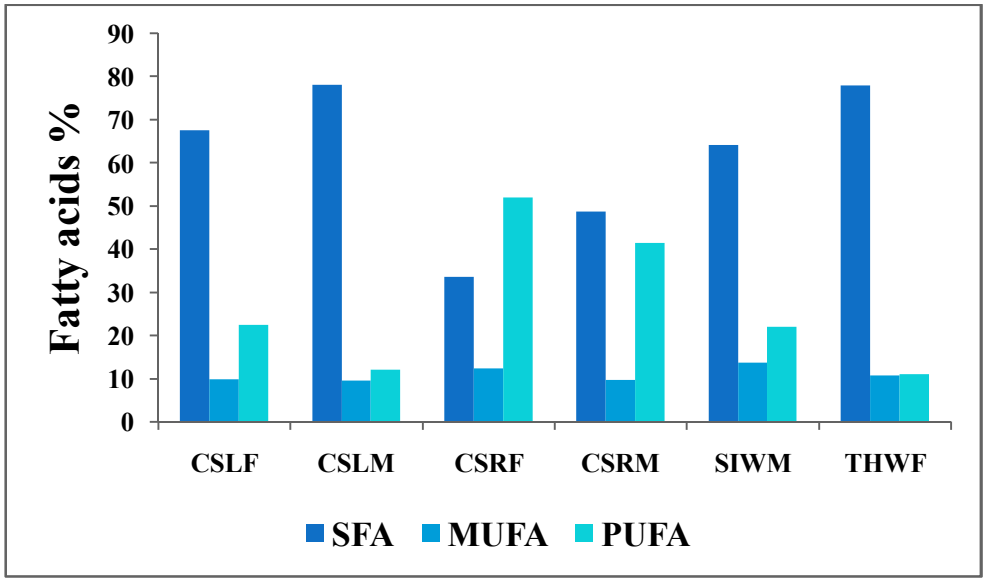

Figure 4.1.2 Fatty acid composition of seagrasses of Palk Bay

Table 4.1.3 Comparison of fatty acid profile in % of total fatty acids.

Sl No.	Species name	Location	Total PUFAs	Total MUFAs	Total SFAs	References	Lipid classes
1	Seagrasses (*C. serrulata*, *H. ovalis*, *H. pinifolia* and *S. isoetifolium*)	Tuticorin	0.68-17.8mg/g	0.13-14.1mg/g	0.45-7.63mg/g	Jeevitha et al., 2013	
2	*Z. marina*	Sea of Japan	63.8-87.5	1.2-4.7	7.7-34.9	Gonocharova et al ., 2000	MGDG,DGDG,PC & PE
3	Seagrasses (*C. serrulata*, *E. acoroides*, *H. uninervis*, *H. ovalis*, *H. ovata* and *T. hemprichii*)	Queensland, Australia	37.18-68.75	5.22-12.73	22.49-54.98	Gillan et al.,1984	
4	*Z. marina* & *Z. asiatica* *Phyllospadix iwatensis*	Sea of Japan	72.3-74.1 68	3.8-4.4 5	21.4-23.7 26.8	Khotimchenko,1993	Phospholipids
5	*T. hemprichii* (leaves) *T. hemprichii* (roots)	North Queensland	40.5 435	12.7 15.9	40.2 42.6	Nichols and Johns, 1985	
6	*Z. marina*	Black Sea	63.1-82.5	Trace-8.1	14.1-30	Milkova etal.,1995	MDGD,DGDG & SQDG
7	Seagrasses (*P. sinuosa*, *Amphibolis griffithii* and *H. ovalis*) Periphyton on *P. sinuosa* Brown algae Freshly red algae Calcareous red algae		36.77-59.44 26.17 23.28-44.24 17.96-36.42 13.31-21.01	5.84-8.08 21.37 23.14-24.74 11.04-22.28 11.23-21.37	32.96-51.60 49.45 31.03-50.49 42.11-59.17 52.17-74.6	Hyndes & Hanson, 2009	....
8	*Z. marina* (live) *Z. marina* (detrital) *Ulva lactuca* (live) *U. lactuca* (detrital) *Gracilaria* Spp. **Eelgrass free sites** *Ulva lactuca* (live) *U. lactuca* (detrital) *Gracilaria* Spp.	South Bay, Virginia	47.9 3.1 11.1 14.8 13.3 9.8 10.8 21.8	11.6 7.5 20.7 6.6 6.1 14.8 2.1 2.6	33.7 79.1 62 66 67.6 62.2 77.1 68.6	Harbeson, 2010	....
9	*Z. marina* (green leaves) *Z. marina* (detritus) *Z. marina* (rhizomes)	Novgorodskaya, Sea of Japan	66.4 12.4 53.8	3.5 23.2 7.2	25.3 38.6 31.4	Kharlamenko et al., 2001	....

10	Algaes, Rhodophyta Pheophyta Green algae Seagrasses (*Z. marina* and *P. iwatensis*)	Yellow Sea, Quingdao	45.4-58.3 45.1-69 52-62.8 72.2-72.6	4.9-16 8.3-15.7 8.3-16.8 1.9-2.8	29.6-37.5 17.9-26.8 20.4-25.1 18.8-21.4	Vaskovssky et al., 1996	----
11	Algae *Z. marina*	Peter the Great Bay	7.1-91.2 63.7-84.3	2.8-24.9 2.1-5.3	3.6-66.8 13.1-30.9	Khotimchenko,2003	MGDG,DGDG & SQDG MGDG,DGDG & SQDG
12	Red algae Brown algae Brown algae Green algae *Z. marina*	Sea of Japan (summer)	25.2-76 16.9-74.5 32.1-67.7 22.3-96.2 63.8-87.6	11-40 14-37.7 11.3-17.4 2.5-17.1 1.3-4.7	13-48 11.5-53.1 21-50.5 1.3-60.6 7.7-34.9	Sanina et al., 2004	PC,PE,PG,MGDG,DGDG&SQDG PC,PE,PG,MGDG,DGDG&SQDG MGDG,DGDG&SQDG MGDG,DGDG,SQDG&DGTS PC,PE,PG,MGDG&DGDG
13	Red algae Brown algae Brown algae Green algae *Z. marina*	Sea of Japan (winter)	27.2-74.4 44.8-83.7 23.8-75.9 25.9-98.5 55.9-94.9	15-27.4 5.5-25.6 5.2-23.6 0.9-21.8 2.2-13.8	10.6-39 6.1-32.3 15.1-52.6 0.6-52.3 2.9-30.3	Sanina et al., 2008	PC,PE,PG,MGDG,DGDG&SQDG PC,PE,PG,MGDG,DGDG&SQDG MGDG,DGDG&SQDG DGTS,MGDG,DGDG&SQDG PC,PE,PG,MGDG&DGDG
14	*Chelonia mydas* Algae Seagrass (*Halophila hawaiiensis*)	Kiholo Ahu-o-Laka Kiholo Ahu-o-Laka	8.15-21.67 16.04-22.4 21.76 26.03	32.4-37.21 33.58-35.93 18.98 22.01	31.43-47.34 31.4-40.99 53.77 47.28	Seaborn et al., 2005	----
15	*C. mydas* (oil) Depot fat Seagrasses (*T. testudinum* and *S. filiforme*)	Italy Hawaiian islands Panama City	6.16-6.17 1.32-17.01 51.29-55	45.5-45.52 38.47-43.89 11.2-11.26	45.85-46.29 40.44-52.58 25.11-34.08	Joseph et al., 1985	----
16	*P. oceanica* (leaves) *P. oceanica* (litter) Epi flora and fauna on leaves	Calvi Bay, Belgium	67.74 26.17 31.01-41.88	3.90 23.21 17.88-22.99	28.35 50.62 39.46-46.01	Michel et al., 2015	----
17	Seagrasses (*E. acoroides*, *C. serrulata* and *S. isoetifolium*)	Gulf of Mannar	6.43-51.28 11.18-51.97	3.51-9.78 9.65-13.76	40.58-90.07 35.65-78.12	Present study Present study	----
18	Seagrasses (*C. serrulata*, *T. hemprichii* and *S. isoetifolium*)	Palk Bay	6.43-51.28 11.18-51.97	3.51-9.78 9.65-13.76	40.58-90.07 35.65-78.12	Present study Present study	----

Comparison of epiphytes of *P. australis* leaves, suspended matter and cultured bacteria from fresh and dead leaves showed that PUFAs found in epiphytes were relatively lower in concentration compared to commonly associated diatoms. The suspended particulate matter adjacent to seagrass beds followed almost similar patterns as that of epiphytes (Nichols et al., 1985). In the present study, no wide differences in PUFAs levels between leaves with roots and rhizomes in *C. serrulata* whereas in *E. acoroides* showed differences among roots with leaves and rhizomes. Moreover the concentrations of C24:0 and C18:1n-9c were relatively higher at roots and rhizomes than leaves. FA profiles of *Z. noltii* showed that C18:3n-3 predominated over C18:2n-6 while vice versa in roots and slightly higher concentrations of C16:0 and C18:1n-9c were found in roots than leaves (Lebreton et al., 2011). Lipid components and its utilization in seagrasses by rock crab *Nectocarcinus integrifons*, rock flathead *Platycephalus laevigatus* and garfish *Hyporhamphus melanochir* showed that MUFAs and PUFAs obtained from the foregut of *H. melanochir* were similar to seagrasses with major acids as C18:1n-9, C18:2n-6, C18:3n-3 and C16:3n-3 (Nichols et al., 1986). FA composition of food sources of amphipods exhibited relatively higher PUFAs content in fresh leaves than litter of *P. oceanica* and major PUFAs were C16:0, C18:0, C20:5n-3, C20:4n-6, C16:1n-7 and C18:1n-9 (Michel et al., 2015).

The variations in the concentrations of FAs arise from the differences in their habitats, including light, temperature, concentrations of nutrients including nitrogen and phosphorous, etc. (Nichols and Johns, 1985; Khotimchenko, 1993). The variations in the level of FAs in the leaves of *Z. noltii* and algae with the presence of their grazers and predators were

studied by Spivak et al. (2007). An increased levels of FAs were positively related with benthic microalgae (chl a) while PUFAs (C18:2n-6 and C18:3n-3) and SFAs (C12:0, C14:0 and C24:0) were decreased by the presence of grazers and predators and positive towards ambient light treatments in *Z. marina*. Phospholipids linked FAs were relatively in higher content in presence of ambient light (without grazers), and also C18:2n-6 and C18:3n-3 decreased by grazers and increased by predators in the presence of ambient light. Among the total fatty acids, percentage of C12:0, C14:0 and C24:0 were directly proportional to the benthic micro algal biomass whereas C18:2n-6 and C18:3n-3 by eelgrass biomass (Spivak et al., 2007). The effect of changes in the environmental parameters of salinity, temperature, pH and sediment characteristics with respect to season showed no significant differences between FA profile of leaves, roots and rhizomes of *Z. noltii* (Sousa et al., 2017).

Major FAs in the three seagrasses from Sea of Japan were C18:2n-6, C18:3n-3 and C16:0 whereas minors were C16:1n-7, C16:3n-3, C18:0 and C18:1n-9 (Khotimchenko, 1993). FA composition of *T. hemprichii* leaves as well as roots showed C18:1n-7 as predominating MUFA in roots than leaves, but were comparatively lower in concentration than other seagrass species (Nichols and Johns, 1985). In the present investigation, relatively higher concentrations of PUFAs were found in leaves and rhizomes than roots in *E. acoroides* whereas *C. serrulata* fatty acid profile revealed more PUFAs in roots and rhizomes than leaves except the species collected from Chinnappalam. Comparison of FA compositions of marine macrophytes of *Sargassum pallidum*, *Ulva fenestrata* and *Z. marina* showed higher ω-6 in *S. pallidum* followed by *Z. marina* and *U. fenestrata* (Popov and Krivoshapko,

2013). The predominated FAs in *Z. marina* from Black Sea were C14:0, C16:0, C16:3n-3, C18:2n-6 and C18:3n-3 and were more comparable to terrestrial plants than algae (Milkova et al., 1995). Sewage discharge on seagrasses affected negatively the habitable area, structural complexity abundance and diversity and, seagrass ecosystems act as an indicator of natural as well as anthropogenic disturbances occurring in the marine ecosystem. Studies on the biofilms of *E. acoroides*, *S. isoetifolium*, *T. hemprichii* and *T. cilliatum* from the polluted and non polluted areas of Tanzania showed large differences in C16:0 between polluted and non-polluted site, and C18:3n-3 was predominated at *T. ciliatum* of polluted site (Daudi et al., 2012).

E. acoroides (leaves and rhizomes) and *C. serrulata* (roots and rhizomes) from Munaikkadu contained more than 50% PUFAs of total FAs. In seagrass species such as *Amphibolis griffithi* and *H. ovalis* PUFAs consisted of 59% and 48% of total FAs respectively while *P. sinuosa* with 51.60% SFAs. The fatty acid profile of *P. sinuosa* followed the order: SFA>PUFA>MUFA (Hyndes and Hanson, 2009). Relatively higher concentrations of PUFAs of C18:3n-3 and 18:2n-6 and SFAs of C24:0, C26:0, C28:0 and C32:0 were obtained in seagrasses than macro algae and periphyton (Hyndes and Hanson, 2009). Wide variations were observed in the total PUFAs and relatively higher PUFAs noticed at brown algae and lower at calcareous red algae. Major PUFAs obtained were C20:4n-6 and C20:5n-3 while MUFAs were C18:1n-9 and C16:1n-7, and C14:0, C16:0 and C18:0 were the major SFAs (Hyndes and Hanson, 2009), and is characteristic to diagnostic diatoms and dinoflagellates (Budge et al., 2008).

Comparison of FAs composition of South Bay, Virginia seagrasses and marine macrophytes of live and decayed, and seagrasses species associated including marine macrophytes, invertebrates and fishes living on the seagrasses meadows showed C18:3n-3, C16:0, C18:2n-6 and C18:1n-9 as most abundant FAs in *Z. marina* (Harbeson, 2010). It is worth mentioning that variations were more observed in the case of PUFAs than MUFAs between live and detrital leaves of *Z. marina* (Harbeson, 2010) while no wide variations observed in the case SFAs. However, more variations were noticed in SFAs and PUFAs than MUFAs in the present study. Marine invertebrates living on the seagrass meadows contained lower concentrations of seagrass biomarkers of C18:3n-3 and C18:2n-6 in blue crab (*Callinectus sapidus*) and skelton shrimp (*Paracaprella spp*), and the biomarkers of C18:2n-6 concentration were relatively lower at seagrass free sites whereas C18:3n-3 was absent (Harbeson, 2010). Among the fishes living in the seagrass meadows, only pig fish (*Orthopristis chrystopera*) contained C18:3n-3 whereas C18:2n-6 was relatively in lower concentrations compared to C20:5n-3 and C22:6n-3 in fishes living on the seagrass meadows as well as free sites. Relatively lower concentrations of C18:2n-6 and C18:3n-3 indicated the possible chain elongation to higher C20 PUFAs (Harbeson, 2010). Green algae and seagrasses are in the same lineage and have same photosynthetic pigments and biochemical pathways with differences in FAs, and C18:2n-6 and C18:3n-3 were the major PUFAs in seagrasses of *Z. marina* and *P. scouleri* (Galloway et al., 2012).

Identification of food sources of invertebrates from the seagrass *Z. marina* community suggested that major FAs of *Z. marina* were C16:0, C22:0, C18:2n-6 and C18:3n-3 while in the detritus of *Z. marina* was C16:0,

C18:0, C18:1n-9 and C16:1n-7. FA compositions of consumer species revealed that none of them assimilated fresh seagrasses (Kharlamenko et al., 2001). Abundant FAs in seagrasses of *Z. marina* and *P. iwatensis* from Yellow Sea, Quingdao were C16:0, C16:3n-3, C18:2n-6, C18:3n-3 and C22:0, and PUFAs of C18:3n-3 predominated in *P. iwatensis* while C18:2n-6 in *Z. marina*. Typical FA C16:3n-3 recorded in seagrass belongs to *Zosteraceae* (Vaskovsky et al., 1996) and relatively higher concentrations of C16:3n-3 found in glycolipids than phospholipids (Sanina et al., 2008). The major fatty acids in *Z. marina* were C16:0, C18:2n-6 and C18:3n-3 and PUFAs were in major portions followed by SFAs and MUFAs (Sanina et al., 2004). One of the uncommon PUFAs of C18:4n-3 (stearidonic acid) have commercial importance (Khotimchenko, 2003; Hong et al., 2002; Callaway et al., 1996). Deviations in FA composition of marine macrophytes might be due to the pathways selected for the biosynthesis of FAs and differences in the utilization of glycolipids in these species. Among the Sulfoquinovosyldiacylglyceride (SQDG) lipids, relatively higher concentration of total PUFAs were observed in *Z. marina* (63.7%) (Khotimchenko, 2003).

Z. marina showed seasonal wise variations in the FA composition irrespective of the lipid classes with C16:0, C16:1, C18:0, C18:1n-9, C18:2n-6 and C18:3n-3 as abundant fatty acids. The concentration of C18:2n-6 was increased irrespective of the lipid classes while C18:3n-3 showed variations with respect to lipid classes and it might be due to a defense role against low temperature photo inhibition of photosynthesis (Sanina et al., 2008). A dietary relationship of immature green turtle of *C. mydas* with dietary components of seagrass and algae from Hawaiian

Islands was carried out on the basis of FA compositions. The FA composition of turtles showed more similarity towards seagrass except PUFAs and it could be due to the modification of dietary fatty acids by de novo biosynthesis (Seaborn et al., 2005). The predominated PUFAs in the crude and refined oils of pen reared green turtle were C18:2n-6 and C18:3n-3 whereas in depot fat contained C20:4n-6 and C22:6n-3. No dietary relationship observed in seagrass with turtle fat in the FA composition, especially in the case of PUFAs while MUFAs were showed an seven fold increase in turtles in comparison with seagrass and SFAs of C14:0 and C12:0 were increased and C16:0 decreased in turtles (Joseph et al., 1985).

Statistical analysis

Pearson correlation matrix

Pearson correlation matrix of fatty acid compositions of seagrasses of Gulf of Mannar and Palk Bay showed differences in interrelations between FAs. Pentadecyclic acid was positively correlated with Lauric acid whereas Linoleic acid was negatively correlated with Palmitic acid (Table 4.1.4). α – Linolenic acid was noticed as strong positive correlations with Stearidonic acid while Lignic acid was positively correlated with Tricosanoic acid, and exhibited highly positive correlations between FAs of Erucic acid and Gadoleic acid. Oleic acid was positively correlated with Linoleic acid. Tricosanoic acid was positively correlated with Behenic acid. Complex lipid substrates in seagrasses are membrane bound and they are phospholipids and glycolipids.

Table 4.1.4 Pearson correlation matrix for fatty acid profile of seagrasses

Fatty acid profile of seagrasses																		
	C12	**C14**	**C15**	**C16:1**	**C16**	**C18:4**	**C18:2**	**C18:3**	**C18:1c**	**C18:1t**	**C18**	**C20:1**	**C20**	**C21**	**C22:1**	**C22**	**C23**	**C24**
C12	1.00																	
C14	0.39	1.00																
C15	*0.64	-0.09	1.00															
C16:1	-0.15	-0.15	-0.02	1.00														
C16	0.43	0.37	0.11	0.33	1.00													
C18:4	0.03	0.18	0.14	0.05	-0.06	1.00												
C18:2	-0.38	-0.33	-0.19	-0.08	* -0.62	-0.40	1.00											
C18:3	-0.25	-0.09	0.09	-0.08	-0.38	**0.79	-0.15	1.00										
C18:1c	-0.40	-0.44	-0.32	0.27	-0.51	-0.30	*0.62	-0.09	1.00									
C18:1t	-0.47	-0.29	-0.12	-0.25	-0.29	-0.03	0.50	0.06	0.06	1.00								
C18	0.00	0.08	-0.19	-0.05	0.39	0.00	-0.52	-0.30	-0.07	0.07	1.00							
C20:1	-0.15	0.23	-0.05	0.25	0.45	0.06	-0.19	0.12	-0.09	-0.09	-0.01	1.00						
C20	-0.19	-0.12	0.03	-0.08	0.02	0.44	-0.35	*0.53	0.05	0.03	0.33	0.53	1.00					
C21	0.01	-0.35	0.35	0.02	-0.31	0.18	0.14	0.11	0.11	-0.08	-0.17	-0.27	-0.22	1.00				
C22:1	0.13	0.07	0.18	0.05	0.53	-0.21	-0.07	-0.17	-0.22	-0.04	-0.11	**0.83	0.30	-0.15	1.00			
C22	0.28	0.44	-0.17	-0.21	0.45	-0.17	-0.48	-0.40	-0.19	-0.46	*0.56	0.10	-0.06	0.12	0.10	1.00		
C23	0.19	-0.01	-0.31	0.00	0.30	-0.33	-0.32	-0.32	0.05	-0.63	0.25	-0.19	-0.13	-0.07	-0.10	*0.60	1.00	
C24	0.31	-0.02	0.01	-0.52	-0.17	*-0.55	0.17	-0.32	0.03	-0.25	-0.14	-0.50	-0.43	0.05	-0.20	0.34	*0.63	1.00

N=14, ** Correlation is significant at the 0.01level (2- tailed).

* Correlation is significant at the 0.01level (2- tailed).

In plants, long chain fatty acids of Palmitic acid and Stearic acid formed through de novo synthesis (acetyl- CoA carboxylase and fatty acid synthase) from photosynthate. A positive correlation of long chain fatty acids from C18:0 to greater than C18:0 is attributed due to the synthesis of higher fatty acids through membrane bound soluble enzyme elongases. Synthesis of unsaturated fatty acids from long chain fatty acids using membrane bound desaturases and elongases are the reasons for a negative correlation between Lignic acid and Stearidonic acid. The relationship between C18:1, C18:2n-6 and C18:3n-3 to C18:4n-3 also due to desaturases (Gunstone et al., 2007; Harwood, 1996).

Principal component analysis

PCA of fatty acid composition of seagrasses revealed that seven components accounting for a total of 89.31% variance (Table 4.1.5&4.1.6). First component accounts for about 26.80% of the total variance characterised by high positive loadings on lauric acid and tricosanoic acid and strong negative loadings on linoleic acid and elaidic acid. Component two accounts for 20.65% of total variance and exhibits strong positive loading on palmitic acid, stearic acid and behenic acid. Variables included in component three accounts for 11.39% of the total variance and show positive loadings on arachidic acid, stearidonic acid and alpha-linolenic acid. Component four accounts for 9.40% of the total variance with strong negative loadings on oleic acid while component six displayed high positive loadings on gadoleic acid and erucic acid and it accounts for 6.32% of total variance. Component five accounts for 8.87% of the total variance characterised by strong positive loadings on palmitoleic acid and high negative loadings on lignoceric acid whereas component seven accounts for

5.84% of the total variance and showed positive loadings on pentadecyclic acid and heneicosanoic acid (Table 4.1.5& 4.1.6).

Table 4.1.5 Total variance explained (Extraction sums)

	Total	% of Variance	Cumulative %
1	4.82573	26.80961	26.80961
2	3.718638	20.6591	47.46871
3	2.051902	11.39945	58.86816
4	1.692017	9.400092	68.26826
5	1.597498	8.874989	77.14324
6	1.137982	6.322119	83.46536
7	1.052611	5.847839	89.3132
8	0.961433	5.341297	94.6545
9	0.54973	3.054055	97.70856
10	0.213278	1.184876	98.89343
11	0.139493	0.774961	99.66839
12	0.059158	0.328653	99.99705
13	0.000532	0.002954	100

Table 4.1.6 Factor loadings of PCA analysis of fatty acid profile of seagrasses

	1	2	3	4	5	6	7
LAU	0.669	0.139	-0.118	0.433	-0.272	0.225	0.272
MYR	0.516	0.131	0.088	0.481	-0.040	0.213	-0.481
PEN	0.138	-0.192	0.143	0.404	-0.039	0.183	0.782
PALOLEIC	0.064	-0.032	-0.018	-0.141	0.921	0.142	0.107
PALMITIC	0.320	0.572	-0.078	0.427	0.322	0.463	-0.132
STEARIDONIC	0.028	0.002	0.774	0.394	0.433	-0.144	0.001
LINOLEIC	-0.566	-0.513	-0.255	-0.504	0.023	0.095	-0.084
aLINOLENI	-0.259	-0.511	0.706	0.095	0.176	0.012	0.015
OLEIC	0.041	-0.200	-0.007	-0.911	0.074	-0.053	-0.005
ELAIDIC	-0.961	-0.078	0.082	0.034	-0.104	0.012	-0.026
STEARIC	0.031	0.932	0.195	0.188	0.094	-0.019	-0.131
GON	0.007	0.048	0.414	-0.076	0.304	0.798	-0.238
ARA	-0.077	0.270	0.861	-0.200	-0.023	0.281	-0.039
HEN	0.000	-0.028	-0.090	-0.148	0.061	-0.135	0.791
ERU	0.026	0.043	-0.045	0.125	0.026	0.971	0.070
BEH	0.421	0.684	-0.173	0.108	-0.164	0.199	-0.086
TRI	0.675	0.450	-0.252	-0.359	-0.144	-0.095	-0.067
LIG	0.169	-0.036	-0.358	-0.058	-0.864	-0.089	0.063
% Variance (Rotation sums)	15.68	14.38	13.34	12.75	12.02	11.83	9.31

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization

4.2 Amino Acid Profile

4.2.1 Introduction

Seagrass meadows are distributed from shallow coastal waters around the World except Antarctica and playing an important role in ecological, geological and economic areas (Rotini et al., 2013). Proteins are complex

molecules and the molecular weight of proteins ranges from 5000 to several thousand daltons. These building blocks of proteins available from nuetraceuticals are classified into two, essential (not synthesized in the body and need to be provided) and non essential amino acids. The amino acids threonine, valine, methionine, leucine, isoleucine, phenyl alanine, histidine, lysine, arginine, tryptophan and cysteine are the essential amino acids whereas aspartic acid, glutamic acid, serine, alanine, proline and glycine belong to non essential amino acids. Amino acids play important roles in humans as well as animal growth, development and health; and it includes protein synthesis, cell signaling, acid-base balance, appetite, body composition, blood flow, antioxidative defense, energy substrates, immunity and health, osmoregulation, RNA and DNA synthesis, ammonia removal, reproduction and metabolic regulation (Wu, 2010). Studies show that amino acids of glutamine, glutamate, and arginine play crucial roles in multiple signaling pathways, nutrient metabolism and oxidative defense (Yao et al., 2008; Bruhat et al., 2009). An external supplement of non essential amino acids is needed for young and gestating mammals for their neonatal growth as well as vascular and intestinal health (Mateo et al., 2007; Zeng et al., 2008). The functional amino acids of glutamine and arginine can prevent diseases of obesity, diabetes, necrotizing enterocolitis and intrauterine growth retardation in both animals and humans (Wu et al., 2009). Seagrasses are reported to have higher nitrogen content and after four weeks of decay, N content was increased (Zieman et al., 1984). So seagrasses and its detritus are used as a source of food for organisms using seagrass meadows as their habitat, and its importance is increased by the presence of essential amino acids present.

4.2.2 Results

Among the total amino acids analysed, essential amino acids (EAAs) predominated over more than 50% of total amino acids in most of the species (Figure 4.2.1 &4.2.2) and total 17 amino acids were quantitated in the seagrasses, and an essential amino acid, cysteine was not detected in seagrasses of Gulf of Mannar and Palk Bay (Table 4.2.1 and 4.2.2). The major essential amino acids were histidine, lysine, leucine and threonine whereas non essential amino acids (NEAAs) were aspartic acid, glutamic acid, glycine and alanine in most of the seagrasses. Among the essential amino acids, the level of histidine was higher followed by lysine, leucine and threonine while in non essential amino acids glutamic acid was the predominating amino acid followed by aspartic acid, glycine and alanine. A comparison of *C. serrulata* leaves with roots and rhizomes showed that leaves have relatively high concentrations as well as number of amino acids. No wide variations in the level of amino acids were observed in *S. isoetifolium* whereas amino acid compositions of *E. acoroides* roots were higher than *T. hemprichii* whole.

Among the leaves of *C. serrulata* total EAAs varied from 338 (Chinnappalam) to 751mg/100g (Mathacovil) whereas roots and rhizomes varied from72 (Mathacovil) to 254mg/100g (Thonithurai), and 1064mg/100g and 940mg/100g respectively for *S. isoetifolium* from Thonithurai and Mathacovil respectively. Total EAAs noticed in *T. hemprichii* were 210mg/100g and 406, 173 and 399mg/100g respectively for leaves, rhizomes and roots of *E. acoroides*. EAAs of threonine in *C. serrulata* leaves varied from 25 (Chinnappalam) to 62mg/100g (Thonithurai), leucine 35 (Thonithurai) to 96mg/100g (Munaikkadu),

histidine 121 (Chinnappalam) to 425mg/100g (Mathacovil) and lysine 31 (Chinnappalam) to 174mg/100g (Munaikkadu) whereas in roots and rhizomes threonine ranged from 1 (Chinnappalam) to 7mg/100g (Thonithurai), leucine 7 (Thonithurai) to 38mg/100g (Munaikkadu) and lysine ND (Mathacovil) to 69mg/100g (Thonithuraii). In *S. isoetifolium*, comparatively higher contents of EAAs like threonine and leucine were noticed at Thonithurai whereas histidine and lysine at Mathacovil.

NEAAs of aspartic acid varied from 40 (Chinnappalm) to 87mg/100g (Mathacovil), glutamic acid 27 (Thonithurai) to 81mg/100g (Mathacovil), glycine 27 (Thonithurai) to 89mg/100g (Munaikkadu) and alanine ND (Chinnappalam) to 147mg/100g (Munaikkadu) at leaves of *C. serrulata* while at roots and rhizomes aspartic acid ranged from 2 (Chinnappalam) to 13mg/100g (Munaikkadu), glutamic acid 2 (Chinnappalam) to 18mg/100g (Munaikkadu), glycine ND (Chinnappalam and Munaikkadu) to 40mg/100g (Thonithurai) and alanine ND (Mathacovil) to 12mg/100g (Munaikkadu). In *S. isoetifolium*, glycine was comparatively higher levels at Mathacovil while glutamic acid, alanine and aspartic acid almost similar. The other free amino acids cysteic acid, taurine and hydroxyl proline were given in Table 4.2.3. Comparatively higher percentage of these amino acids were noticed in *C. serrulata* from Gulf of Mannar rather than from Palk Bay except taurine while no such variations were observed in *S. isoetifolium*. Relatively higher contents of these amino acids were obtained in the roots and rhizomes than leaves and these amino acids were not detected in *C. serrulata* from Mathacovil. Among these amino acids, hydroxyl proline was a non essential amino acid, taurine non-protein amino acid and cysteic acid an intermediate to cysteine metabolism.

Table 4.2.1 Amino acid composition in seagrasses of Gulf of Mannar (mg/100g)

Amino acids	*C. serrulata* (leaf)		*C. serrulata* (roots & rhizomes)		*S. isoetifolium*	Leaf	Rhizomes	Roots
	Thonithurai	Chinnappalam	Thonithurai	Chinnappalam	Thonithurai	*E. acoroides* (Chinnappalam)		
Asp	43±0.48	40±0.52	9±0.18	2±0.08	4±0.16	84±0.78	44±0.42	64±0.58
Thr	62±0.58	25±0.36	7±0.18	1±0.04	92±0.88	31±0.36	11±0.18	33±0.42
Ser	26±0.36	28±0.38	11±0.22	1±0.08	89±0.92	42±0.40	39±0.44	34±0.36
Glu	27±0.24	48±0.32	6±0.14	2±0.08	319±4.24	114±1.32	54±0.48	50±0.48
Pro	14±0.18	ND	ND	ND	64±0.72	19±0.24	17±0.22	ND
Gly	27±0.26	40±0.36	40±0.38	ND	90±0.88	56±0.42	79±0.84	58±0.62
Ala	31±0.28	ND	3±0.11	2±0.10	102±1.12	60±0.48	28±0.24	5±0.12
Val	24±0.22	23±0.40	6±0.16	ND	68±0.78	136±1.44	2±0.08	24±0.18
Met	1±0.08	5±0.16	ND	ND	59±0.64	3±0.11	27±0.30	3±0.10
Ile	25±0.32	19±0.26	2±0.10	ND	85±0.88	30±0.40	15±0.24	23±0.28
Leu	35±0.44	48±0.56	7±0.22	11±0.24	172±2.68	78±0.66	30±0.36	47±0.44
Tyr	6±0.12	79±0.68	8±0.20	ND	56±0.52	33±0.28	16±0.16	23±0.18
Phe	37±0.24	43±0.28	10±0.18	4±0.12	89±0.76	41±0.34	14±0.18	44±0.32
His	363±4.54	121±1.22	120±1.56	49±0.52	310±4.12	29±0.32	23±0.38	134±1.68
Lys	120±1.12	31±0.40	69±0.72	3±0.14	147±2.32	34±0.44	21±0.40	31±0.48
Arg	18±0.30	23±0.34	33±0.44	9±0.21	42±0.58	24±0.36	30±0.46	60±0.88

Table 4.2.2 Amino acid composition in seagrasses of Palk Bay (mg/100g)

Amino acids	*C. serrulata* (leaf)		*C. serrulata* (roots & rhizomes)		*S. isoetifolium* (Mathacovil)	*T. hemprichii* (Munaikkadu)
	Munaikkadu	Mathacovil	Munaikkadu	Mathacovil		
Asp	84±0.72	87±0.66	13±0.18	8±0.16	4±0.12	44±0.56
Thr	54±0.64	43±0.58	4±0.12	4±0.14	88±0.68	26±0.34
Ser	59±0.78	50±0.72	9±0.18	3±0.12	86±0.76	39±0.48
Glu	72±0.52	81±0.68	18±0.24	4±0.16	314±4.32	56±0.42
Pro	28±0.20	19±0.16	ND	8±0.20	54±0.42	ND
Gly	89±0.60	60±0.42	ND	3±0.15	107±1.15	45±0.38
Ala	147±2.12	40±0.36	12±0.16	ND	105±1.22	31±0.25
Val	21±0.28	47±0.54	25±0.22	3±0.12	82±0.54	20±0.32
Met	ND	4±0.12	ND	ND	56±0.62	1±0.04
Ile	43±0.58	39±0.54	ND	7±0.18	55±0.76	20±0.36
Leu	96±1.02	70±0.88	38±0.42	25±0.34	27±0.44	28±0.46
Tyr	34±0.40	256±3.42	ND	ND	60±0.52	44±0.32
Phe	70±0.48	53±0.40	28±0.20	1±0.04	86±0.68	3±0.11
His	225±3.56	425±5.36	35±0.28	32±0.30	319±4.44	50±0.72
Lys	174±2.52	48±0.68	18±0.24	ND	154±2.24	40±0.64
Arg	ND	22±0.36	7±0.20	ND	73±0.84	21±0.42

Table 4.2.3 Free amino acids in % total amino acids.

Species name	Location	Station	Cysteic acid	Taurine	Hydroxy proline
C. serrulata (leaf)	Gulf of Mannar	Thonithurai	0.29±0.06	0.11±0.04	0.36±0.08
C. serrulata (leaf)	Gulf of Mannar	Chinnappalam	0.08±0.02	0.64±0.14	ND
C. serrulata (leaf)	Palk Bay	Munaikkadu	0.21±0.04	0.06±0.02	0.18±0.06
C. serrulata (leaf)	Palk Bay	Mathacovil	0.09±.02	0.05±0.02	0.44±0.09
C. serrulata (R&R)	Gulf of Mannar	Thonithurai	2.32±0.26	0.14±0.04	1.18±0.18
C. serrulata (R&R)	Gulf of Mannar	Chinnappalam	6.21±0.44	2.38±0.22	7.18±0.58
C. serrulata (R&R)	Palk Bay	Munaikkadu	2.04±0.24	3.56±0.30	3.19±0.34
C. serrulata (R&R)	Palk Bay	Mathacovil	ND	ND	ND
S. isoetifolium	Gulf of Mannar	Thonithurai	0.58±0.12	4.05±0.36	0.04±0.02
S. isoetifolium	Palk Bay	Mathacovil	0.68±0.14	4.23±0.32	0.10±0.02
E. acoroides (leaf)	Gulf of Mannar	Chinnappalam	0.41±0.10	0.24±0.04	0.07±0.02
E. acoroides (Rh)	Gulf of Mannar	Chinnappalam	2.69±0.30	ND	0.13±0.04
E. acoroides(Rt)	Gulf of Mannar	Chinnappalam	0.64±0.16	0.57±0.11	0.51±0.12
T. hemprichii	Palk Bay	Munaikkadu	0.33±0.08	0.20±0.04	0.42±0.10

4.2.3 Discussion

An increase in salinity from 15 (Gulf of Mannar side) to 28*psu* (Palk Bay side) among the stations showed an increase in the concentrations of proline, alanine, aspartic acid and glutamic acid in the leaves of *C. serrulata* whereas in *S. isoetifolium* no remarkable variations were noticed in the present study. These variations in amino acids with respect to salinity were similar to the findings of Pulich Jr. (1986). The subtropical seagrasses *H. wrightii*, *H. engelmani*, *T. testudinum*, *S. isoetifolium* and *R. maritima* showed a stress related response to salinity. There is a decrease in the concentration of most of the amino acids with increase in salinities (20, 32 and 47*psu*) while proline as well as non protein amino acids increased with rise in salinity from 20 to 32*psu* and maximum change in concentration was noticed in *R. maritima* and *H. wrightii* at a salinity of 20 to 47*psu* (Pulich Jr., 1986). Proline and betaine in *H. wrightii* and *T. hemprichii* functioned as osmetic agents in highly salt-tolerant plants (Pulich Jr., 1986).

Histidine, lysine, glycine, leucine, aspartic acid and glutamic acid were the major amino acids in most of the seagrasses of Gulf of Mannar and Palk Bay whereas methionine and proline was minor in this study. The amino acids in *Amphibolis Antarctica* contained higher concentrations of glycine and lysine whereas tyrosine and methionine at lower concentrations (Harris et al., 1994). Species wise variations were observed in the amino acid profile of seagrasses of the present study and similar findings were reported by Jeevitha et al. (2013) in seagrasses from Tuticorin Bay, southeast coast of India (Table 4.2.4). The major essential amino acids recorded were isoleuine and tryptophan whereas non essential amino acids were tyrosine and glutamine found in *H. pinifolia* (Jeevitha et al., 2013).

Table 4.2.4 Comparison of amino acid profile.

Sl No	Species Name	Location	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Ile	Leu	His	Lys	Phe	References
1	Seagrasses (*C. serrulata*, *H. ovalis*, *H. pinifolia* and *S. isoetifolium*) mg/g DW	Tuticorin	0.01-3.3	0.11-3.3	0.05-2.1	0.02-1.1	0.04-1.1	0.12-3.2	0.14-2.3	0.04-8.9	0.01-2	0.31-1.1	0.03-4.1	0.02-1.1	Jeevitha et al., 2013
2	*H. wrightii* (µmol/g DW)	Texas	6.41-7.85	1.35-3.16	4.84-8.55	11.30-13.23	71.28-268.82	.90-2.57	4.44-5.11						Pulich Jr., 1986
	H. engelmannii (µmol/gDW)		7.92-10.71	3.10-3.53	10.32-16.14	11.57-12.87	4.25-95.95	6.13-14.08	102.85-383.30						
	R. maritima (in µmol/g DW)		10.22-11.07	2.91-3.60	3.70-4.13	25.31-50.25	60.86-182.60	0.86-1.80	4.19-4.78						
	T. testudinum (in µmol/g DW)		2.42-3.17	.62-1.48	1.03-1.80	1.26-2.81	57.32-93.75	0.92-1.96	1.03-2.18						
	S. filiforme (in µmol/g DW)		2.95	1.05	3	5.25	1.30	1.58	2.75						
3	*P. australis* in g\Kg	South Australia	1.49-7.08	2.05-2.79		4.86-7.43	2.05-2.83	.03-3.70	2.31-3.06	1.95-2.84	2.80-4.06	.64-.84	1.80-2.50	2-2.49	Torbatinejad et al., 2007
4	*Amphibolis antarctica* (in mol%)	Melbourne, Australia	9.1	4	8.7	9.2	6.9	12	9.1	3.9	6.9	2.2	10.4	2.7	Harris et al., 1994
5	*P. australis*	South Australia	4.16-7.02	2.05-2.79	2.10-3.50	4.86-7.43	2.05-2.83	2.78-3.7	2.31-3.06	1.95-2.84	2.80-4.06	.64-.84	1.80-2.50	2-2.49	Torbatinejad & Sabine, 2001
6	Green algae in % Brown algae in % Red algae in %	Palk Bay & Gulf Of Mannar	8.3-10.7 10.9-12.7 15.7	4.9-6.7 5.4-5.7 5.9	6.7-7.2 6.7 4.3	11.9-15.3 13.2-13.9 17.4	3.9-5.8 4.0-4.3 5.7	6.2-7.2 5.6-7.0 4.3	6.9-9.2 6.7-8.5 5.5	3.2-3.8 4.6-5.3 3.6	7.8-8.6 7.3-7.9 7.8	3.3 2.6-2.7 2.2	5.9-6.3 6.2-6.4 8.6	5.2-5.5 4.7-5.3 5.3	Rameshkumar et al., 2012
7	*Rhizophora mangle* (green) in µmol/g	Rookery Bay , Florida	78.7	49	64.8	98.6		93.3	82.5	38.4	84.6	22.4	35	43.3	Zieman et al., 1984
	R. mangle (Yellow)		98.3	46.7	70.1	79.6		86.1	73.3	39.1	80.5	18.7	41.9	34.1	
	Seagrasses (green)		158.9-188.3	64.4-74.2	105.4-120.8	147.1-152.4		165-193.2	139.6-176.1	84.5-85.4	213.7-219.9	27.7-41.1	49-68.7	86.3-93.9	
	R. mangle (green)		79.8	52.1	60.3	84.1		81	76.5	41.4	98.9	28.5	34.5	39	
	R. mangle (Yellow)		75	42.7	53.9	75.7		70.8	68	35.7	84.8	15.4	33.6	32.3	
	Seagrasses (green)	Pine Channel, Florida	107.1-307.8	42.7-135.7	135.3-207.9	179.6-267.9		344.2-405	158.5-270.7	93.2-109.6	211.8-234.8	79.9-104.6	151.8-154.9	99.2-104.2	
8	*Chelonia mydas* mg/100g	Nicaragua	705-2333	332-1242	409-1248	665-2076	531-905	853-2738	651-1968	372-1340	677-1971	547-1420	850-2620	347-1102	Thayer and Engel,1982
9	Seagrasses (*E. acoroides*, *C. serrulata* and *S. isoetifolium*) mg/100g wet weight	Gulf of Mannar	2-43	1-92	1-89	2-319	0-64	0-90	2-102	0-85	7-172	49-363	3-147	4-89	Present study
10	Seagrasses (*C. serrulata*, *T. hemprichii* and *S. isoetifolium*) mg/100g wet weight	Palk Bay	4-87	4-88	3-86	4-314	0-54	0-107	0-147	0-55	25-96	32-425	0-174	1-86	Present study

The amino acid profile of *P. australis* showed high levels of amino acids namely glutamic acid, aspartic acid, leucine, serine, valine and arginine, and low concentrations of histidine, methionine, tyrosine and cystein. Variations in the level of amino acids could be due to place and depth at which seagrasses collected, growth level of seagrasses and season (Torbatinejad and Sabine, 2001).

The dissolved free amino acids (DFAA) in the surface and bottom water samples of seagrass beds were serine, alanine and glycine and which might have been released from seagrasses as well as sediments. The free extractable amino acids exhibited higher concentrations during light than dark and difference in concentrations were observed between different body parts, and followed the order young leaves>roots>old leaves> rhizomes (Jorgensen et al., 1981). Fertilization with nitrogen alone and nitrogen with phosphorous in seagrasses were noticed, an increase in the concentrations of glutamine and aspargine were several times higher in *S. isoetifolium* than *H. uninervis* (Udy et al., 1999). Relatively high total nitrogen and phosphorous concentrations were noticed in the surrounding environments of Gulf of Mannar than Palk Bay and no such relations were noticed in this study.

There are reports indicating significant changes in amino acid profile of seagrasses of *Z. caprocorni* and *H. uninervis* on exposure to heavy metals like iron (Fe) and copper (Cu) and a chelator (ethylene diamine tetra acetic acid, EDTA) (Prange and Dennison, 2000). On the exposure of Fe, significant differences were seen on the basis of dominant amino acids proline and glutamine in *H. uninervis* though total amino acid profile did not

show much difference. Further a marginal difference in the concentration of aspargine was observed. Similarly total free amino acids were decreased with treatment of Cu in seagrasses (*H. uninervis* and *Z. capricorni*) and more affected amino acids were glutamine in *Z. capricorni* and proline in *H. uninervis*. Relatively lower concentrations of total amino acids noticed in the present study in Gulf of Mannar than Palk Bay and the absence of proline and alanine in *C. serrulata* from Chinnappalam could be related to higher concentrations of heavy metals at Gulf of Mannar.

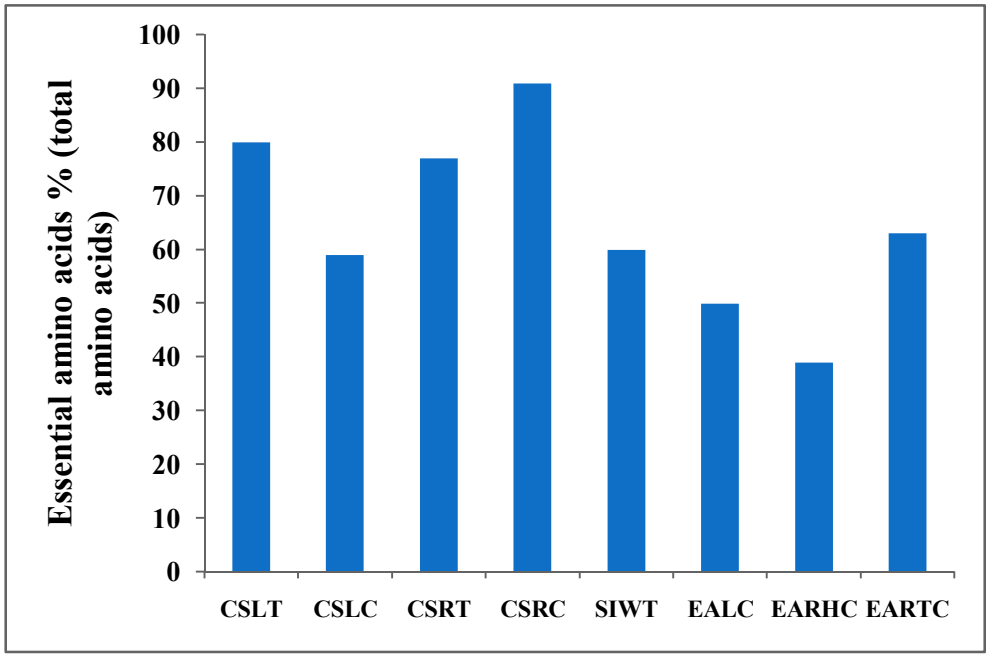

Figure 4.2.1 % of Essential amino acids at Gulf of Mannar

The growth and physiological responses of three seagrasses viz; *Z. caprocorni*, *H. uninervis* and *C. serrulata* of Moreton Bay, Australlia was studied by Udy and Dennison (1997), and among these seagrasses, *H. uninervis* with higher levels of proline exhibited a physiological

tolerance mechanism against Cu toxicity. Seagrass namely *T. hemprichii*, *H. uninervis* and *C. roundata* and macro algae of *Sargassum* sp. and *Padina* sp. were able to take up dissolved amino acids and urea using their above ground tissues and roots. The major N substrates were ammonium (NH_4^+), nitrate (NO^{3-}), urea and amino acids and rate of uptake followed the order: NH_4^+>NO^{3-}>urea>amino acids. Relatively high uptake rates for macro algae than seagrasses were observed. The leaves of *C. roundata*, *T. hemprichii* and *H. uninervis* have higher uptake rate at roots for inorganic N sources and lowest for amino acids. The ^{15}N labeled experiment showed that quantity of ^{15}N recovered from roots of seagrasses were negligible indicating the transfer of ^{15}N from roots to leaves (Vonk et al., 2008).

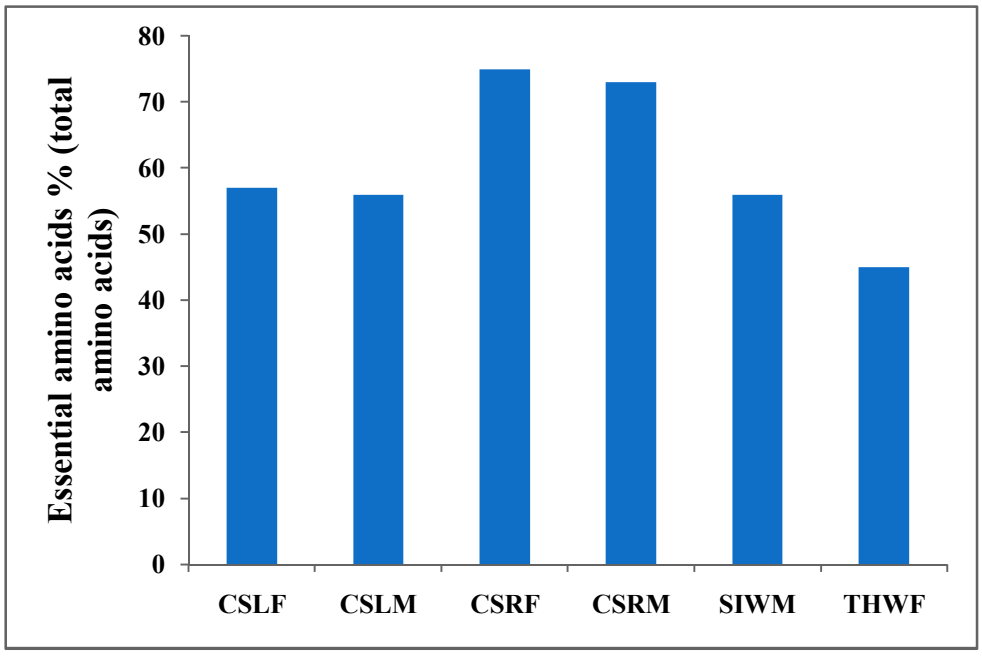

Figure 4.2.2 % of Essential amino acids at Palk Bay

The total amino acids as well as proline concentrations in *P. oceanica* revealed an increase in seagrasses at hyper saline (43*psu*) stress than the control (37*psu*) (Marin-Guirao et al., 2013). Limited toxicity of reduced nitrogen (NHx) pulses on tropical seagrass species were strongly influenced by the interactions of pH and light, and the level of uptake of nitrogen was more in *H. uninervis* compared to *T. hemprichii* and thereby an increase in free amino acids contents relative to total N%. Differences in amino acids content were observed between species during metabolisation of excess nitrogen (Christianen et al., 2011). It's worth mentioning that the plants assimilate ammonium rapidly into free amino acids on ammonia toxicity and first glutamine is synthesized from ammonia which in turn synthesizes other amino acids. In *H. uninervis*, aspargine increased to prevent the glutamate accumulation (Britto and Kronzucker, 2002). An increased NHx could be used for the conversion of glycine to serine during photorespiration (Guo et al., 2007). Equilibrium could be created between limited toxicity of NHx pulses and ammonia under high pH condition and if the concentrations were high, ammonia can diffuse through outer membranes of chloroplasts followed by uncoupling of photophosphoryl. It reduced the photosynthetic efficiency which retards the NHx detoxification through amino acid synthesis (Britto and Kronzucker, 2002). The synthesis of free amino acids from NHx during detoxification was varying with respect to species. Similar findings were observed in the case of *Z. capriconi* (Udy and Dennison, 1997) and *Z. marina* (Van der Heide et al., 2008). The detoxification process assumes importance in the sense as anthropogenic pressure along the coast by land run off, use of fertilizers, deforestation, shrimp farming, etc. (Green and Short, 2003).

The amino acid deamination in the sediments of *Z. capriconi* beds were conducted using ^{15}N experiments showed that the rate of generation of ^{15}N glycine and assimilated ^{15}N ammonium (from deamination of the amino acid) took place unpredictably (Boon et al., 1986). The bacteria in the rhizosphere of seagrasses deaminated the amino acids of glutamic acid directly with the uptake of ammonium by seagrass roots (Smith et al., 1984). It is known that seagrasses could directly take up low molecular weight compounds such as amino acids, mainly glycine (McRoy et al., 1977). The differences in ammonium turn over in the sediments of seagrass bed and other areas showed importance of seagrasses on nitrogen cycling. Evaluation of amino acid composition of a major vertebrate which feeds on seagrasses such as green turtle (*Chelonia mydas*) revealed arginine, lysine, leucine as major essential amino acids and methionine as minor. The predominated non essential amino acids were glycine, glutamate and aspartate while tyrosine at lower concentrations (Thayer and Engel, 1982). Similarly the marine algae species from Gulf of Mannar and Palk Bay revealed alanine, aspartic acid, glutamic acid, leucine, lysine and serine as predominating amino acids (Rameshkumar et al., 2012). Compound specific stable isotopic analysis was used to study the diet composition of organisms in a complex seagrass meadow by Choi et al. (2017), and it was based on the differences in concentrations of phenylalanine and glutamic acid in the seagrass consuming organisms. The diets of polychaeta and crustaceans contained seagrass as major component while fishes varied with respect to species and bivalves dominated by algae.

Degradation process in vascular plants happens through three stages and they are loss of liable compounds, microbial colonization and utilization followed by mechanical fragmentation. The rate loss depends on habitat as well as season and showed a consistency with habitat (single) or regions. The rate of decay was directly related to the amount of structural tissues as well as nitrogen content. Seagrass possess higher nitrogen content and it may increase during decay while mangroves have lower nitrogen content and decrease slowly with decay. Both free amino acid and protein decay in seagrasses might be due to leaching of water soluble components or consumption of protein by bacterial action whereas in mangroves through both processes. Total essential amino acid contents in seagrasses and mangroves were less than 50% of total amino acids except green *Rhizophora mangle* from Pine Channel (50.3%), and relatively higher amino acid contents were observed in seagrasses than mangroves (Zieman et al., 1984).

Statistical analysis

Pearson correlation matrix

Pearson correlation matrix of amino acid compositions of seagrasses shows differences in interrelations between amino acids. A strong positive correlation was exhibited by essential amino acids viz; isoleucine, phenylalanine, lysine and arginine, and non essential amino acids, serine, proline, glycine and alanine with threonine, valine and methionine while highly positive correlations were observed between threonine and valine, and all the above mentioned amino acids except arginine showed significant positive correlations towards phenylalanine whereas aspartic acid obtained

strong significant positive correlations with leucine (Table 4.2.5). Highly significant variations were observed in serine with phenylalanine, lysine and arginine while proline, glycine and alanine with lysine and serine, and highly significant correlations were observed for glutamic acid with serine. Glycine exhibited correlations with aspartic acid and proline, and tyrosine with aspartic acid. Alanine showed highly significant correlations with proline and glycine. Amino acids are not only used for building blocks for proteins but also serve as precursors for the synthesis of many metabolites. Among these, most important aromatic amino acids are phenylalanine, tyrosine and tryptophan, and play an important role in plant mechanism (Tzin and Galili, 2010). The amino acid metabolism includes the synthesis of phenolic acids, alkaloids, lignans, coumarins, flavonoids and glucosinolates (Subhashini et al., 2013), phenylpropenes, lignins, stilbenes, isoflavonoids, terpenoids and folic acid (Dewick, 2002), pigments, hormones and cell wall components (Maeda and Dudareva, 2012). Plants and bacteria have the ability to produce all the 20 amino acids (Miles, 2003). The multiple functions of amino acids in growth and other biological process of a living organism is the reason for high correlations between the amino acids in seagrasses.

Table 4.2.5 Pearson correlation matrix for amino acid profile of seagrasses

Amino Acid Composition of Seagrasses																
	THR	**VAL**	**MET**	**ILE**	**LEU**	**PHE**	**HIS**	**LYS**	**ARG**	**ASP**	**SER**	**GLU**	**PRO**	**GLY**	**ALA**	**TYR**
THR	1.00															
VAL	0.95	1.00														
MET	** 0.94	** 0.96	1.00													
ILE	** 0.98	** 0.94	** 0.94	1.00												
LEU	0.02	-0.05	-0.22	0.06	1.00											
PHE	** 0.93	** 0.92	** 0.90	** 0.93	0.13	1.00										
HIS	0.07	0.07	-0.09	-0.05	0.27	0.09	1.00									
LYS	** 0.77	* 0.61	* 0.66	** 0.81	0.08	* 0.72	-0.13	1.00								
ARG	* 0.64	* 0.68	* 0.63	0.58	-0.10	* 0.63	0.12	0.30	1.00							
ASP	0.52	0.45	0.25	0.53	** 0.76	0.51	0.34	0.43	0.45	1.00						
SER	** 0.97	** 0.95	** 0.91	** 0.97	0.10	** 0.90	0.01	** 0.74	** 0.66	0.59	1.00					
GLU	0.48	0.50	0.29	0.44	0.58	0.41	0.39	0.17	0.48	0.84	** 0.60	1.00				
PRO	** 0.94	** 0.92	** 0.97	** 0.95	-0.10	** 0.92	-0.02	** 0.75	0.49	0.30	** 0.91	0.27	1.00			
GLY	** 0.76	* 0.65	* 0.62	** 0.82	0.29	* 0.72	-0.11	** 0.87	0.54	* 0.72	** 0.81	0.47	* 0.65	1.00		
ALA	** 0.88	** 0.80	** 0.80	** 0.87	0.19	** 0.84	0.04	** 0.80	0.35	0.47	** 0.89	0.48	** 0.89	* 0.68	1.00	
TYR	0.37	0.47	0.28	0.42	0.36	0.36	0.56	0.20	0.19	* 0.61	0.44	0.59	0.34	0.39	0.32	1.00

N=14, ** Correlation is significant at the 0.01level (2- tailed).

* Correlation is significant at the 0.01level (2- tailed).

Principal component analysis

PCA of amino acid composition of seagrasses of Gulf of Mannar and Palk Bay revealed three components accounting for a total variance of 86.37%. First component accounts for about 61.44% of total variance characterised by a strong positive loadings on threonine, valine, methionine, isoleucine, phenyl alanine, serine, proline and alanine while component two exhibited strong positive loadings on aspartic acid and leucine, and histidine was the only variable displayed in component three. Components of two and three accounted for 17.04% and 7.88% of total variance respectively (Table 4.2.6&4.2.7).

Table 4.2.6 Total variance explained (Extraction sums)

	Total	% of Variance	Cumulative %
1	9.831	61.441	61.441
2	2.727	17.043	78.484
3	1.262	7.886	86.37
4	0.871	5.444	91.815
5	0.501	3.133	94.948
6	0.447	2.797	97.744
7	0.249	1.559	99.303
8	0.067	0.421	99.724
9	0.03	0.19	99.914
10	0.014	0.086	100

Table 4.2.7 Factor loadings of PCA analysis of amino acid profile of seagrasses

Amino acids	Component		
	1	2	3
THR	0.965	0.15	0.142
VAL	0.943	0.029	0.278
MET	0.982	-0.126	0.075
ILE	0.969	0.207	0.045
LEU	-0.146	0.92	0.154
PHE	0.922	0.174	0.137
HIS	-0.101	0.131	0.84
LYS	0.784	0.342	-0.32
ARG	0.633	-0.034	0.374
ASP	0.348	0.838	0.357
SER	0.946	0.246	0.153
GLU	0.316	0.612	0.571
PRO	0.968	-0.006	0.035
GLY	0.738	0.551	-0.118
ALA	0.853	0.3	-0.007
TYR	0.273	0.395	0.659
% Variance (Rotation sums)	56.35	17.38	12.64

4.3 Carbohydrate Profile

4.3.1 Introduction

Seagrasses are the only angiosperms (flowering plants) adapted to the marine environment and permanent immersion at shallow water. Seagasses contain high cellulose content, low nitrogen levels and a good source of phenolic acids (Davies et al., 2007). Generally, seagrass seeds are used as a source of direct food but not seagrass as a whole. Carbohydrates are one of the major constituents of these vascular plants and among these, neutral

sugars have more importance than others. Seagrass meadows of *P. oceanica* in the Mediterranean Sea act as a source of transparent exopolymer particles (around 0.10TgC annually) (Iuculano et al., 2017). Major monosaccharides which form structural polymers are cellulose, hemicelluloses and pectin. Besides, cyclitols present in seagrasses which are important membrane constituents as well as intermediates in cellular metabolism (Opsahl and Benner, 1993). Non structural carbohydrates present in seagrasses allow the plants to sustain respiration and rebuild damaged tissue in response to disturbance. The reserve formation process involves through normal metabolic formation of carbohydrates while reserve accumulation occurs through the formation of carbohydrates due to environmental factors (Campbell et al., 2012). Total carbohydrates in seagrasses are distributed to stem, roots, leaf and rhizome. Comparatively higher contents are observed in rhizomes than rest of the parts and carbohydrate reserve in roots reduces carbon loss from herbivores. The soluble carbohydrates noticed in seagrasses include sucrose, glucose, galactose, fructose, apiose, arabinose, fucose, mannose, rhamnose and xylose (Touchette and Burkholder, 2000).

4.3.2 Results

The predominated carbohydrates were ribose, xylose, glucose and galactose in seagrasses and its composition were given in Table 4.3.1&4.3.2. Xylose was one of the major carbohydrates among the seagrasses, and highest concentration was noticed in *C. serrulata* (65.44%) (Chinnappalam), and *E. acoroides* account for the lowest (2.05%). This was followed by ribose predominated in *C. serrulata* (Thonithurai), *S. isoetifolium* (Mathacovil) and *T. hemprichi* (Munaikkadu). A station wise variation was higher at Gulf of Mannar than Palk Bay in the case of *C. serrulata*. Glucose was the

predominant carbohydrate in *S. isoetifolium* of Mathacovil followed by xylose and arabinose whereas *S. isoetifolium* of Thonithuraii followed the order; ribose (41.04%)>xylose (36.17%)>galactose (17.93%). *E. acoroides* contained glucose (91.44%) as major carbohydrate. The predominated carbohydrates in *T. hemprichi* were xylose (61.99%) and ribose (24.95%).

Table 4.3.1 Carbohydrate composition in seagrasses of Gulf of Mannar (% of total carbohydrates)

Carbohydrates	*C. serrulata*	*C. serrulata*	*S. isoetifolium*	*E. acoroides*
	Thonithurai	Chinnappalam	Thonithurai	Chinnappalam
Ribose	24.78±0.48	2.12±0.22	41.04±0.60	5.12±0.22
Xylose	60.02±0.82	65.44±0.88	36.17±0.51	2.05±0.19
Arabinose	1.82±0.14	5.61±0.22	0.29±0.06	1.05±0.10
Fructose	ND	ND	ND	ND
Mannose	3.77±0.18	8.36±0.32	ND	0.21±0.06
Glucose	5.43±0.24	12.14±0.38	2.71±0.28	91.44±0.89
Galactose	4.05±0.20	4.03±0.18	17.93±0.40	ND
Sucrose	0.03±0.01	ND	0.87±0.10	0.13±0.04
Maltose	0.09±0.02	2.29±0.20	0.99±0.12	ND
Lactose	ND	ND	ND	ND

Table 4.3.2 Carbohydrate composition in seagrasses of Palk Bay (% of total carbohydrates).

Carbohydrates	*C. serrulata*	*C. serrulata*	*S. isoetifolium*	*T. hemprichii*
	Munaikkadu	Mathacovil	Mathacovil	Munaikkadu
Ribose	15.09±0.36	1.33±0.16	24.51±0.46	24.95±0.48
Xylose	41.62±0.64	26.41±0.44	26.79±0.50	61.99±0.86
Arabinose	4.91±0.26	4.60±0.28	5.06±0.32	2.56±0.20
Fructose	ND	ND	ND	ND
Mannose	1.39±0.14	2.21±0.21	1.81±0.18	3.78±0.26
Glucose	ND	ND	41.06±0.68	3.64±0.28
Galactose	34.71±0.40	63.84±0.89	0.41±0.06	2.95±0.24
Sucrose	0.42±0.08	1.39±0.18	0.36±0.06	ND
Maltose	1.87±0.18	0.22±0.04	ND	0.04±0.01
Lactose	ND	ND	ND	0.08±0.02

4.3.3 Discussion

The major carbohydrates in the present study were ribose, glucose, xylose and galactose (Figure 4.3.1) and it was similar to other studies carried out (Table 4.3.3) (Knox, 1984; Opsahl and Benner, 1993; Torbatinejad et al., 2007). The dominant monosaccharides other than glucose were arabinose, xylose and galactose in the angiosperms belonging to dicotyledon genus and monocotyledons genus (Knox, 1984). The structural constituents of the seagrass *P. australis* of green, fresh, dry wash and dry unwashed were compared and the major carbohydrates were glucose, galactose, xylose and mannose in the soluble as well as insoluble non starch polysaccharides (NSP) (Torbatinejad et al., 2007). Xylose was the predominated carbohydrate in *C. serrulata* followed by ribose except from Mathacovil where galactose predominated over xylose while *S. isoetifolium* from Gulf of Mannar contained arabinose and xylose, and glucose and xylose at Palk Bay in the present study. The total insoluble NSP concentration were relatively higher in green *P. australis* and lower in dry unwashed whereas soluble NSP exhibited a maximum at fresh *P. australis* and minimum at dry unwashed indicating the loss of sugars on treatment (Torbatinejad et al., 2007). Composition analysis of sugars in the walls of pollen grains of seagrass *A. antarctica* suggested that hydrolysis of polysaccharides is related to method and mono saccharides such as rhamnose and galactose were the most abundant in triflouro acetic acid hydrolysis while galactose (32.6mol%) and glucose (32.8mol%) in sulphuric acid hydrolysis (Harris et al., 1994).

Table 4.3.3 Comparison of carbohydrate profile

Sl No	Species name	Location	Ribose	Xylose	Glucose	Galactose	Arabinose	Reference
1	*Z. marina* (Boiled water)	German Baltic coast	-----	30 mol%	47	5.5	1.4	Davies et al., 2007
	Z. marina (TFA)		-----	50 mol%	6.5	5.1	3.3	
2	*H. wrightii* (green)	Laguna Madre, USA	-----	17.1 mg/g	169.9	11.1	6.1	Opsahl & Benner,1993
	H.wrightii (senescent)		-----	17.3 mg/g	176.4	12.2	6.8	
3	*Amphibolis Antarctica* (TFA)	Melbourne, Australia	-----	7 mol%	6.8	40.1	7	Harris et al.,1994
	A. antarctica (H_2SO_4)		-----	8 mol%	32.8	32.6	5	
4	*P. australis*	South Australia	2.5-3.3 g/Kg	43.1-59.4 g/Kg	122-157 g/Kg	3.9-10.9 g/Kg	3-5 g/Kg	Torbatinejad et al., 2007
5	*P. australis*	South Australia	0.25-0.33%	4.13-5.94%	12.2-15.7%	0.39-1.09%	0.30-0.50%	Torbatinejad & Sabine, 2001
6	Seagrasses (*E. accordies*, *C. serrulata* and *S. isoetifolium*)	Gulf of Mannar	2.12-41.04%	2.05-65.44%	2.71-91.44%	0-17.93%	0.29-5.61	Present study
7	Seagrasses (*C. serrulata*, *T. hermprichii* and *S. isoetifolium*)	Palk Bay	1.33-24.95%	26.41-61.99%	0-41.06%	0.41-63.84	2.56-5.06	Present study

The major monosaccharides of soluble NSP were mannose, glucose and galactose whereas insoluble NSP contained xylose and glucose in *P. australis* (Torbatinejad and Sabine, 2001). Major sugars noticed in boiling water extraction of *Z. marina* were glucose, xylose and galacturonic acid while using TFA, xylose and galactouronic acid as major followed by glucose and glucouronic acid (Davies et al., 2007). Xylose was the major carbohydrate in most of the seagrasses except *E. acoroides* and *S. isoetifolium* (Mathacovil) in this study. Cell wall compositions of *Z. marina* fibers were comparable to other terrestrial fibers and contained nearly 57% of cellulose (Davies et al., 2007). Pectic polysaccharides are a complex mixture of acidic polysaccharides and neutral polysaccharides, which on hydrolysis leads to arabinose or galactose with galacturonic acid (Selvendran, 1985).

Glucose was the most abundant neutral sugar noticed at a concentration of 169.9mg/g ash free dry weight and 176.4mg/g respectively for green and senescent blades of *H. wrightii* followed by xylose and galactose (Opsahl and Benner, 1993). In *H. wrightii*, cellulose and hemicelluloses formed nearly 20% of the total organic mass while lignin and cutin together present in very low concentration (Opsahl and Benner, 1993). Similar trends were observed in biopolymers of herbaceous plant tissues and the major constituents were polyuronic acids of pectin and other polymers (Benner et al., 1990). The sum of cellulose, hemicelluloses and lignin comprised of more than 75% of the biomass of woody plant tissues (Sjostrom, 1981). Seagrasses of *Zannicheliaceae* contained glucose, fructose and sucrose with comparatively higher concentrations of soluble sugar in *C. nodosa* and *S. filiforme* whereas sucrose was predominating sugar in *Posidoniaceae* family. *P. torreyi* was the only seagrass species

which have four soluble sugars under the genera *Zosteraceae*, and relatively high contents of glucose, fructose and sucrose were reported in *Z. marina* (Drew, 1983). *C. serrulata* from Gulf of Mannar and *T. hemprichii* of Palk Bay in this study (Table 4.3.1) showed comparatively higher carbohydrates. *E. acoroides* of family *Hydrocharitaceae* have high concentrations of soluble sugars while lowest at *H. decipiens*. Among the different body parts of seagrasses considered, most of the seagrasses have high sugars in rhizomes and roots than leaves (Touchette and Burkholder, 2000; Lee and Dunton, 1996). Myo-inositol was the common cyclitol in all genera of seagrasses, and relatively low concentrations of myo-inositol in seagrasses might be due to its utilization for next elongation through cell wall biosynthesis whereas high cyclitol content in marine plants used for osmoregulation (Drew, 1983).

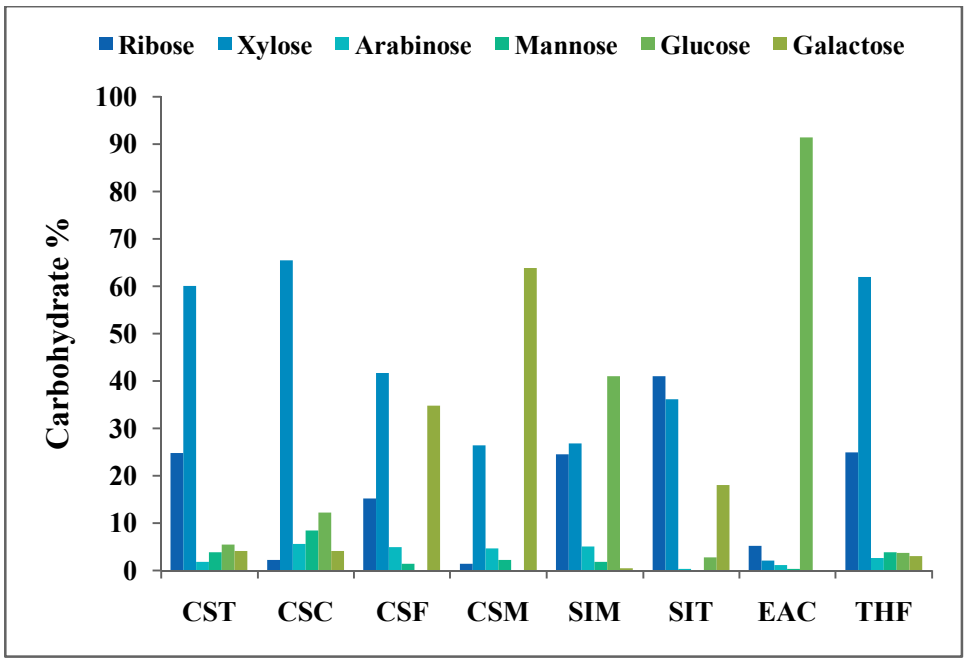

Figure 4.3.1 Carbohydrate profile of seagrasses

It is also reported that the non structural carbohydrate acts as an organic osmolytes against the saline stress (Marin-Guirao et al., 2013). Sucrose was the dominant carbohydrate in the total soluble carbohydrate pool and others were glucose, fructose, apiose, arabinose, fucose, galactose , mannose, rhamnose and xylose. Xylose was the most predominated carbohydrate in most of the seagrasses of Gulf of Mannar and Palk Bay and others were ribose, arabinose, mannose, glucose, galactose, maltose and sucrose (Table 4.3.1). The soluble carbohydrates in *H. engelmannii* were higher in December (11.4 -28.2%) than September (5.2 -20.2%) of Indian Bluff Island and also higher values at Homosassa River Bay, Florida (12.4 - 34.7%) (Dawes et al., 1987). The carbohydrate fraction (nitrogen free extract) of the seagrass *P. torreyi* ranged from 45.4 (autumn) to 65.8% dry matter (summer) (Serviere-Zaragoza et al., 2002).

Average carbohydrates carbon content in *T. testudinum* showed slight seasonal variations (Lee and Dunton, 1996). Among the total carbohydrate carbon content in seagrass, 50% account for non structural carbohydrates and the accumulated non structural carbohydrate can be used as carbon source when photosynthesis exceeds the needs from respiration and growth. The variations in carbohydrate carbon content related to high temperature and irradiance in the water column, and no remarkable seasonal wise variations in carbohydrate carbon content were obtained (Lee and Dunton, 1996). Also related to differences in nutrient loading rates and abiotic factors of sediment mineralogy, sediment grain size, water clarity as well as depth, temperature, light, salinity, etc, and variations can be affected to large scale. (Campbell et al., 2012). Level of water soluble carbohydrates (WSC) in seagrasses of *H. ovalis*, *Z. capriconi* and *C. serrulata* were showed

variations and it was predominent at rhizomes of *H. ovalis* and *C. serrulata* (Kuiper-Linley et al., 2007). Differences observed in the carbohydrate content from two sites might be due to the nutrient concentrations, currents, substrates and bed structure (Rey and Stephens, 1996). This might be the reason for variations in carbohydrate composition of seagrasses of four stations and also same species of *C. serrulata* and *S. isoetifolium* from Gulf of Mannar and Palk Bay.

Statistical analysis

Pearson correlation matrix

Pearson correlation matrix of carbohydrate composition of seagrasses Gulf of Mannar and Palk Bay revealed differences in interrelations between carbohydrates. A positive correlation was observed between xylose and mannose while negative correlation between xylose and glucose, and a strong positive correlation noticed in sucrose with galactose. The stability of individual sugars in seagrasses followed the order: mannose>fucose>arabinose=rhamnose=galactose=xylose>glucose>myo-inositol (Opsahl and Benner, 1993). This might be the reason for a positive correlation between xylose and mannose and also a negative correlation between xylose and glucose. Sucrose was the dominant carbohydrate in seagrasses as a major storage of carbohydrate (initially stored in rhizomes), and it occupied more than 90% of the total soluble carbohydrate pool. Sucrose is presented mostly in fresh seagrasses and also it accounts for loss of neutral sugars (Touchette and Burkholder, 2000) (Table 4.3.4).

Table 4.3.4 Pearson correlation matrix for carbohydrate profile of seagrasses

Carbohydrate composition of Seagrasses								
	Ribose	**Xylose**	**Arabinose**	**Mannose**	**Glucose**	**Galactose**	**Sucrose**	**Maltose**
Ribose	1.00							
Xylose	0.26	1.00						
Arabinose	-0.51	0.24	1.00					
Mannose	-0.29	*0.78	0.52	1.00				
Glucose	-0.33	*-0.79	-0.26	-0.44	1.00			
Galactose	-0.25	-0.04	0.30	-0.12	-0.51	1.00		
Sucrose	0.02	-0.22	0.07	-0.32	-0.39	**0.87	1.00	
Maltose	-0.15	0.46	0.44	0.50	-0.44	0.18	0.00	1.00

N=8, ** Correlation is significant at the 0.01level (2- tailed).

* Correlation is significant at the 0.01level (2- tailed).

Principal component analysis

PCA of carbohydrate composition of seagrasses of Gulf of Mannar and Palk Bay revealed three components accounting for a total of variance 84.98% (Table 4.3.5&4.3.6). First component accounts for about 42.34% of the total variance and exhibited a strong positive loadings on galactose and sucrose and a high negative loadings on glucose. Component two accounts for 25.79% of the total variance and displayed positive loading on xylose and mannose whereas variables included in component three showed a positive loadings on arabinose and strong negative loadings on ribose accounting for 16.85% of the total variance.

Table 4.3.5 Total variance explained (Extraction sums)

	Total	**% of Variance**	**Cumulative %**
1	3.387331	42.34163	42.34163
2	2.063315	25.79144	68.13307
3	1.348295	16.85369	84.98676
4	0.695606	8.695078	93.68184
5	0.318382	3.979779	97.66162
6	0.164394	2.054929	99.71655
7	0.021189	0.264867	99.98141
8	0.001487	0.018588	100

Table 4.3.6 Factor loadings of PCA analysis of carbohydrate profile of seagrasses

Carbohydrates	Component		
	1	2	3
Ribose	0.042	0.134	-0.955
Xylose	0.272	0.921	-0.224
Arabinose	0.032	0.470	0.763
Mannose	-0.093	0.874	0.330
Glucose	-0.838	-0.457	0.082
Galactose	0.949	0.285	0.005
Sucrose	0.893	-0.267	-0.028
Maltose	0.415	0.499	0.135
% Variance (Rotation sums)	33.22	30.77	20.99

4.4 Conclusions

The FA compositions of seagrasses from Gulf of Mannar and Palk Bay, South East coast of India revealed that SFAs were the predominated FAs followed by PUFAs and MUFAs in most of the seagrasses except *E. acoroides* (leaves and rhizomes) and *C. serrulata* (roots and rhizomes) from Munaikkadu where the order was PUFAs>SFAs>MUFAs. The most abundant fatty acids noticed in seagrasses were C16:0, C18:0, C18:2n-6, C18:3n-3, C18:1n-9c and C24:0. *C. serrulata* contained comparatively higher PUFAs at roots and rhizomes than leaves except from Chinnappalam whereas in *E. acoroides*, leaves followed by rhizomes and roots indicating the habitat difference and availability of nutrients in the environment. Total 18 FAs were identified and quantitated in this study. PUFAs of C18:2n-6 predominated over C18:3n-3 in most of the seagrasses except leaves and rhizomes of *E. acoroides*. Among the FAs, species wise and different body parts variations were noticed in C16:0, C18:0, C18:2n-6, C18:1n-9c and

C24:0. C24:0 noticed relatively higher concentrations in roots and rhizomes than leaves in most of the seagrasses. Total PUFAs were more than 20% of total FAs in most of the seagrasses and its importance were again increased because more than 20% were used for extraction of PUFAs.

Total amino acid contents were relatively higher in *S. isoetifolium* followed by *C. serrulata*, *E. acoroides* and *T. hemprichii*. Leaves of *C. serrulata* contained comparatively large quantities of amino acids than roots and rhizomes. *C. serrulata* (both leaves and roots and rhizomes) from Palk Bay predominated over Gulf of Mannar and vice versa obtained in the case of *S. isoetifolium*. Among the seagrasses, most of them exhibited higher histidine levels and lower methionine concentration. Total 17 amino acids were quantified in the seagrasses and 50% of total amino acids were found to be essential amino acids, suggesting seagrasses as a possible source of essential amino acids for humans.

Variations in carbohydrate composition observed between seagrasses of different species as well as same species (*C. serrulata* and *S. isoetifolium*) collected from Gulf of Mannar and Palk Bay. A total of ten carbohydrates were quantified and the dominated carbohydrates were ribose, xylose, glucose and galactose. Most of the carbohydrates noticed in *C. serrulata* from Gulf of Mannar and *T. hemprichii* from Palk Bay and namely ribose, xylose, glucose, arabinose, mannose, sucrose, lactose and galactose. Among different seagrasses, *E. acoroides* contained glucose more than 90% of the total carbohydrates.

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Chapter 5

BIO POTENTIAL OF SEAGRASSES OF *C. SERRULATA* AND *S. ISOETIFOLIUM*

Contents

5.1 Introduction

Marine plants and organisms are a source of structurally diverse secondary metabolites and they are reported to play an important role in the maintenance of human body. Large numbers of life saving drugs are developed from the plants of marine origin (Ravikumar et al., 2011a). Among the marine plants, seagrasses are one of the specialized groups of marine flora which are poorly known in India compared to seaweeds and mangroves particularly with reference to bioactive potential studies (Mani et al., 2012a; Kannan et al., 2013a). Seagrasses are submerged marine angiosperms that grow abundantly in tidal and sub tidal coastal areas of the World (Chanthini et al., 2015). People living along the coastal areas use seagrasses as folk medicine against fever, muscle pains, wounds, skin diseases, stomach problems, heart diseases and blood pressure (Kannan et al., 2013c). Antioxidants in biological systems have the ability to prevent

cell damage caused by the action of reactive oxygen species (Kannan et al., 2012). Phenolic compounds from seagrasses such as phenols, flavonoids and tannins showed a major role in antioxidant activity. The antioxidant activity of phenolic compounds may be due to their capacity to scavenge toxic free radicals as well as reactive species such as superoxide radical (O_2^-, hydroxyl radical (OH^-), peroxide radical (ROO^-) and nitric oxide radical (NO^-) (Rengasamy et al., 2012).

Seagrasses are reported to have variety of biological activities which include antibacterial (Bhosale et al., 2002; Engel et al., 2006; Qi et al., 2008; Choi et al., 2009; Kannan et al., 2010 a; Ravikumar et al., 2011b; Mani et al., 2012 a, b; Berfad and Alnour, 2014; Supaphon et al., 2014), antialgal (Harrison, 1982), antifungal (Jensen et al., 1998), antiviral (Rowley et al., 2002), antiprotozal (Orhan et al., 2006), anti-inflammatory (Hua et al., 2006), antidiabetic (Gokee and Haznedaroglu, 2008), antiinsecticidal (Mani et al., 2012a), anti larval and antifeedant (Qi et al., 2008), and antifouling (Iyapparaj et al., 2014) activities. Around 65-80% of the people in developing countries use traditional medicine (majority of them consumed as plant extracts) for primary health care (Politi et al., 2012) and has been used against a wide range of diseases (both chronic and infectious) (Duraipandiyan et al., 2006). Besides the biological activities seagrass meadows have major ecological role (act as a source of organic carbon, food and shelter for its surrounding organisms, improves water quality and reduces sediment erosion) (Mckenzie, 2008; Choi et al., 2009; Mani et al., 2012a, b). Seagrass meadows play an important role in maintaining the integrity of the coast (CRZ, 2011). Physio-chemical parameters such as dissolved oxygen and salinity would influence the antioxidant activity of

seagrasses (Ramah et al., 2014). Even though environmental laws are strict, seagrass meadows are declined due to anthropogenic activities, urban and industrial waste disposal, bottom trawling fisheries, etc., and due to this, several countries have started transplantation of seagrasses. Most of the antioxidant and antimicrobial studies carried out with seagrasses were those grew at high salinities (above 33*psu*). This study investigated the level of bio potentials of seagrasses of *C. serrulata* and *S. isoetifolium* growing at low salinities (15*psu*) and is conducted by measuring the antioxidant activities (DPPH, H_2O_2, Hydroxy radical, FRAP and TAA assays), phytochemicals and their level, and antimicrobial activity.

5.2 Results

5.2.1 Antioxidant activities (DPPH, H_2O_2, OH^-, FRAP and TAA)

DPPH free radical savenging activity of seagrasses of Thonithurai, Gulf of Mannar revealed that *S. isoetifolium* were more active than *C. serrulata* when whole as well as root and rhizome extracts were concerned. There is no remarkable difference in the activity between different body parts of *C. serrulata* (Table 5.2.1). Hydrogen peroxide activity in seagrasses followed the order: *S. isoetifolium* (whole)>*C. serrulata* (whole)>*C. serrulata* (roots and rhizomes) (Figure 5.2.1). Hydroxy radical activity followed the same order but the activity of roots and rhizomes of *C. serrulata* were much lower in comparison to whole seagrasses while activity of standard gallic acid was almost similar to seagrassses of *S. isoetifolium* (whole) (Figure 5.2.2). FRAP assay followed the order: *S. isoetifolium* (whole)>*C. serrulata* (roots and rhizomes)>*C. serrulata* (whole). Total antioxidant activities were higher in the roots and rhizomes of *C. serrulata* and are comparable to *S. isoetifolium* but much higher than *C. serrulata* (whole) (Table 5.2.1).

5.2.2 Antimicrobial activity

The bacteriostaic activity was observed and inhibition zones were 2.7 and 3.3 mm respectively for *C. serrulata* and *S. isoetifolium* (whole) against pathogens of *Pseudomonas aeruginosa* while 1.7 and 2.3mm against *Staphylococcus aureus* (Table 5.2.2). The roots and rhizomes of *C. serrulata* showed inhibition of 1.7mm only towards *Pseudomonas aeruginosa*, and rest of the pathogens were resistant against extracts irrespective of body parts. No zone of inhibition was obtained towards solvent methanol.

5.2.3 Phytochemicals, Phenolics and Flavonoids

A total of seven phytochemicals namely phenols, sterols, flavonoids, reducing sugars, alkaloids, tannins and terpenoids were noticed in the seagrasses studied while cardiac glycosides, saponins and anthraquinones were absent (Table 5.2.3). Total phenolics in seagrasses followed the trend: *S. isoetifolium* (whole)>*C. serrulata* (roots and rhizomes)>*C. serrulata* (whole) (Figure 5.2.3) while flavonoids was in the order; *C. serrulata* (roots and rhizomes)>*S. isoetifolium*>*C. serrulata* (whole) (Figure 5.2.3).

5.2.4 Chemical characteristics of seagrasses (using GCMS, FTIR and UV-VIS)

GCMS analysis of seagrasses indicated that major components were saturated fatty acids, polyunsaturated fatty acids, sterols, long chain alkanes and terpenes. Relatively large numbers of components were found in *S. isoetifolium* (39 components) followed by *C. serrulata* (whole) (34) and *C. serrulata* (roots and rhizomes) (20), and components with greater than 0.20% of total components were given in Table 5.2.4. The predominating

compounds (17) identified in *S. isoetifolium* were Octadecanoic acid, Methyl 11,14,17-Eicosatrienoate, Tetradecanoic acid-10,13-dimethyl methyl ester, Phytol, Ergost-5, 22-en-3 ol-(3 beta, 22E) and Gama Sitosterol. *C. serrulata* (roots and rhizomes) exhibited only 10 major components under similar conditions with Methyl-9-cis,11-trans Octadecadienoate, Furancarboxylaldehyde,5-(hydroxyl methyl), Methyl 11,14,17-Eicosatrienoate, Tetradecanoic acid-10,13-dimethyl methyl ester, n-Hexanoic acid and Ethyl 9,12-Hexadienoate while *C. serrulata* (whole) contained 17 components with Methyl 12,15-Octadiecanoate, Tetradecanoic acid-10,13-dimethyl methyl ester, Methyl 11,14,17-Eicosatrienoate, Methyl 16-methylHeptadecanoate, n-Hexanoic acid, Phytol, Gama Sitosterol,Trans-2-methyl-4-pentythiane and 9,12- Octadecanoic acid (Z,Z). The components of Tetradecanoic acid-10,13-dimethyl methyl ester, Octadecanoic acid, Methyl 11,14,17-Eicosatrienoate, Heptacosanoic acid-25-methyl methyl ester and Stigma sterol were identified in all the three extracts.

Among the two seagrasses studied, there are twelve major absorptions peaks identified in *C. serrulata* (roots and rhizomes) while ten and eight peaks respectively for *S. isoetifolium* (whole) and *C. serrulata* (whole). Major absorption peaks noticed in *C. serrulata* (whole) were at 1062, 1405, 1606, 2925 and 3296cm^{-1} while *C. serrulata* (roots and rhizomes) contained 1099, 1416, 1606, 2853, 2925 and 3293cm^{-1} and *S. isoetifolium* (whole) showed at 1078, 1396, 1594, 2850, 2925 and 3320cm^{-1} (Table 5.2.5) corresponding to absorptions of amides, aromatics, carboxylic acids, esters, alcohols and phenols functional groups (Table 5.2.6). Major absorptions at UV region were at 220-280nm, 270-290nm and 310-331nm for phenolic compounds, phenolic acid and flavonoid derivatives respectively whereas

visible region contained absorptions at 401-411 and 661-671nm. No wide variation in absorption maxima was obtained between *C. serrulata* (roots and rhizomes) and *S. isoetifolium* in the UV region while in visible region, the absorption maxima of *S. isoetifolium* predominated over *C. serrulata* (Figure 5.2.4, 5.2.5 and 5.2.6).

Table 5.2.1 Antioxidant activities of Seagrasses

SI. No.	Sample name	DPPH Activity (%)	Reducing Power Assay mg/g *	Total Antioxidant Activity mg/g**
1	*C. serrulata* (whole)	56 ± 0.45	38 ± 0.65	48 ± 0.55
2	*C. serrulata* (roots &rhizomes)	48 ± 0.35	65 ± 0.81	217 ± 2.52
3	*S. isoetifolium* (whole)	56 ± 0.30	168 ± 1.53	210 ± 1.53

* Gallic acid equivalent and **Ascorbic acid equivalent

DPPH activity exhibited extract concentrations were at 3.38, 2.56 and 1.42mg respectively for *C. serrulata* (whole), *C. serrulata* (roots &rhizomes) and *S. isoetifolium* (whole).

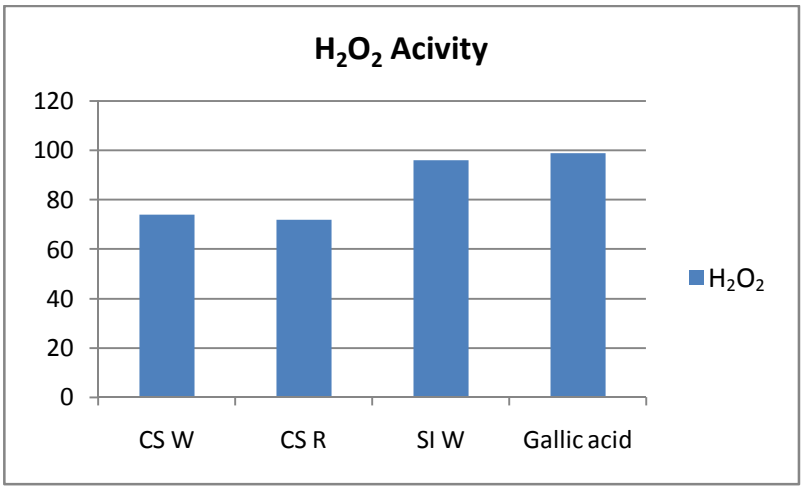

Figure 5.2.1 Hydrogen peroxide radical activity

Gallic acid concentration - 200µg, *C. serrulata* (whole) - 338µg, *C. serrulata* (roots & rhizomes) - 511µg and *S. isoetifolium* (whole) - 282µg

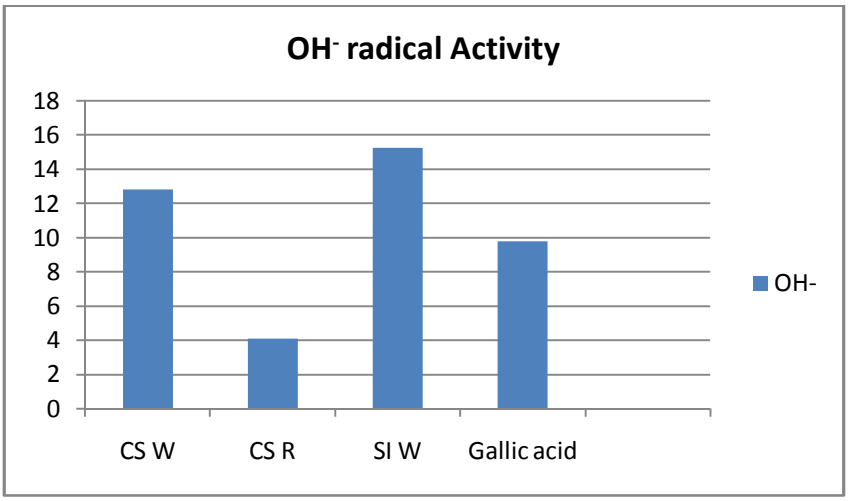

Figure 5.2.2 Hydroxy radical activity

Gallic acid concentration - 40µg, *C. serrulata* (whole) -169 µg, *C. serrulata* (roots & rhizomes) - 255µg and *S. isoetifolium* (whole) - 70µg

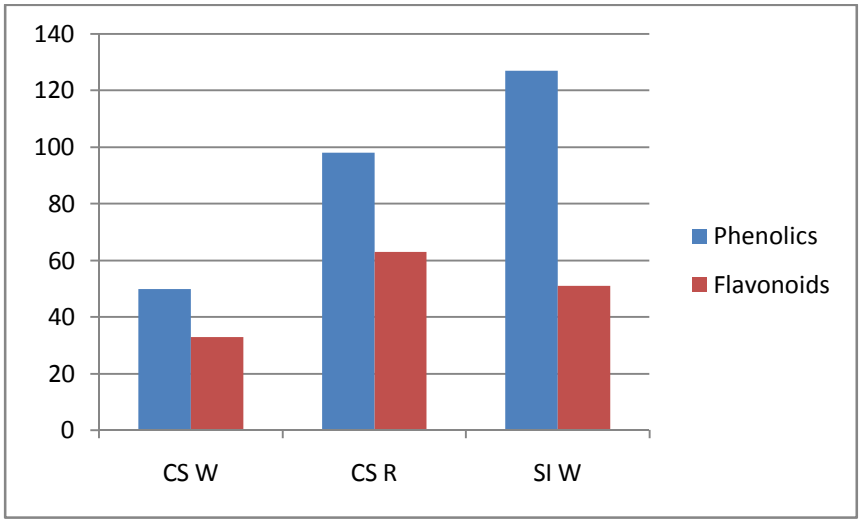

Figure 5.2.3 Total phenolics and flavonoids in seagrasses

Table 5.2.2 Antimicrobial activity of seagrasses

Sl. No.	Pathogens	*C. serrulata* (Whole)	*C. serrulata* (Roots and rhizomes)	*S. isoetifolium* (Whole)
		Zone of inhibition in mm		
1	*Pseudomonas aeruginosa*	2.7±0.58	1.7 ±0.58	3.3±0.58
2	*Staphylococcus aureus*	1.7 ±0.58	Resistant	2.3±0.58
3	*Escherichia coli*	Resistant	Resistant	Resistant
4	*Enterococcus faecalis*	Resistant	Resistant	Resistant
5	*Yersinea enterocolifica*	Resistant	Resistant	Resistant

Sample concentrations were 0.338, 0.511 and 0.141µg respectively for *C. serrulata* (whole), *C. serrulata* (roots and rhizomes) and *S. isoetifolium* (whole) respectively.

Table 5.2.3 Phytochemicals in seagrasses

Sl.No	Sample name	Phenols	Flavonoids	Tannins	Sterols	Saponins	Alkaloids	Terpenoids	Reducing sugars	Cardiac glycosides	Anthraq-uinone
1	C. S (W)	+	+	+	+	-	+	+	+	-	-
2	C. S (R)	+	+	+	+	-	+	+	+	-	-
3	S. I (W)	+	+	+	+	-	+	+	+	-	-

Table 5.2.4 Major components in GC MS analysis in % of total components

SI. No.	Compounds name	C. S (Whole)	C. S (Roots & rhizomes)	S. I. (Whole)
1	3,7,11,15-Tertamethyl-2-hexadecen-1-ol	0.89±0.09	-	0.75±0.08
2	Butanoic acid 3-methyl-3,7-dimethyl-6-octenyl ester	-	-	0.54±0.04
3	Tetradecanoic acid-10,13-dimethyl methyl ester	17.34±0.88	12.13±0.49	16.03±0.81
4	Octadecanoic acid	1.57±0.12	1.52±0.11	35.13±0.98
5	Methyl 11-Hexadecanoate	-	-	2.65±0.20
6	Methyl 11,14,17-Eicosatrienoate	13.80±0.62	12.95±0.54	24.42±0.89
7	Phytol	5.35±0.42	-	7.17±0.61
8	Heptacosanoic acid-25-methyl methyl ester	0.29±0.06	4.60±0.31	0.21±0.04
9	Methyl tricontanoate	-	-	0.22±0.04
10	Methyl octasanoate	-	-	0.38±0.06
11	13-Docosenamide	-	-	0.64±0.09
12	Ergost-5-en-3 ol (3 beta)	-	-	1.52±0.16
13	Ergost-5, 22-en-3 ol-(3 beta, 22E)	-	-	5.75±0.32
14	Gama sterol	4.50±0.34	-	3.13±0.28
15	4,22-Stigmastadiene-3-one	2.37±0.28	-	0.87±0.08
16	Stigma sterol	0.84±0.10	0.50±0.06	0.29±0.05
17	Vitamin A aldehyde	-	-	0.29±0.08
18	n-Hexadecanoic acid	10.16±0.55	5.21±0.38	-
19	Methyl 12,15-Octadiecanoate	20.19±0.90	-	-
20	Methyl 16- methyl Heptadecanoate	12.81±0.66	-	-
21	9,12- Octadecanoic acid (Z,Z)	3.20±0.31	-	-
22	1-Propyl-9,12-Octadecadienoate	2.27±0.28	-	-
23	Trans-2-methyl-4-pentythiane	3.31±0.34	-	-
24	Hop-22(29)-en-3-beta-ol	0.28±0.04	-	-
25	Stigmast-4-en-3-one	0.80±0.10	-	-
26	Furancarboxylaldehyde,5-(hydroxyl methyl)	-	24.67±0.89	-
27	Methyl-9-cis,11-trans Octadecadienoate	-	31.06±0.96	-
28	n-Propyl-11- Octadecenoate	-	2.36±0.18	-
29	Ethyl 9,12-Hexadienoate	-	4.99±0.36	-

Table 5.2.5 FTIR Absorption peaks of seagrasses in cm^{-1}

SI.No.	C. S (W)	C. S (R)	S. I. (W)
1	1020	1045	-
2	1062	1099	1078
2	-	1201	-
4	-	1254	1396
5	1405	1416	1594
6	1606	1606	1982
7	-	1995	2094
8	2033	2018	2158
9	2133	2138	2175
10	-	2853	2850
11	2925	2925	2925
12	3296	3293	3320

Table 5.2.6 FTIR functional groups comparison

SI No.	Origin	Range of wave number cm^{-1}	Assignment
1	O-H	3570-3200 (braoad)	Hydroxy group, H- bonded OH stretch
2	O-H	1410-1310	Phenol or tertiary alcohol, OH bend
3	O-H	1350-1260	Primary or secondary, OH in plane bend
4	C-O	~ 1050	Primary alcohol, C-O stretch
5	C-O	~ 1100	Secondary alcohol, C-O stretch
6	C-O	~ 1200	Phenol C-O stretch
7	$\diagdown\!\!\diagup CH_2$	2935-2915/ 2865-2845	Methylene C-H asym./sym. stretch
8	C=C-C	1615-1580	Aromatic ring stretch
9	C=O	1610-1550	Carboxylate (carboxylic acid salt)
10		1420-1370	Organic sulfates
11	Combination	2000-1660	Aromatic ring combination bands

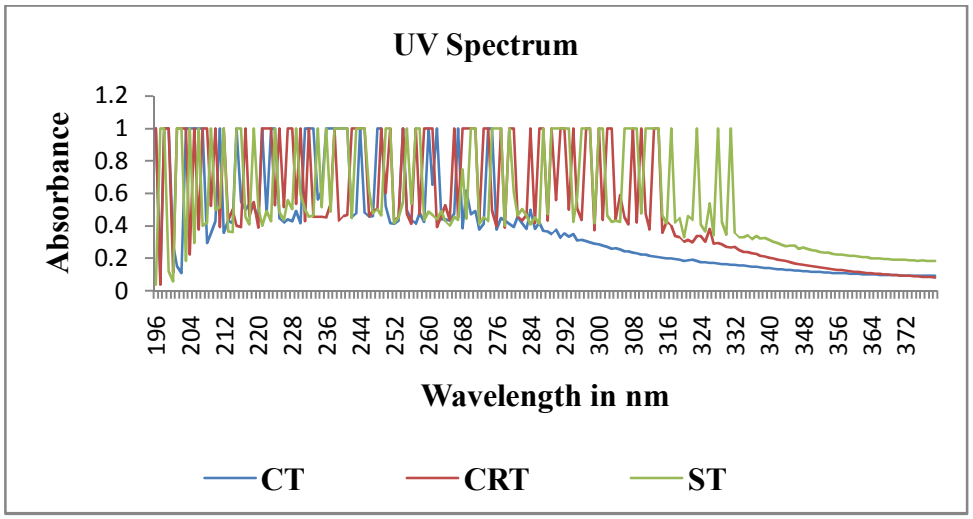

Figure 5.2.4 Visible Region (380-430 nm)

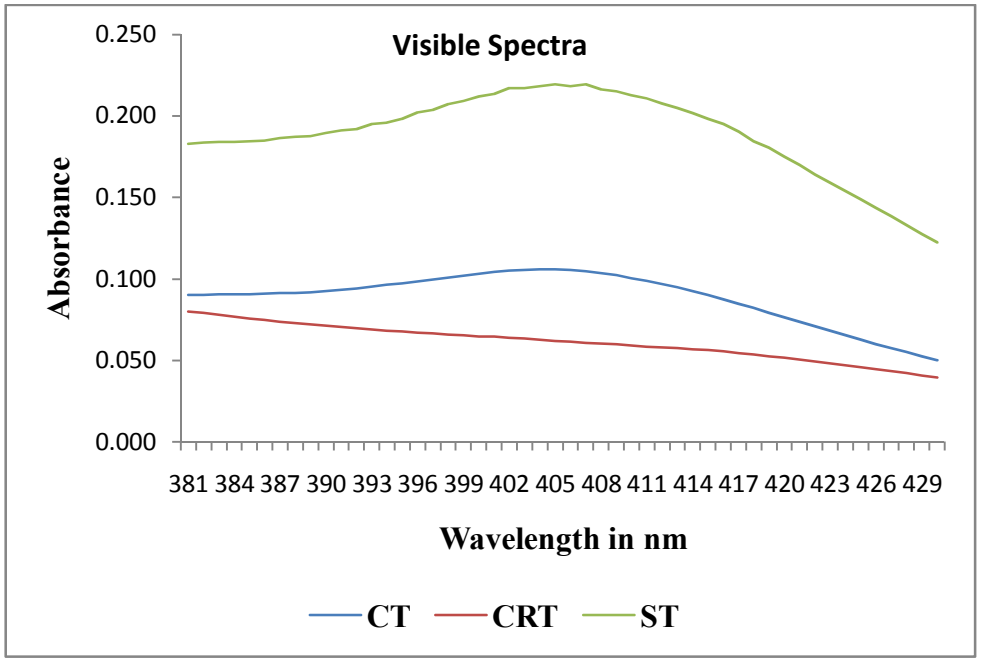

Figure 5.2.5 Visible Region (640-680nm)

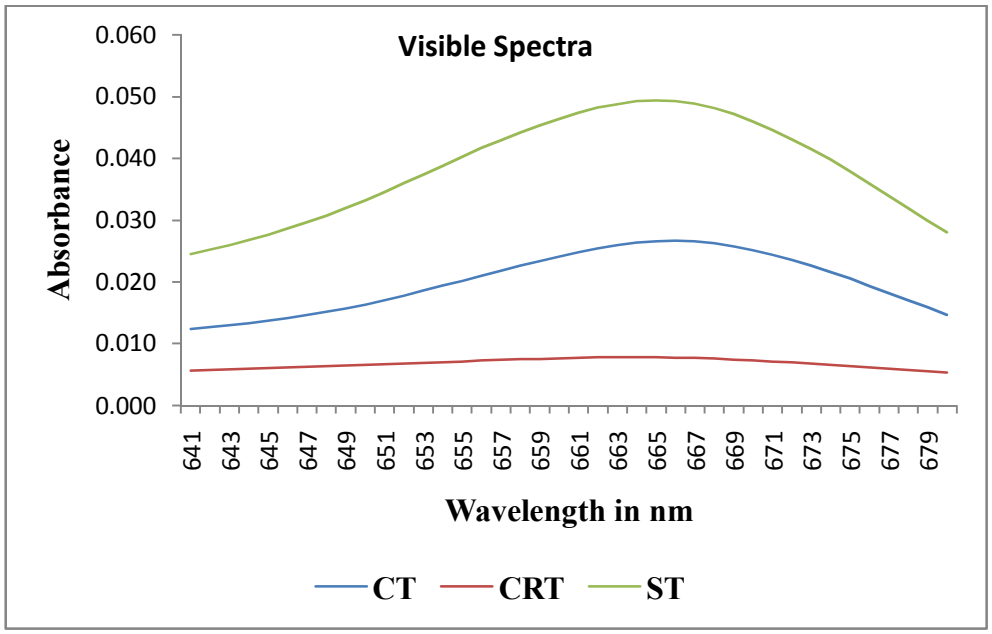

Figure 5.2.6 Visible Region (640-680nm)

5.3 Discussion

5.3.1 Antioxidant activities (DPPH, H_2O_2, OH^-, FRAP and TAA)

DPPH free radical scavenging activity

In the present study, both the species *C. serrulata* and *S. isoetifolium* (whole) exhibited good activity (56%) and it was comparable while medium activity (48%) was obtained in *C. serrulata* (roots and rhizomes). Greater than 50% DPPH activity was mentioned as good activity (Indu and Sreenivasan, 2013). Based on the antioxidant constituents in seagrassses, relatively higher antioxidant activity was obtained in *H. pinifolia* followed by *T. hemprichii*, *E. acoroides* and *S. isoetifolium* (Kannan et al., 2010c). DPPH free radical activity data provides information on the capability of reducing substances to accept an electron or hydrogen radical to form a

stable diamagnetic molecule in different matrices. Higher DPPH scavenging activity was reported in *H. pinifolia* (68.066%) and lower in *H. ovata* (16.926%) (Rengasamy et al., 2012). Methanolic extracts of seaweeds showed activity higher than hexane and ethyl acetate extracts, and DPPH free radical activities were accounted by the presence of phytochemicals present in the extract (Chejara et al., 2014). DPPH free radical activity was widely used to determine the reducing substances in different matrices and this stable free radical accept an electron or hydrogen radical to form a stable diamagnetic molecule.

DPPH scavenging radical activity of *H. uninervis* extract was increased with an increase in the concentration of the extract (Baehaki et al., 2016) and *S. isoetifolium* was 50 and 45% respectively for rhizomes and leaves (Jeyapragash et al., 2016). Crude methanol as well as sub fractioned solvents of *Z. marina* showed maximum DPPH free radical scavenging activity (Choi et al., 2009) and these results were much higher than the present study. Among the seagrasses, comparatively higher DPPH radical scavenging activity obtained for methanol extracts of *H. pinifolia* (87.81%), *S. isoetifolium* (83.03%) and *H. ovalis* (82.55%) whereas the acetone extraction of the same showed higher activities for *H. ovalis* (73.55%) over *H. pinifolia* and *S. isoetifolium*. The activity was greater than that of the seagrasses of Gulf of Mannar and Palk Bay of the present study. DPPH radical scavenging activity was positively correlated with phenol content (Choi et al., 2009) and was attributed to the redox potentials of polyphenols; their ability to absorb and neutralize the free radical, quenching singlet oxygen and decomposition of peroxides (Girija et al., 2013). In this study seagrass extract concentrations were higher than the earlier reports (Kannan

et al., 2013c) and the concentrations were 3.3, 2.5 and 1.4mg/ml respectively for *C. serrulata* (whole), *C. serrulata* (roots and rhizomes) and *S. isoetifolium* (whole). DPPH radical scavenging activity of a fucoidan like sulphated polysaccharide crude extract of *H. pinifolia* revealed a maximum of 90.94% at a concentration of 1mg/ml (Kannan et al., 2013c).

DPPH radical scavenging activity of seagrasses from Chinnapalam revealed that activity varied from *E. acoroides* (35.80%) to *C. rotundata* (70.30%). In comparison to the present study, *S. isoetifolium* was almost same while *C. serrulata* was lower (Kannan et al., 2013a). *C. serrulata* and its silver nano particles revealed that DPPH free radical scavenging activity of silver nano particles (87.99%) was higher than *C. serrulata* aqueous extract (65.68%) at a concentration of 100µg/ml (Chanthini et al., 2015) and it was much higher than the present study. DPPH free radical scavenging activity of four seagrassses of Indonesia expressed by IC_{50} values (the concentration of the substrate to reduce the DPPH activity to 50%) showed highest activity for *S. isoetifolium* (ethyl acetate) followed by methanol extracts and lowest activity noticed in the hexane extract, and showed a positive relationship with the phenol content (Santoso et al., 2012). In the present study, no wide variations were observed in the level of DPPH activity between leaves as well as roots and rhizomes. Leaf, root and rhizome ethanol extracts of *E. acoroides* revealed that comparatively higher DPPH activity exhibited by leaf followed by root and rhizome, no wide variations in activity between roots and rhizomes were observed (Kannan et al., 2010b). Apigenin-7-0-b-D-glucoside, Chrysoerial and Luteolin were isolated from *Z. marina* and the IC_{50} values of Luteolin were much higher than others and also higher than standards of BHA, vitamin E and vitamin

C, probably due to suppression of the effect of matrix metalloproteinase -1 (Kim et al., 2004). Wide variations were observed in DPPH radical scavenging activity in seaweeds of Gulf of Mannar (Meenakshi et al., 2012). Comparatively higher DPPH activity was noticed in seaweeds of Pondicherry and Mandapam coast (Kokilam and Vasuki, 2014).

Ferric reducing antioxidant power (FRAP) assay

The reducing power assay varied from 38 (*C. serrulata*) to 168mg gallic acid/g (*S. isoetifolium*) and these values were much higher than other studies carried out (Kannan et al., 2010b; Rengasamy et al., 2012; Girija et al., 2013; Kannan et al., 2013a; Baehaki et al., 2016; Jeyapragash et al., 2016). FRAP assay in seagrasses of Mandapam showed higher activity in *H. stipulacea* (46.289mg gallic acid/g) and lower in *E. acoroides* (3.373mg gallic acid/g). A positive correlation was not observed with FRAP assay and phenolic content except *H. stipulacea* (Rengasamy et al., 2012). The non polar fractions of ethyl acetate and n-butanol showed relatively higher reducing power than crude methanol extract as well as other extracts, and relatively lower reducing power assay found in aqueous extract. The reducing power assay was much higher in the present study similar to Choi et al. (2009). Reducing power assay was showed a higher activity for seagrasses from Tuticorin following the order; *H. pinifolia*>*H. ovalis*> *S. isoetifolium* with methanol extraction predominated over rest of them (Girija et al., 2013). In *H. pinifolia* reducing power of crude sulphated polysaccharides increased with increasing concentration (Kannan et al., 2013c). An increase in reducing power with increased concentrations of extracts was noticed in seagrasses of Chinnappalm and among the six seagrasses, *C. rotundata* dominated over other species (Kannan et al.,

2013a). FRAP assay in different body parts of *E. acoroides* ethanol extract followed the order: leaf>root>rhizome (Kannan et al., 2010b). In the present study, species to species variations in FRAP assay was observed and it was in line with seaweeds of Gulf of Mannar (Meenakshi et al., 2012). The activity of low-therified pectin extracted from *Z. marina* were higher compared to medicines of Emoxipin and Mildronat while during inhibition study displayed relatively higher inhibitory capacity towards Mildronant, and both pectin and Emoxipin exhibited comparable inhibitory capacity (Kolechenko et al., 2005).

Total reducing power in seagrasses of *S. isoetifolium* from Palk Bay revealed that maximum reducing capacity noticed in rhizomes than leaves (Jeyapragash et al., 2016) and *H. uninervis* activity increased with an increase in the concentration of the extract (Baehaki et al., 2016). Higher FRAP assay was noticed in bark extracts of mangroves and a positive correlation was observed with total phenolic content. Among the phytochemicals, polyphenols have the capacity to act as a reductone (Krishnamoorthy et al., 2011). The total antioxidant activity of an extract is based on single electron transfer (SET) and hydrogen atom transfer mechanisms. The reducing potential depends on the plant species and also the medium of extraction. Different extracts of seaweeds showed that ethyl acetate had more activity than methanol and hexane in *B. plumosa* and *D. australis* while *G. pudumadamensis* followed the order methanol>ethyl acetate>hexane. The antioxidant activity of phenolics depends on competitive functions of other phytochemicals which could be responsible for the non linear relationship with phenolic content (Chejara et al., 2014). The reducing power in brown seaweeds from Gulf of Mannar was

comparable and a positive correlation was observed with phenolic content (Kayalvizhi et al., 2014).

Hydroxy radical activity

In the present study, the hydroxyl group activities in the seagrass extracts were much lower than other studies carried out but marginally higher than the standard gallic acid used (200µg/ml). Among the free radicals, hydroxyl radical was the most reactive radical and have the ability to damage various cells which leads to carcinogenesis, mutagenesis and cytotoxicity (Krishnamoorthy et al., 2011). The crude sulphated polysaccharide effect on scavenging hydroxyl radicals showed a maximum activity of 52% at a concentration of 1mg/ml (Kannan et al., 2013c) which was much higher than the present study. Scavenging effect of hydroxyl radicals in six seagrass extracts of Chinnapalam revealed that the percentage inhibition was high at *C. rotundata* and lowest at *T. hemprichii* (Kannan et al., 2013a).

Hydrogen peroxide radical scavenging (H_2O_2) activity

The hydrogen peroxide activity of *S. isoetifolium* was much higher in the present study compared to previous reports (Athiperumalsami et al., 2010; Girija et al., 2013; Kannan et al., 2013a; Jeyapragash et al., 2016). Antioxidant activity of seagrasses and seaweeds of Gulf of Mannar revealed greater H_2O_2 scavenging antioxidant activity towards methanolic extracts than water extracts (Athiperumalsami et al., 2010). Comparatively higher hydrogen peroxide activity was observed in *H. pinifolia* over *H. ovalis* and *S. isoetifolium* in the hexane extract followed by methanol and acetone. The activity for methanol extract also followed in *H. ovalis* in the same order as that of *H. pinifolia* while *S. isoetifolium* displayed more activity (Girija et

al., 2013). An increase in the concentration of H_2O_2 was observed in seagrass of *P. oceanica* with an epiphyte *L. Lallemandii* than *P. oceanica* alone (Sureda et al., 2008). Hydrogen peroxide activity of *S. isoetifolium* in the present study was comparable whereas lower in *C. serrulata* than the study carried out by Kannan et al. (2013c). Hydrogen peroxide activity of crude sulphated polysaccharide reached nearly 100% at a concentration of 250µg/ml (Kannan et al., 2013c). The study also indicated lower activities for *C. serrulata* while *S. isoetifolium* demonstrated almost same in activity comparison with the earlier study of Kannan et al. (2013a). Aqueous methanol extracts of six seagrasses of Chinnappalam showed relatively higher hydrogen peroxide activity for the species *C. rotundata* and lower at *E. acoroides* (Kannan et al., 2013a). Hydrogen peroxide activity in *S. isoetifolium* was 38 and 42% respectively for leaves and rhizomes (Jeyapragash et al., 2016).

Total Antioxidant Activity (TAA)

The TAA among the species studied shows higher activities (210mg/g) for *S. isoetifolium* (whole) and 48mg/g for *C. serrulata* (whole). However, *C. serrulata* (roots and rhizomes) demonstrated higher or equivalent activity (217mg/g) than *S. isoetifolium* (whole) (Table 5.1). The activity was very much higher in the present study compared with similar works carried out (Rengasamy et al., 2012; Girija et al., 2013; Kannan et al., 2013a; Jeyapragash et al., 2016). Total antioxidant activity varied from *E. acoroides* (8.43) to *H. pinifolia* (132.38mgAE/g) (Rengasamy et al., 2012). Variations were observed between species to species as well as differences in solvent polarity. Total antioxidant activity was higher in methanol and acetone extracts of *H. pinifolia*; while reverse order noted in *S. isoetifolium*.

The minimum activity was exhibited by *H. ovalis* in both the extracts (Girija et al., 2013). Total antioxidant activity in seagrasses of Palk Bay varied from 2.54 to 12.83mgAE/g and 2.20 to 17.63mgAE/g in leaves and rhizomes respectively (Jeyapragash et al., 2016).

TAA in aqueous methanol extracts of six seagrasses collected from Chinnapalam ranged from 3.19 (*T. hemprichii*) to 15.75mgAE/g (*H. pinifolia*) (Kannan et al., 2013a). TAA of the methanol extracts of *Phillanthus* species ranged from 245 to 376mgAE/g (Kumaran and Karunakaran, 2007) with higher activity noticed in seaweeds of *S. wightii* (123mgAE/g) (Meenakshi et al., 2012). Total antioxidant activity in seaweeds was much lower than that of seagrasses of this study (Kokilam and Vasuki, 2014). Antioxidant properties of brown seaweeds of Gulf of Mannar revealed a strong correlation between total antioxidant activity and phenolic content, and it is attributed to their capacity to act as reducing agents, hydrogen donors and free radical quenchers (Kayalvizhi et al., 2014).

5.3.2 Antimicrobial Activity

The methanol extracts demonstrated medium to higher activities against five important fish pathogens (Table 5.3.1). *Z. marina*, *H. stipulacea*, *C. nodosa* and *R. cirrhosa* exhibited activity at higher concentrations (extracts) than the present study (Abd El-Hady et al., 2007; Choi et al., 2009; Abd El-Hady et al., 2012). Antibacterial activities of *C. serrulata* and *S. isoetifolium* indicated the high efficiency of methanol extracts in this study (Arumugam et al., 2010; Kannan et al., 2012; Mani et al., 2012a; Kannan et al., 2013b; Iyapparaj et al., 2014). Antibacterial activity of human and fish pathogens

revealed more activity towards acetone than methanol extract of *S. isoetifolium* (Mani et al., 2012a). *C. rotundata* extracts were tested against 10 human pathogens and activity of the extracts followed the order: methanol>ethanol and acetone>butanol, and no activity towards aqueous extract. The differences in efficiency are related to differences in the cell wall structure of bacteria and distribution of active components, and the potency which varied from species to species (Mani et al., 2012b). Phytopathogenicity of extracts of *H. stipulacea* and *Halodule pinifolia* showed high activity against all pathogens in comparison with *C. serrulata*. The relatively lower activity of hexane extracts might be due to pathogenic strains responsible for some kind of resistance mechanisms such as enzymatic inactivation, target site modification and decrease intracellular drug accumulation (Arumugam et al., 2010) or to the chemistry of the active principle in hexane.

Variation in antibiotic activities depend upon the solvents used for extraction, condition or state of the sample, season in which samples were collected, age of the plant, duration of storage, temperature, preparation of media and pH (Umamaheshwari et al., 2009; Arumugam et al., 2010). Crude aqueous ethanol extracts of roots of *C. serrulata* and its sub fractions were tested against bacterial fish pathogens and comparatively more activity was detected in hexane fraction against *Vibrio parahaemolyticus* and *Bacillus subtilis* (Ravikumar et al., 2011a). *C. nodosa* and *R. cirrhosa* showed relatively more antifungal activity towards the extracts of *C. nodosa* while bacteria showed more inhibition towards *R. cirrhosa*. Activity increased with increasing concentration of secondary metabolites (Abd El- Hady et al., 2007). *H. stipulacea* aqueous extract showed antimicrobial activity against

B. subtilis, *Candida albicans* and *Aspergillus niger* (gram positive) whereas no activity towards gram negative bacteria of *E. Coli* and *P. aeruginosa* (Abd El-Hady et al., 2012). Comparison between *C. serrulata* and *S. isoetifolium* observed that *S. isoetifolium* was more active than *C. serrulata* in the present study. Antibacterial activity of seagrasses aqueous methanol extracts against urinary tract pathogens noticed that seagrasses were resistant towards all the pathogens, and relatively higher activity found in *H. pinifolia* and *C. rotundata*, and zone inhibition ranged from 10.3 to 14.3mm and 9.7 to 12mm respectively (Kannan et al., 2012).

Crude methanol extract of *Z. marina* and its different partitioned solvents were treated against three human skin pathogens, and demonstrated a minimum inhibitory concentration (MIC) was 1mg/ml found in the ethyl acetate (*S. aureus*), n-butanol (*C. albicans*) 4mg/ml towards chloroform (*C. albicans*) and no inhibition obtained in the aqueous fraction (Choi et al., 2009). Five human pathogens were tested against aqueous methanolic extracts of seagrasses and *S. aureus* pathogen noticed maximum zone of inhibition in *H. pinifolia* and lower inhibition obtained in *S. isoetifolium* and *C. serrulata* (Kannan et al., 2013b). Aqueous ethanol extracts of *C. serrulata* root were fractionated and acetone fraction showed activity against all poultry pathogens except *Salmonella sp*. and no activity was observed in other solvents (except first fraction) (Ravikumar et al., 2011b). Strongest antibacterial activity of *P. oceanica* was observed in the cyclohexane extract and it showed excellent inhibition against all tested organisms except *P. mirabilis* while no activity observed in the ethyl acetate extract (Berfad and Alnour, 2014).

Table 5.3.1 Comparison of antimicrobial activity of seagrasses

Species name	Conce-ntration	Zone of inhibition in mm					References
		P. aerug-inosa	*S. aureus*	*E.coli*	*E. fae-calis*	*Y. enter-ocolifica*	
H. pinifolia (Methanol) *H. ovalis*	-	18 NA	N A NA	-	-	-	Umamaheswari et al., 2009
C. serrulata (Acetone) *C. serrulata* (Hexane)	1 mg	-	7-12	7-8	-	-	Ravikumar et al., 2011
C. rotundata (Methanol)	50 µl	-	3-4	5-7	-	-	Mani et al., 2012 b
S. isoetifolium (Methanol) (Acetone)	50 µl	 -	1 4	NA 1	NA NA	 -	Mani et al., 2012 a
H. pinifolia (Methanol) *H. stipulacea* *C. serrulata* *C. serrulata* (Hexane)	5 mg/ml	12-13 4-5 0-1 1-2	-	-	-	-	Arumugam et al., 2008
H. stipulacea (Aqueous)	-	NA	-	NA	-	-	Abd El-Hady et al., 2012
C. serrulata (Aqueous Methanol) *S. isoetifolium*	50 µg	6.3 R	-	R R	-	-	Kannan et al., 2012
Z. marina (Methanol)	MIC	8 mg/ml	-	-	-	-	Choi et al., 2009
C. nodosa (Ethanol) *R. cirrhosa*	10 mg	10 11	11.5 10	NA 19.5	-	-	Abd El-Hady et al., 2007
C. serrulata (Methanol) *S. isoetifolium*	MIC	1 mg/ml 10 mg/ml	-	-	-	-	Iyapparaj et al., 2014
C. serrulata (Methanol) *S. isoetifolium*	10 mg/ml	-	2-3 3-4	-	-	-	Kannan et al., 2013
P. oceanica (Ethanol)	-	9	8	NA	-	-	Berfad and Alnour, 2014
Halodule sp. (Methanol) *H. ovalis*	10 µg	NA	NA	NA	-	-	Devi et al., 2011
H. pinifolia (Methanol) *H. stipulacea* *C. serrulata*	5mg/ml	13 7 1	- - -	- - -	- - -	- - -	Arumugam et al., 2010
C. serrulata *H. pinifolia* *H. ovalis*	1mg/ml	NA NA NA	NA NA NA	7.33-9 8-9 7-7.6	NA NA NA	- - -	Sangeetha and Asokan, 2016
C. serrulata (W) (Methanol)	0.338 mg	2-3	1-2	R	R	R	Present study
C. serrulata (R & R) (Methanol)	0.511 mg	1-2	R	R	R	R	Present study
S. isoetifolium(W) (Methanol)	0.141 mg	2-3	1-2	R	R	R	Present study

Methanol and hexane extracts of *Halodule* sp. and *H. ovalis* showed antifungal activity with more activity found in *Halodule* sp. (Devi et al., 2011). Similarly, crude methanol and hexane extracts of marine algae and *E. acoroides* exhibited higher activity for gram positive bacteria (Alam et al., 1994). Crude organic extracts are complex mixtures of primary and secondary metabolites including fatty acids which are responsible for antimicrobial activities, and further altering in the composition of the crude extract may result in false negative or positive results (Engel et al., 2006).

This might be the reason for relatively lower zone of inhibition in the extracts having different chemical constituents in the present study. Ecological studies showed that antimicrobial metabolites may target most of the marine organisms and they might not be active against human pathogens. The inhibited growth of all microbes in assays noted more lipophilic than hydrophilic extracts, and lipophilic secondary metabolites playing important role in defending host tissues from harmful organisms (Engel et al., 2006). In the present study, *C. serrulata* (whole) contained comparatively higher activity than roots and rhizomes. An aqueous ethanolic extract of *S. isoetifolium* (root) showed higher larvicidal activity at lower concentrations followed by *S. isoetifolium* (leaf) and *C. serrulata* (root), and no activity was observed in *H. beccarii* and *C. serrulata* (leaf) (Ali et al., 2012).

Methanol extracts of *C. serrulata* and *S. isoetifolium* showed better antibacterial activity than other solvents of dichloromethane and acetone, and an excellent antimicrobial activity was observed in *C. serrulata* at a MIC of 1mg/ml and 10mg/ml in *S .isoetifolium*. Also methanolic extracts of

C. serrulata and *S. isoetifolium* exhibited 0% fouling (100% inhibition) at 6mg/ml (Iyapparaj et al., 2014). Antifouling potential tested against 37 microorganisms revealed that jelly fish, soft coral, seagrass (*C. rotundata*) and brown algae showed inhibition against bacterial strains at different degrees (Bhosale et al., 2002). Anti-inflammatory activity was found in the hexane fraction and has the highest capacity to inhibit proIL-1â expression as compared to other fractions in lipopolysaccharides (LPS)-stimulated J774A.1 murine macrophages (Hua et al., 2006). Among the three extracts of *C. serrulata*, higher antimicrobial activity exhibited in ethyl acetate followed by chloroform and ethanol and no activity towards hexane. Pathogens of *P. aeruginosa*, *E. faecalis* and *S. aureus* were not inhibited by any of the extracts (Sangeetha and Asokan, 2016). Antimicrobial activity of *H. uninervis* exhibited inhibition against both gram positive and negative bacteria and highest inhibitory activity against gram negative bacteria (Supriadi et al., 2016).

5.3.3 Phytochemicals, Phenolics and Flavonoids

Phytochemicals present in the methanol extracts of *S. isoetifolium* and *C. serrulata* were relatively lower compared with other studies carried out (Athiperumalsami et al., 2008; Kannan et al., 2010a; Girija et al., 2013; Kannan et al., 2013a;). But a total of 15 phytoconstituents were in seagrass of *S. isoetifolium* from Gulf of Mannar and relatively higher numbers were obtained in methanol extract than acetone (Mani et al., 2012a). Athiperumalsami et al. (2008) reported that phytochemicals present in the extracts were the leading factor for antioxidant activity. Kannan et al. (2010c) reported the presence of antioxidant constituents of four seagrasses (*H. pinifolia*, *T. hemprichii*, *E. acoroides* and *S. isoetifolium*) and relatively lower

antioxidants were reported in *S. isoetifolium*. *C. rotundata* contained phytochemicals in all the extracts but limited in numbers (Mani et al., 2012b). Higher numbers of phytoconstituents detected in *C. serrulata* than other species (Kannan et al., 2010a). Comparatively more number of phytochemicals were detected in this study than other studies carried out (Lakshmanan and Dhanalakshmi, 1988; Kannan et al., 2010c; Mani et al., 2012b; Baehaki et al., 2016; Hardoko et al., 2016; Sangeetha and Asokan, 2016).The chemical constituents present in plants might be different at different place and substrate, water and other physical as well as physiological factors affect its composition. Saponins, alkaloids, steroids and terpenes were present in all the species studied whereas flavonoids occured only in *H. ovalis* and *T. hemprichii* while anthroquinone heterosides were absent in all the samples. Marine plants have the ability to adopt inorganic salts and they undergo conjugation with phenolic compounds, particularly flavonoids (Lakshmanan and Dhanalakshmi, 1988).

C. serrulata contained more number of phtochemicals in ethyl acetate extract and no differences were obtained between ethanol and aqueous (Sangeetha and Asokan, 2016), and the methanol extract of *H. uninervis* revealed the presence of flavonoids, alkaloids and steroids (Baehaki et al., 2016). Phytochemicals present in the fresh leaves of *C. serrulata* were alkaloids, terpenoids, polyphenols and flavonoids (Hardoko et al., 2016). An aqueous methanolic extracts of six seagrasses collected from Gulf of Mannar recorded 10 phytochemicals, and alkaloids and glycosides were absent in all the seagrasses (Kannan et al., 2013a). Seagrasses of *H. pinifolia* and *H. ovalis* were reported to contain more phytochemicals (Girija et al., 2013) than the present study while the phytochemicals in *S. isoetifolium*

were comparable. Phytoconstituents present in six seagrasses were phenols, flavonoids, tannins, vitamin C and vitamin E (Kannan et al., 2013b; Athiperumalsami et al., 2010). Phenols and alkaloids occurred only in the ethanol extract of *P. oceanica* whereas saponins found only in acetone (Berfad and Alnour, 2014). In this study, no differences were obtained in the number of phytochemicals between species as well as body parts considered. The aqueous methanol extracts of three seagrasses revealed comparatively higher numbers of phytochemicals in the roots than leaves and *S. isoetifolium* predominated over *C. serrulata* and *H. beccarii* (Ali et al., 2012).

Total phenolics

In the present study, phenolics were at much higher levels recorded in both species as well as different body parts in comparison with earlier reports (Table 5.3.2) (Gulf of Mannar as well as Worldwide). Relatively higher concentrations of phenolics reported in the ethyl acetate fraction (Choi et al., 2009), and total phenol contents were higher during summer preferably due to environmental pressures of turbidity, metal contamination, pollution, ocean acidification, competition with invasive seaweed, infection by *Labyrinthula*, etc, (Rotini et al., 2013). A comparison between leaves and rhizomes showed that rhizomes have long life time and less changes in the physiological process and hence, lower phenolic content in rhizomes (Migliore et al., 2007). Phenol content in the leaves as well as rhizomes of seagrasses taken from Palk Bay contained relatively lower concentrations in *H. beccarii* and higher in *E. acoroides* (Pradheeba et al., 2011). Higher phenol contents in leaves may be due to the defensive mechanisms of seagrass leaves against epiphytes because they are more exposed than rhizomes (Pradheeba et al., 2011). *C. serrulata* of Gulf of Mannar observed

higher phenol content in roots and rhizomes than leaves, and it was similar to terrestrial plants as well as angiosperms found to exhibit higher phenol content in rhizomes than leaves due to longer life span (Pradheeba et al., 2011).

Table 5.3.2 Comparison of phenolic content

Seagrass Species	Solvent used for extraction	Phenol Content in mg/g GAE	Location	References
Z. marina	Methanol	204.63	Korea	Choi et al., 2009
Z. marina	Ethyl acetate fraction	968.50	Korea	Choi et al., 2009
P. oceanica	-----	22.31-27.62 (FW)	Italy	Rotini et al., 2013
C. serrulata (leaves)* *S. isoetifolium*	-----	6.5 6.0	Palk Bay	Pradheeba et al., 2011
C. serrulata (rhizome)* *S. isoetifolium*	----	6.0 5.5	Palk Bay	Pradheeba et al., 2011
S. isoetifolium	Methanol	0.58	Tuticorin	Girija et al., 2013
S .isoetifolium	Methanol	0.94	Indonesia	Santoso et al., 2012
C. rotundata	Methanol	3.35	Indonesia	Santoso et al., 2012
C. serrulata (leaves)* *S. isoetifolium*		0.226 0.209	Gulf of Mannar	Athiperumalsami et al., 2008
C. serrulata (leaves)* *S. isoetifolium*	Aqueous methanol	13.27 3.94	Chinnapalm	Kannan et al., 2013 a
S. isoetifolium *	Aqueous methanol	0.4	Mandapam	Kannan et al., 2010 a
E. acoroides leaf *	Ethanol	0.378	Chinnapalm	Kannan et al., 2010 b
E. acoroides root *	Ethanol	0.301	Chinnapalm	Kannan et al., 2010 b
E. acoroides rhizome *	Ethanol	0.121	Chinnapalm	Kannan et al., 2010 b
C. serrulata (leaves) *S. isoetifolium*	Ethanol	0.545 0.463	Mandapam	Rengasamy et al., 2012
C. serrulata (W)	Methanol	50	Gulf of Mannar	Present study
C. serrulata (R &R)	Methanol	98	Gulf of Mannar	Present study
S. isoetifolium (W)	Methanol	127	Gulf of Mannar	Present study

GAE= Gallic acid equivalents.

* $A^{1\%}$ gallic acid = 970.8, $A^{1\%}$ tannic acid = 830, $A^{1\%}$ Catechin = 1188.7 and $A^{1\%}$ pyrogallol = 1551.8. Blainski A., Lopes G. C. and de Mello J. C. P. (2013). Application and analysis of the Folin Ciocalteu method for the determination of the total phenolic content from Limonium Brasilience L. Molecules, 18, 6852 -6865.

Methanol, acetone and hexane extracts of seagrasses of *H. pinifolia*, *H. ovalis* and *S. isoetifolium* from Tuticorin showed comparatively higher concentrations in the methanol extract. However in *S. isoetifolium* different trend was noticed (Girija et al., 2013). Total phenolic content in *H. stipulacea* from El-Bradawil Lake, Egypt was 0.611mgGAE/g (Abd El-Hady et al., 2012). As the carbon dioxide content increased, a decrease in the concentration of total phenolic acids at *C. nodosa* leaves were noticed while total alkalinity was not changed throughout. There was also a decrease in the concentration of total reactive phenolics with increase in carbon dioxide content in roots and rhizomes of *P. perfoliatus* and *R. maritime*. The total decrease in concentration was 61% and 45% respectively for the two species while in *C. nodosa*, 59% decrease was noticed (Arnold et al., 2012). Seagrasses of *T. hemprichii*, *S. isoetifolium*, *C. rotundata* and *E. acoroides* from Indonesia showed comparatively higher phenolic contents in the methanol extract followed by ethyl acetate and n-hexane. However in the case of *S. isoetifolium*, ethyl acetate fraction showed high phenolic content. These results indicated that phenolic compounds have solubility in polar as well as semi polar solvents depending on the nature of the species (Santoso et al., 2012).

There are reports indicating relatively higher concentrations of phenolic acid content in the vegitatives propagules in *Potamogeton* species and species to species variation has been observed (Spencer and Ksander, 1994). Total phenolic acid content in fresh leaves were higher in *Z. noltii* from Bay of Cadiz and lower in Alfaces Bay, and wide variations were observed between different locations as well as samplings times which depend on abiotic and biotic factors (Grignon-Dubois et al., 2012). An

increase in phenolic contents was noticed when *C. taxifolia* coexisted with *P. oceanica*. Further higher total phenolic content were noticed in the intermediate leaves than adult at all the stations which could be related to higher metabolic rate. The major phenolic compounds investigated included 4-hydroxybenzoic acid, 4-coumaric acid, trans-cinnamic acid, caffeic acid and ferulic acid (Dumay et al., 2004). Higher total phenolic content in seagrasses were noticed in *H. pinifolia* and lower in *E. acoroides*, and rest of the species demonstrated similar concentrations (Kannan et al., 2010a). *E. acoroides* from Mandapam coast contained relatively higher concentrations of phenol in leaf followed by root and rhizome (Kannan et al., 2010b). Species to species variations were also observed and *Halophila* genera (*H. ovalis* and *H. ovata* with *H. stipulacea*) exhibited large variations (Rengasamy et al., 2012).

Total flavonoids

The aqueous crude extract of *Z. noltii* showed higher Apigenin 7-sulfate (71.3-82.7%), Diosmetin 7- sulfate (85.1-92.9%) and Lutcollin 7-sulfate as major flavonoids (Grignon-Dubois and Rezzonico, 2012). Flavonoids were concentrated at the leaf surfaces and extracellular cuticular layer in the epidermal cells. No significant differences in the total flavonoid content were noticed in *H. johnsonii* between intertidal and subtidal while relatively lower concentrations in *H. decipiens* (Gavin and Durako, 2011). Flavonoids localized in the cytoplasm and cuticle of leaf tissue of *H. johnsonii* displayed no remarkable differences under the treatment of salinity and light/shade during a period of one day to 21 days, and these flavonoids act as sun green pigments or compatible solutes (Gavin and Duracko, 2012). Comparatively higher concentrations of flavonoids

reported in *C. serrulata* and lower at *H. pinifolia* and wide variations were observed between species. Flavonoid level in seagrasses of Gulf of Mannar followed the order: *C. serrulata*>*C. rotundata*>*T. hemprichii*>*S. isoetifolium*> *E. acoroides*>*H. pinifolia*. Flavonoids present in the plants help them to interact with other organisms (Kannan et al., 2013a).

5.3.4 Chemical characteristics of seagrasses (using GCMS, FTIR and UV-VIS)

GCMS

Chemical constituents other than fatty acids obtained in the present study were sterols, phytol, furancarboxylaldehyde, long chain alkanes, vitamin A aldehyde and an acyclic diterepene alcohol. 2-pentadecea-none, 6, 10, 14 -trimethyl and 1, 2 -benzene dicarboxylic acid di isooctyl ester were the common active compounds noticed in the extracts of *S. isoetifolium* and *C. serrulata*. Only five components were detected in both the extracts and all were fatty acids and its esters (Iyapparaj et al. 2014). Saturated fatty acids of hexadecanoic acid methyl ester, octadecanoic acid methyl ester, oleic acid and erucic acid were reported to have antibacterial and antifungal activities (Wagh et al. 2006; Khoobchandani et al. 2010). Major chemical components were saturated, monounsaturated and polyunsaturated fatty acids and along with phytol and an acyclic diterepene alcohol investigated in *S. isoetifolium* and *C. serrulata* (Kannan et al. 2012).

Fatty acids of tridecanoic acid, tetradecanoic acid and n-hexadecanoic acid showed antioxidant as well as antimicrobial activities (Bodoprost and Rosemeyer, 2007) whereas anti-inflammatory and antiarthritic activity exhibited by 9, 12-octadecadienoic acid (Z, Z) (Kalaivani et al., 2012). In all

the extracts 3,7,11,15-tetramethyl -2-hexadecan-1-al a acyclic diterpene alcohol was found in lower concentration which showed antimicrobial and anti-inflammatory activity and the same was reported in earlier study in Kannan et al. (2012). Total 11 compounds were responsible for anti-inflammatory activity of *Z. japonica* and in which predominated chemical constituents were hexadecanoic acid, hexadecanoic acid methyl ester, linoleic acid, linoleic acid methyl ester and oleic acid, and components other than fatty acids were β-elemene and testosterone (Hua et al. 2006).

FTIR

FTIR technique is usually used for elucidation and identification of pure organic compounds by spectral interpretation and it is based on the absorption bands (related to specific functional groups) and their intensities (strong, medium or weak). Several naturally occurring phytochemicals such as polyphenolic compounds were used as alternative therapeutic agent. The absorptions bands responsible for phenolic compounds noticed in this study were in line with other studies carried out (Kannan et al., 2011; Kayalvizhi et al., 2014; Khan et al., 2015). The leaf extracts of ethanol, methanol, isopropanol and acetone confirmed the presence of phenolic compounds and among these, more comparison towards standard gallic acid noticed in ethanol extract (Khan et al., 2015). The major absorption bands observed in *C. serrulata* and *H. pinifolia* were 3415, 2954, 1649, 1408 and 1025cm^{-1} and this indicated the presence of functional groups such as alcohols, phenols, primary amines, aromatics, carboxylic acids, ethers and esters (Prabhakaran et al., 2012). FTIR studied in mangroves and seaweeds absorption bands corresponding to functional groups alkyl halides, aromatics and alkenes (Prabhakaran et al., 2012). The inhibitory activity of

the extracts was correlated with major functional groups and ethyl acetate fractions of *Terrapene ornata* and *Padina tetrastromatica* showed the presence of phenolic acids and major absorption bands were found at 3346, 1400, 1029 and 800-540cm^{-1} (Kayalvizhi et al., 2014). Major peaks in *Enteromorpha linza* correspond to amides, phosphorous and halogen compounds, alcohols and phenols (Paul and Sheeba, 2014) and it is similar to the present study. FTIR comparison of *C. serrulata* aqueous extract and its silver nano particles showed the presence of bonded alcohols and phenols, N-H bond of primary and secondary amines, C-C stretch (in ring) aromatics, C-Cl stretch of alkyl halides and C-H bearing of alkanes (Chanthini et al., 2015).

Kannan et al. (2011) compared major functional groups present in the aqueous methanol extracts of six seagrasses with standards namely phenolic acids of gallic acid, tannic acid, p-coumaric acid and vanillin. The common peaks found in these seagrasses were at 669cm^{-1} (C-OH in alcohols of C-O-H bonding), 863cm^{-1} (primary amine), 1065cm^{-1} (CH2 and CH-OH in alcohols), 1250-1289cm^{-1} (Ar –o in alkyl and aryl ethers, 1404cm^{-1} (C-N in primary amide), 1636cm^{-1} (N-H in primary amides), 1508-1534cm^{-1} (benzene ring in aromatic compounds), 1653cm^{-1} (C=O stretch in enol form), 2852 and 2929cm^{-1} (CH3 and CH2 in aliphatic compounds) and 3392cm^{-1} (OH in alcohols, O-H stretch or N-H stretch). Among the solvents of methanol, ethanol, hexane, chloroform and water, phenolic compounds were absent in aqueous extract and more comparison with respect to gallic acid was observed in ethanol extract (Vijayalakshmi and Ravindhran, 2012). The absorption peaks obtained in gallic acid and seaweeds of Gulf of Mannar were comparable, and more similarity towards *S. wrightii* than *U.*

lactuca (Meenakshi et al., 2012) while the seagrases in this study were compared more towards *U. lactuca*. Absence of peak at 2260cm^{-1} indicated the absence of cyanide group which explained the nontoxic nature of plants, and the present study also demonstrated similar observation (Singh and Mendhulkar, 2015). Major peaks correspond to polyphenols were at 900, 1500, 1714, 3000 and 3100cm^{-1}. Absence of peak at 1635cm^{-1} revealed the absence of moisture or water (Geethu et al., 2014).

UV-VIS Spectrum Analysis

UV-VIS spectroscopy is used for identification of individual compounds and rarely used to characterize particular class of organic compounds. This also provides limited amount of qualitative information and generally produces narrow bands. This technique is not applicable for the identification of mixture of components, even though several researchers followed UV-VIS spectroscopy for screening phenolic compounds in seagrasses, seaweeds and other marine organisms. The UV-VIS absorption peaks were between 270-290nm for phenolic acid derivatives whereas 317-340nm for flavonoid derivatives (Vijayalakshmi and Ravindhran, 2012), and intensities of absorption maxima in the present study followed the order; *S. isoetifolium*>*C. serrulata* (roots and rhizomes)>*C. serrulata* (whole). UV-VIS fingerprint profile of the methanol extract of *E. linza* showed maximum absorptions at 269, 329, 410 and 425nm (Paul and Sheeba, 2014). Root extracts of *Diospyrus ferrea* was showed an absorption maxima at 260 and 227nm towards methanol solvent whereas 271 and 227nm for ethanol solvent. The ethanolic extract showed higher absorptions at 270 and 275nm due to the presence of phenols and poly phenolic compounds at higher concentrations (Vijayalakshmi and Ravindhran, 2012). The UV-VIS spectra

results of seagrasses of Gulf of Mannnar showed the presence of phenolic acids in the extracts (Kannan et al., 2013b; Kannan et al., 2011). It is also reported that the highest absorption was found at 270 and 321nm and these were characteristic of flavonoids derivatives, and the two spectra lies between 230-290nm and 300-350nm were related to flavonoids in the earlier report (Singh and Mendhulkar, 2015). More intensified absorption spectrum was obtained in ethanol than rest of the solvents (methanol, acetone and isopropanol) respective to phenolic compounds and higher absorptions were at 270 and 275nm (Khan et al., 2015).

5.4 Conclusions

DPPH free radical scavenging activity of seagrasses revealed that *S. isoetifolium* were more active than *C. serrulata* whole as well as root and rhizome extracts. A positive correlation between DPPH radical activity and phenol content was attributed to the redox potentials of polyphenols; they can absorb and neutralize the free radical, quenching singlet oxygen and decomposition of peroxides. FRAP assay depends on the presence of reductones and it breakdown the free radical chain by donating a hydrogen atom. Among the phytochemicals, polyphenols have the capacity to act as a reductone. FRAP assay followed the order: *S. isoetifolium* (whole)> *C. serrulata* (roots and rhizomes)>*C. serrulata* (whole). Among the free radicals, hydroxyl radical was the most reactive radical and have the ability to damage various cells which may lead to carcinogenesis, mutagenesis and cytotoxicity. Hydroxy radical activity followed the order: *S. isoetifolium* (whole)>*C. serrulata* (whole)>*C. serrulata* (roots and rhizomes) but the activity of roots and rhizomes were much lower in comparison to whole seagrasses while no wide variations were observed between whole

seagrassses of *C. serrulata* and *S. isoetifolium*. Weak oxidizing capacity of H_2O_2 was attributed due to its lesser interaction to enzymes directly (oxidation of essential thiols) and also cross cell membranes rapidly which leads to the formation of hydroxyl radicals, and these radicals could damage various cells. Hydrogen peroxide activity also follows the same order of hydroxy radical activity. A strong correlation was noticed between total antioxidant activity and phenolic content, and it was due to their capacity to act as reducing agents, hydrogen donors and free radicals quenchers. Total antioxidant activities were higher in the roots and rhizomes of *C. serrulata* and the differences between different body parts were also high whereas no wide variations were observed between *C. serrulata* (roots and rhizomes) and *S. isoetifolium* (whole).

Generally phytochemicals present in the extracts were the leading factor for antioxidant activity. The chemical constituents present in plants might be different at different places and factors responsible were substrate, water, and other physical as well as physiological factors. Total of seven phytochemicals out of ten were noticed in all the seagrasses, and they were phenols, sterols, flavonoids, reducing sugars, alkaloids, tannins and terpenoids while cardiac glycosides, saponins and anthraquinones were absent. The degree of intensities of most of the phytochemicals in seagrasses followed the order: *S. isoetifolium* (whole)>*C. serrulata* (roots and rhizomes)>*C. serrulata* (whole). Total phenolics in seagrasses followed the order: *S. isoetifolium* (whole)>*C. serrulata* (roots and rhizomes)>*C. serrulata* (whole) while total flavonoids followed the order; *C. serrulata* (roots and rhizomes)>*S. isoetifolium* (whole)>*C. serrulata* (whole).

GCMS analysis of seagrasses showed that major components were saturated fatty acids, polyunsaturated fatty acids, sterols, long chain alkanes and terpenes. Relatively large number of components were found in *S. isoetifolium* (whole) followed by *C. serrulata* (whole) and *C. serrulata* (roots and rhizomes). FTIR analysis facilitates the characterization and identification of series of components and their interpretation, and this technique offers a qualitative rapid non destructive convenient investigation. Major common absorptions were found at 1606, 1982, 2133, 2853, 2925 and 3296cm^{-1}, and it was similar to phenolic compounds present in seagrasses, seaweeds and mangroves. Simple phenolic compounds showed an absorption maximum between 220-280nm and it was comparable to marine plants. The absorption peaks were between 270-290nm for phenolic acid derivatives whereas 317-340nm for flavonoid derivatives similar to other studies carried out, and intensities of the absorption maxima followed the order; *S. isoetifolium* (whole)>*C. serrulata* (R&R)>*C. serrulata* (whole).

Factors which indirectly affect the intensity of antibacterial activity were solvents used for extraction, condition or state of the sample, season of sampling, age of plant, duration of storage, temperature, preparation of media and pH. The relatively lower activity of extracts was due to pathogenic strains which were responsible for some kind of resistance mechanisms such as enzymatic inactivation, target site modification and decrease intracellular drug accumulation. Further variations in activity of the extracts were noticed which could be due to the distribution of active components and this varied from species to species. Ecological studies showed that antimicrobial metabolites in most of the marine organisms might not be active against human pathogens. The inhibited growth of

microbes in all assays were noted which was more lipophilic than hydrophilic extracts and in lipophilic, secondary metabolites were playing an important role in defending host tissues from harmful organisms. The antimicrobial activity of seagrasses grown under low salinity showed that *C. serrulata* and *S. isoetifolium* (whole) were comparable while *C. serrulata* (roots and rhizomes) exhibited zone of inhibition against pathogen of *P. aeruginosa*, and rest of the pathogens were resistant against extracts irrespective of body parts. Even though higher antioxidant activities, phenolic and flavonoid contents and phytochemicals exhibited relatively lower antimicrobial activity due to their combined effect and their inability to break the bacterial cell wall. Major components present in seagrasses as well as antioxidant activities showed that *C. serrulata* and *S. isoetifolium* are a good source of bio active substances from our ecosystem.

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Chapter 6

SUMMARY AND CONCLUSIONS

Seagrasses are marine flowering plants which complete the life cycle in water and have the ability to survive at fresh water, estuarine, marine and hyper saline conditions. Seagrass beds act as a carbon sink due to its absorbing capacity of carbon dioxide from atmosphere which leads to a decrease in the effects of global warming. The fact is that seagrasses distributed only 0.1% in the marine environment but it contributes 12% of the organic carbon in the ocean and it is estimated to produce approximately 10 litres per day from one square meter. Seagrasses provide the nursery feeding grounds for marine organisms including fishes, molluscs, prawns, crabs, sea horses, green turtles, dugongs, sea urchins, etc. Seagrasses reduce the sediment erosion and increase the clarity of water by settling the suspended material to the bottom. Marine organisms noticed in seagrass blades contained more than 153 species of microalgae, 359 species of macro algae and 178 species of invertebrates as epiphytes and epizooties. The three dimensional structure of seagrasses provides hiding places for marine organisms to escape from their predators. Decomposition of seagrasses occur slowly and a major part of carbon is retained for a long periods in sediments. The awareness of the economic value of seagrasses to humans increased towards last third of twentieth century and it was $1583 ha^{-1}\ Y^{-1}$, two orders of magnitude higher than crop lands.

Seagrasses are rich in secondary metabolites which possess antibacterial, antiviral, antifungal and antialgal activities, and are a source of antioxidants and bioactive compounds. The medicinal uses of seagrasses are categorized into maintenance of general health, mental disorder, heart diseases, dermatological problems, infections and gastro intestinal diseases. Antioxidant and antimicrobial activities exhibiting secondary metabolites are generated in seagrasses due to their interactions with micro and macro algae, epiphytes, other organisms used for prey and habitat, and these activities are decreased with an increase in the content of pollutants. In this aspect, antimicrobial and antioxidant studies are more important if it is obtained from an unpolluted environment. Phenolics in seagrass are performed as protection of the plant against photosynthetic stress, reactive oxygen, anthropogenic pressures and inter specific competition, and these are presented in seagrasses as phenolic acids, sulfated phenolic acids, phenolic acids conjugates, flavones, tannins (condensed not hydrolysable) and lignins, are varied widely and dependent upon species, population and tissue.

Although a variety of secondary metabolites are produced by seagrasses which have pharmaceutical significance, they are one of the specialized groups of marine flora which are poorly known in India compared to seaweeds and mangroves especially for chemical characterisation and bio active potential studies. The present study is an attempt to evaluate the biochemical components in seagrasses collected from Gulf of Mannar and Palk Bay. The objectives of the present study were to study of the general ecological characteristics of seagrass meadows along Gulf of Mannar and Palk Bay, biochemical evaluation and profile of seagrasses of Gulf of Mannar and Palk Bay and bio potential of seagrasses grown at low salinities.

The hydrogen ion concentration was noticed as alkaline and variations in pH, surface temperature and salinity were observed in this study. Variations in the concentrations of nitrate, nitrite, silicate, phosphate and DO were observed along the Gulf of Mannar and Palk Bay, and relatively higher nutrients contents were also noticed at Gulf of Mannar than Palk Bay. Heavy metals content in sea waters of Gulf of Mannar were slightly higher than Palk Bay except Pb, and the toxic pollutants Hg and As were not detected in this study. The pH of the sediment was acidic in nature at Palk Bay and slightly higher values were observed at Gulf of Mannar. The sediment texture revealed that sand predominated over silt followed by clay. Most of the macro elements as well as heavy metals were observed higher concentrations in the sediments of Gulf of Mannar than Palk Bay, and metals Cu, Cr, Ni, Fe, Zn, Co and Pb at lower concentrations than similar findings in sediments from different regions of the World. The Hg, As and Cd levels were comparable and slightly higher than other studies carried out. These results indicated that these stations were less contaminated.

Species as well as location wise variations in biochemical composition were observed in seagrasses of Gulf of Mannar and Palk Bay. Comparatively higher carbohydrate content was found in roots and rhizomes than leaves. Seagrasses of larger leaf of *E. acoroides* with high lipid content followed by *C. serrulata* and lowest with spherical shaped *S. isoetifolium*, and also lipid content in leaves predominated over roots and rhizomes except *C. serrulata* from Thonithurai. Comparatively higher concentrations of phenolics, flavonoids and tannins were observed in seagrasses of Palk Bay than Gulf of Mannar, and also comparatively higher contents were noticed at leaves than roots and rhizomes of Gulf of Mannar and Palk Bay,

and the content varied with reference to the surrounding environmental conditions. Relatively higher levels of phenolic compounds were obtained in *C. serrulata* (Palk Bay) and *E. acoroides* (Gulf of Mannar). Species to species variations in the concentrations of phenolics, flavonoids and tannins were noticed both at Gulf of Mannar and Palk Bay.

Chlorophyll content in seagrasses of Gulf of Mannar and Palk Bay showed species as well as station wise variations, and comparatively higher contents of chl a and b obtained from Gulf of Mannar. Seagrasses with cylindrical leaf of *S. isoetifolium* showed higher chls concentrations than non cylindrical leaves (*C. serrulata* and *E. acoroides*), and chl content in leaves were much higher than roots and rhizomes. Among the different body parts, relatively higher concentrations of N, C and P were noticed in leaves rather than roots and rhizomes except P content. Species to species variations were noticed in C and N content of seagrasses from Gulf of Mannar while P content at Palk Bay. Macro elements in seagrasses followed the order Na>Ca>K>Mg, and also station wise variations in concentration of these elements were noticed higher levels at Gulf of Mannar rather than Palk Bay and roots and rhizomes were predominated over leaves. The results showed that seagrasses of Gulf of Mannar and Palk Bay were a good source of macro elements. Species to species as well as location wise variations in the concentrations of heavy metals were observed and species to species variations were more observed in Gulf of Mannar than Palk Bay. Among the different body parts, concentrations of metals in leaves, roots and rhizomes varied with respect to stations. Most of the metals were at higher concentrations in seagrasses of Gulf of Mannar than Palk Bay. Toxic

metals of As was investigated only in the roots of *E. acoroides* whereas Hg in the roots and rhizomes of *C. serrulata*.

The FA compositions of seagrasses from Gulf of Mannar and Palk Bay demonstrated that SFAs were the predominated FAs followed by PUFAs and MUFAs in most of the seagrasses except *E. acoroides* (leaves and rhizomes) and *C. serrulata* (roots and rhizomes) from Munaikkadu where the order is PUFAs>SFAs>MUFAs. *C. serrulata* contained comparatively higher PUFAs at roots and rhizomes than leaves except from Chinnappalam whereas in *E. acoroides* leaves followed by rhizomes and roots. PUFAs of C18:2n-6 predominated over C18:3n-3 in most of the seagrasses except leaves and rhizomes of *E. acoroides*. More than 50% of essential amino acids were noticed in most of the seagrasses of Gulf of Mannar and Palk Bay. Total amino acids contents were relatively higher in *S. isoetifolium* followed by *C. serrulata*, *E. acoroides* and *T. hemprichii*. *C. serrulata* (both leaves and roots and rhizomes) from Palk Bay predominated over Gulf of Mannar and vice versa obtained in the case of *S. isoetifolium*. Variations in carbohydrate composition observed between seagrasses of different as well as same species (*C. serrulata* and *S. isoetifolium*) collected from Gulf of Mannar and Palk Bay. The maximum number of carbohydrates found in *C. serrulata* from Gulf of Mannar and *T. hemprichii* from Palk Bay, and in *E. acoroides* glucose content was more than 90% of the total carbohydrates.

DPPH free radical scavenging activity of seagrasses revealed that *S. isoetifolium* (whole) was more active than *C. serrulata* whole as well as roots and rhizomes extracts. FRAP assay followed the order *S. isoetifolium* (whole)>*C. serrulata* (roots and rhizomes)>*C. serrulata* (whole). Among

the free radicals, hydroxyl radical was the most reactive radical and had the ability to damage various cells which may leads to carcinogenesis, mutagenesis and cytotoxicity. Hydroxy radical and hydrogen peroxide activity followed the order *S. isoetifolium* (whole)>*C. serrulata* (whole)> *C. serrulata* (roots and rhizomes). Total antioxidant activities were higher in the roots and rhizomes of *C. serrulata* and the differences between different body parts were also high whereas no wide variations were observed between *C. serrulata* (roots and rhizomes) and *S. isoetifolium* (whole). Total of seven phytochemicals out of ten were noticed in all the seagrasses, and they were phenols, sterols, flavonoids, reducing sugars, alkaloids, tannins and terpenoids while cardiac glycosides, saponins and anthraquinones were absent. Total phenolics in seagrasses followed the order *S. isoetifolium* (whole)>*C. serrulata* (roots and rhizomes)>*C. serrulata* (whole) while total flavonoids followed the order *C. serrulata* (roots and rhizomes)>*S. isoetifolium*(whole)>*C. serrulata* (whole).

GCMS analysis of seagrasses showed that major components were saturated fatty acids, polyunsaturated fatty acids, sterols, long chain alkanes and terpenes. Relatively large numbers of components were found in *S. isoetifolium* (whole) followed by *C. serrulata* (whole) and *C. serrulata* (roots and rhizomes). Major FTIR absorption peaks corresponding to amides, aromatics, carboxylic acids, esters, alcohols and phenols functional groups. The UV absorption peaks region corresponded to phenolic acids, flavonoids and their derivatives, and intensities of the absorption maxima were followed the order *S. isoetifolium* (whole)>*C. serrulata* (R&R)>*C. serrulata* (whole). The antimicrobial activity of seagrasses grown under low salinity showed that *C. serrulata* and *S. isoetifolium* (whole) were comparable

while *C. serrulata* (roots and rhizomes) exhibited zone of inhibition against pathogen of *P. aeruginosa*, and rest of the pathogens were resistant against extracts irrespective of body parts.

The major findings of this study were summarised as, water and sediment quality parameters had strong influence on the biochemical components as well as bio potentials of seagrasses, and most of the toxic metals were absent or lower concentrations in the present study area. Species as well as location wise variations in biochemical compositions, phenolic compounds, pigments, elemental compositions and macro elements were observed in seagrasses of Gulf of Mannar and Palk Bay, and were related with the surrounding environmental characteristics of the seagrass meadows. Seagrasses of Gulf of Mannar and Palk Bay were can be used as a source of macro elements. Even though one of the most toxic metals of As were slightly higher concentrations in the sediments were not influenced in the level of As in seagrasses irrespective of body parts except *C. serrulata* (roots and rhizomes) from Thonithurai. Total PUFAs were more than 20% of total FAs in most of the seagrasses and can be used for extraction of PUFAs. More than 50% of essential amino acids in seagrasses suggests as a possible source of essential amino acids for humans. Major components presented in seagrasses as well as antioxidant activities showed that *C. serrulata* and *S. isoetifolium* are a good source of bio active substances as well as neutraceuticals.

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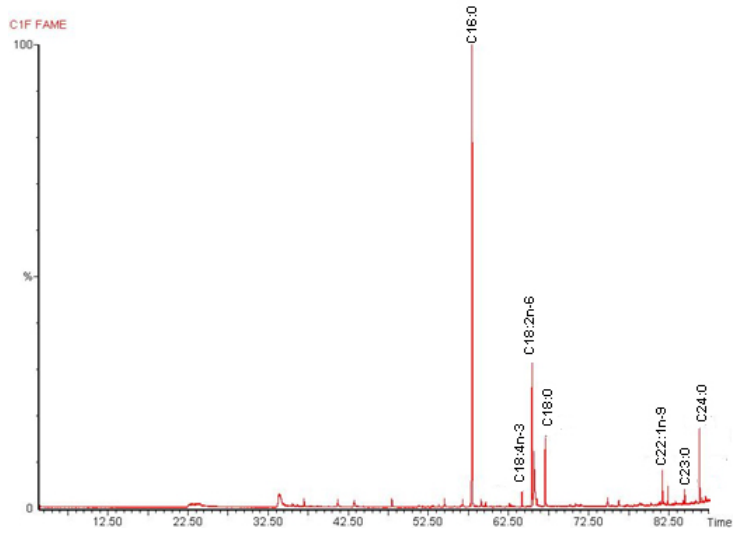
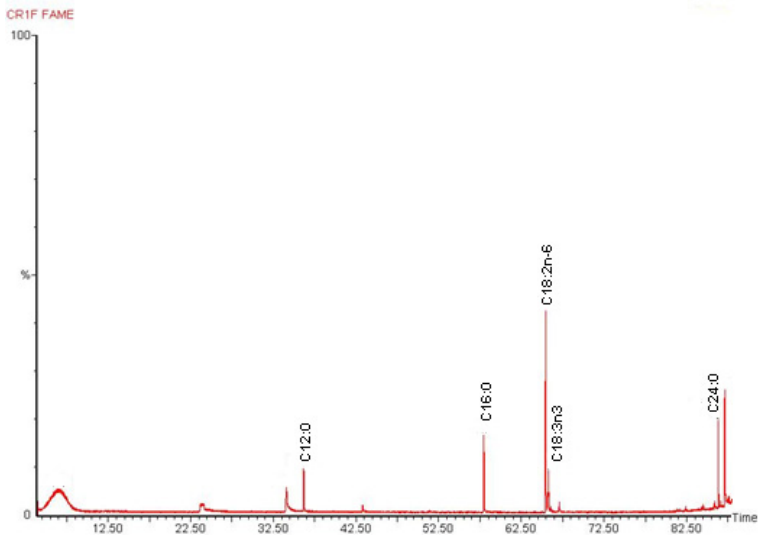
Appendices

Appendix 1

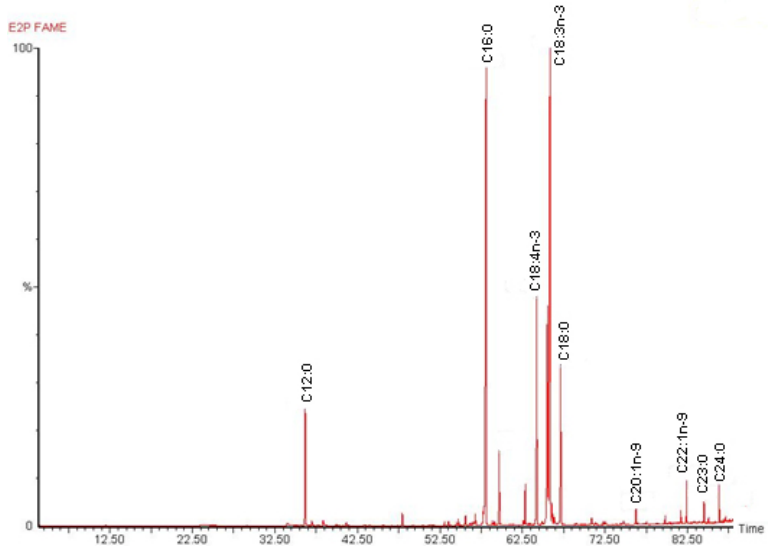
Total ion chromatogram of fatty acids in *C. serrulata* leaves (Farm Pond)



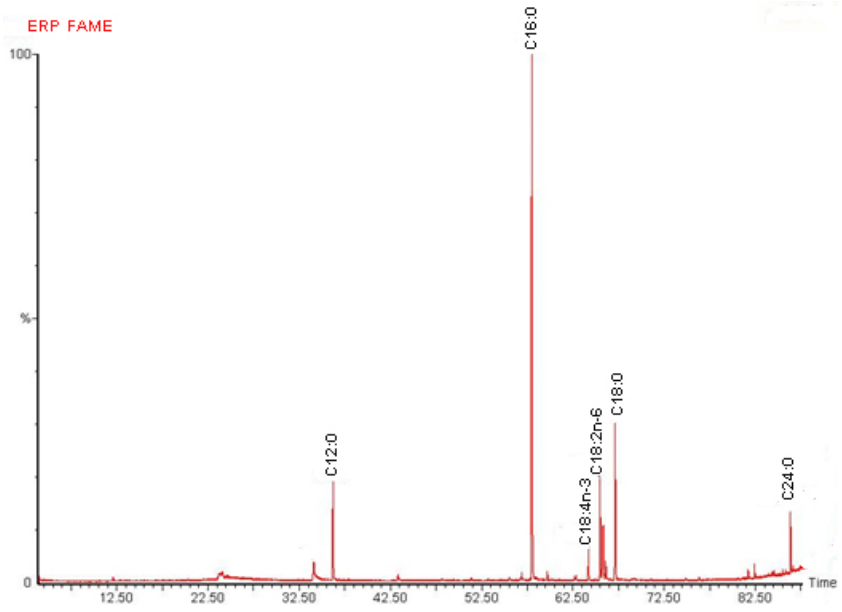
Total ion chromatogram of fatty acids in *C. serrulata* roots and rhizomes (Farm Pond)



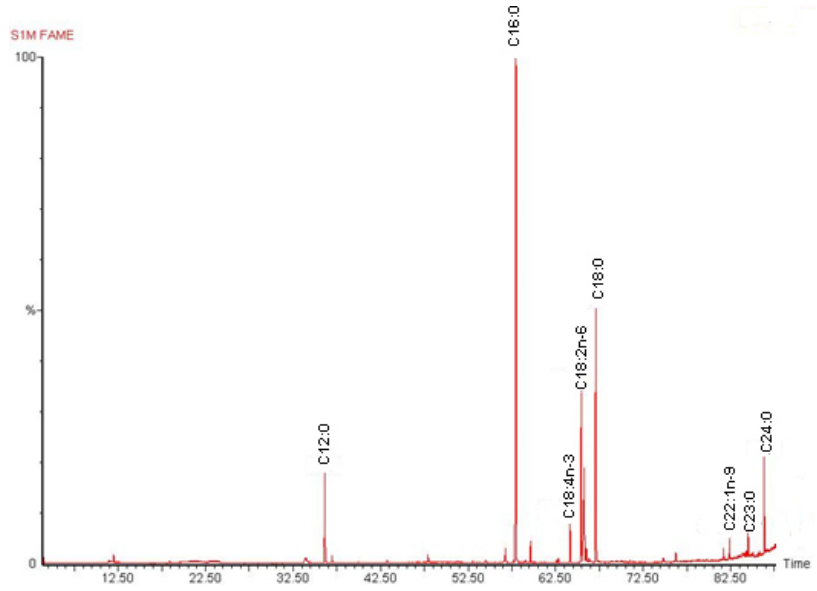
Total ion chromatogram of fatty acids in *E. acoroides* leaves (Chinnappalam)



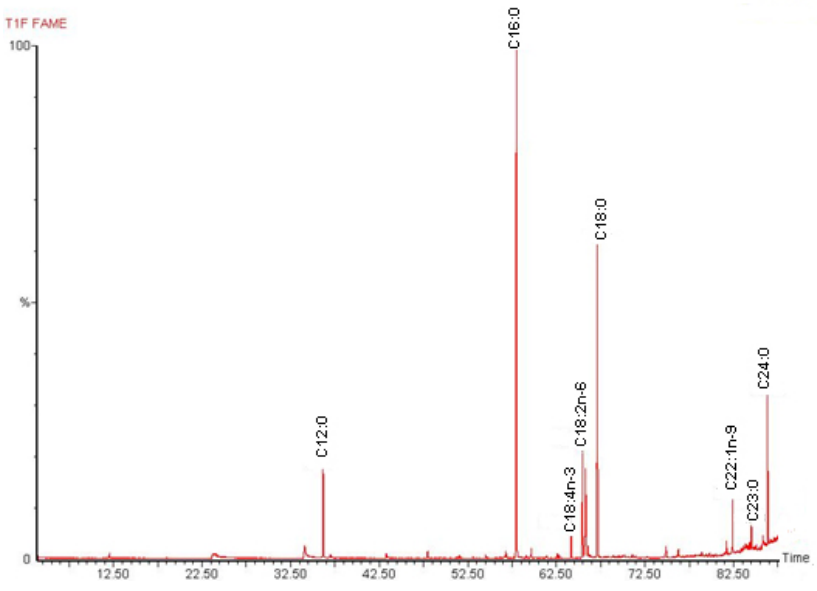
Total ion chromatogram of fatty acids in *E. acoroides* roots (Chinnappalam)



Total ion chromatogram of fatty acids in *S. isoetifolium* whole (Mathacovil)

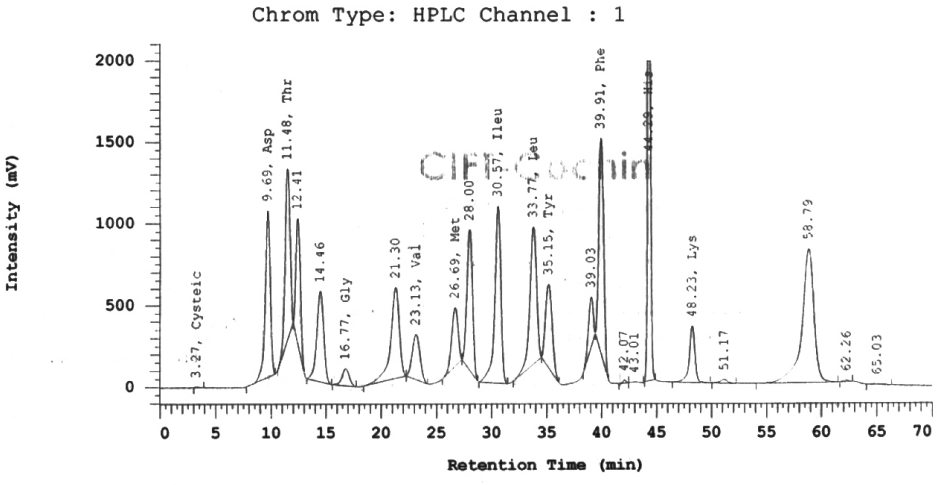


Total ion chromatogram of fatty acids in *T. hemprichii* whole (Farm Pond)



Appendix 2

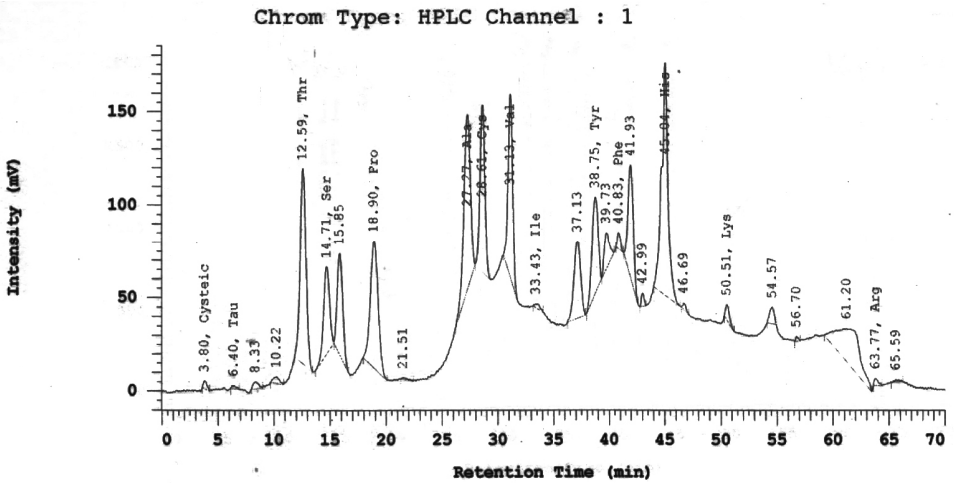
AMINO ACIDS STD



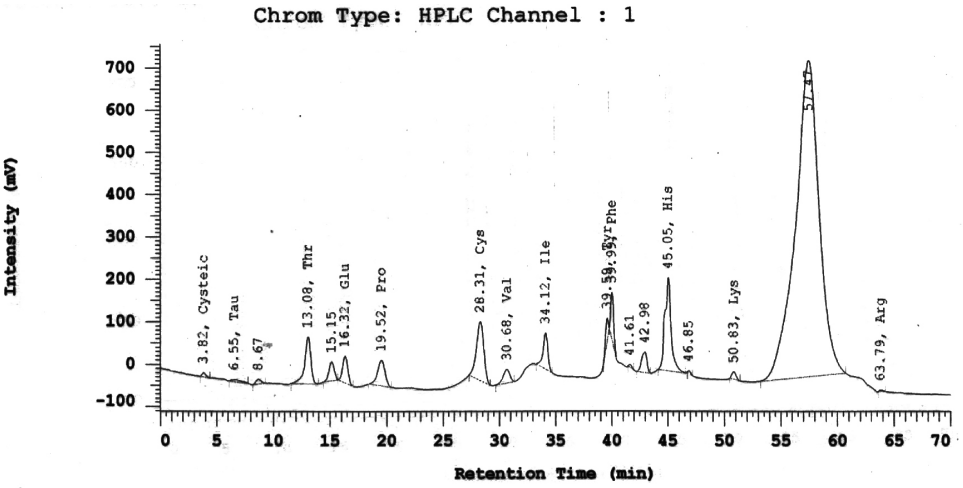
Details of amino acids standard concentration of 1.25µmoles

SI. No.	Name of amino acid	Retention time	Area
1	Aspartic acid	9.69	32501240
2	Threonine	11.48	31108275
3	Serine	12.41	20724220
4	Glutamic acid	14.46	23886228
5	Proline	16.77	4840318
6	Glycine	21.30	30014461
7	Alanine	23.13	13283027
8	Cysteine	26.69	14103564
9	Valine	28.00	29828372
10	Methionine	30.57	40910366
11	Isoleucine	33.77	35188904
12	Leucine	35.15	21170533
13	Tyrosine	39.03	8671815
14	Phenyl alanine	39.91	38998617
15	Histidine	44.29	44871654
16	Lysine	48.23	11938654
17	Arginine	58.79	61650800

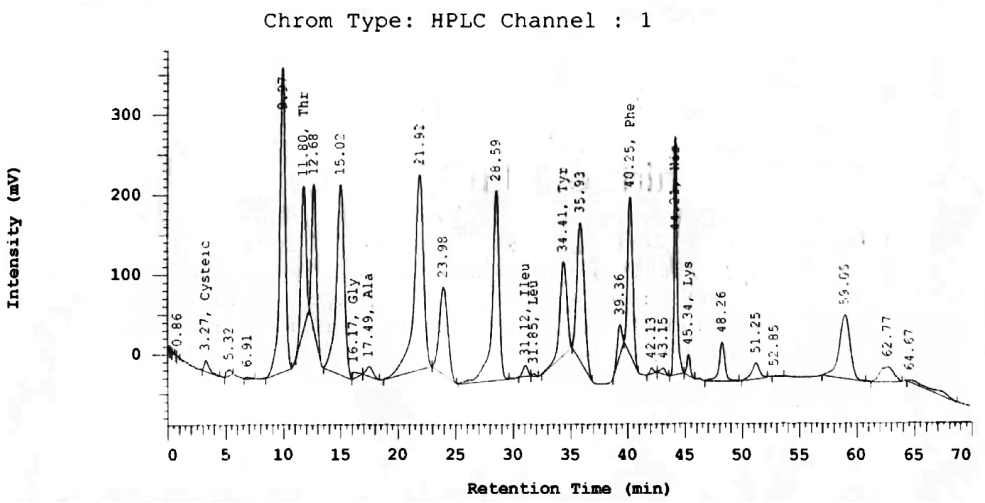
Amino acids chromatogram of *C. serrulata* leaves from Thonithurai



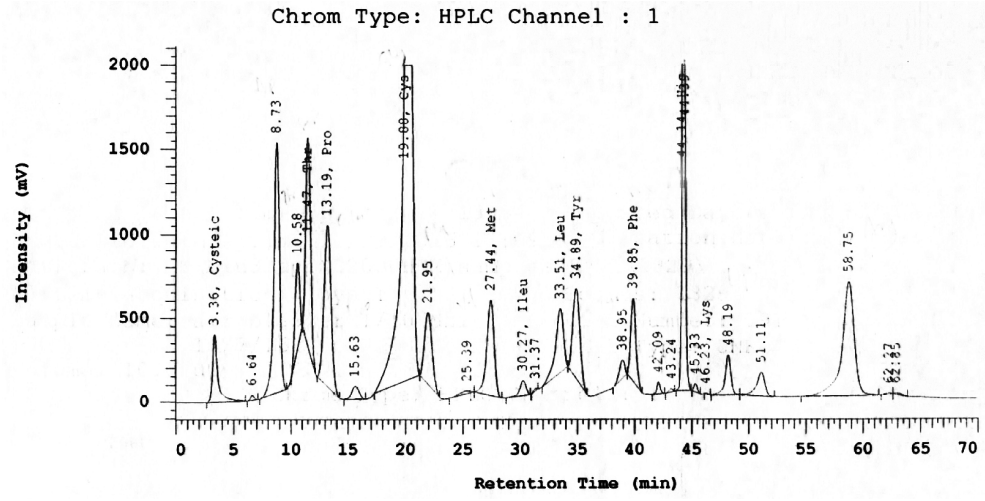
Amino acids chromatogram of *C. serrulata* roots and rhizomes from Farm Pond



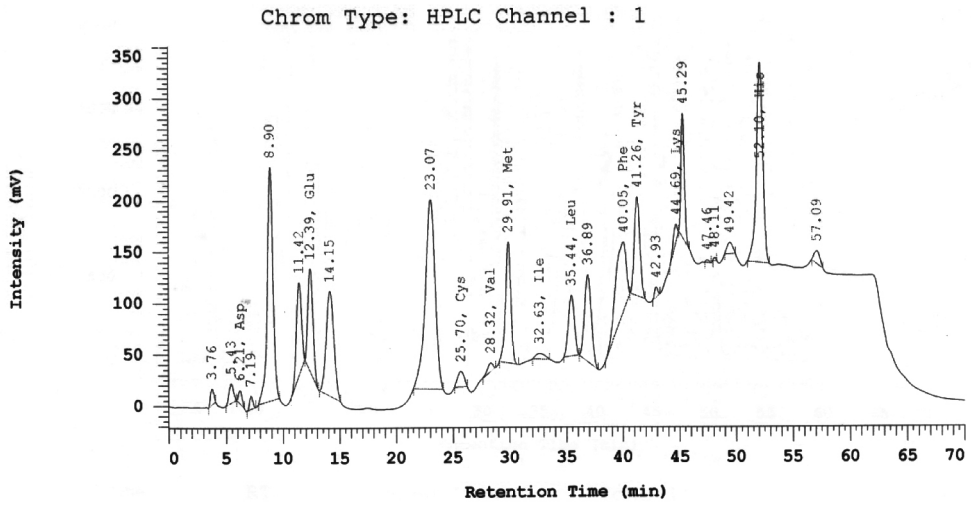
Amino acids chromatogram of *E. acoroides* leaves from Chinnappalam



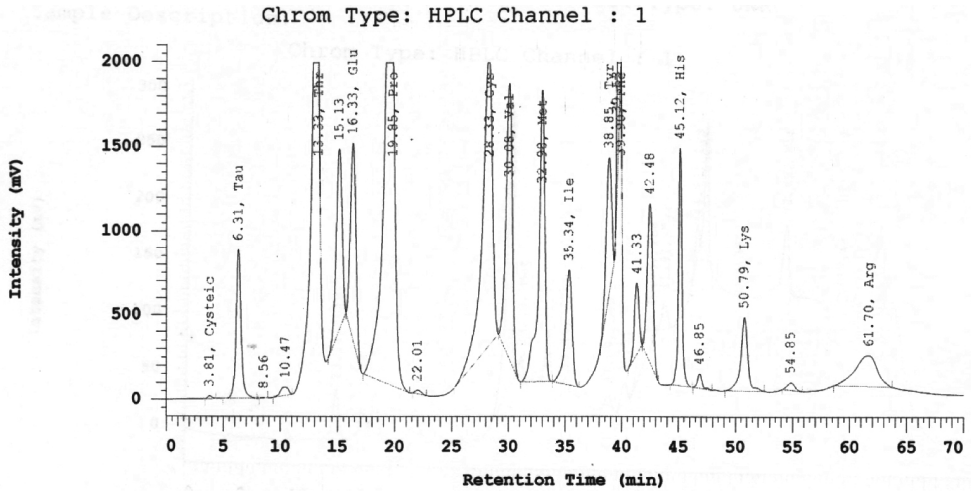
Amino acids chromatogram of *E. acoroides* rhizomes from Chinnappalam



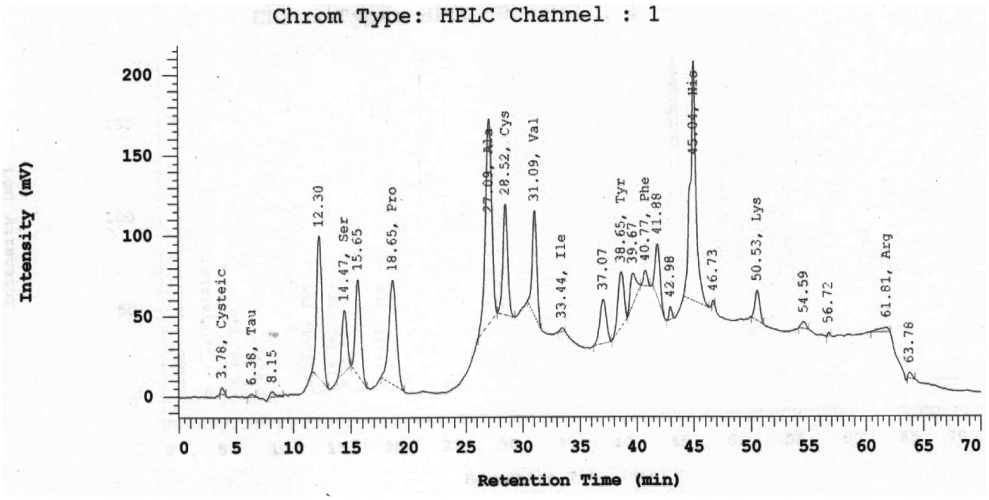
Amino acids chromatogram of *E. acoroides* roots from Chinnappalam



Amino acids chromatogram of *S. isoetifolium* whole from Mathacovil

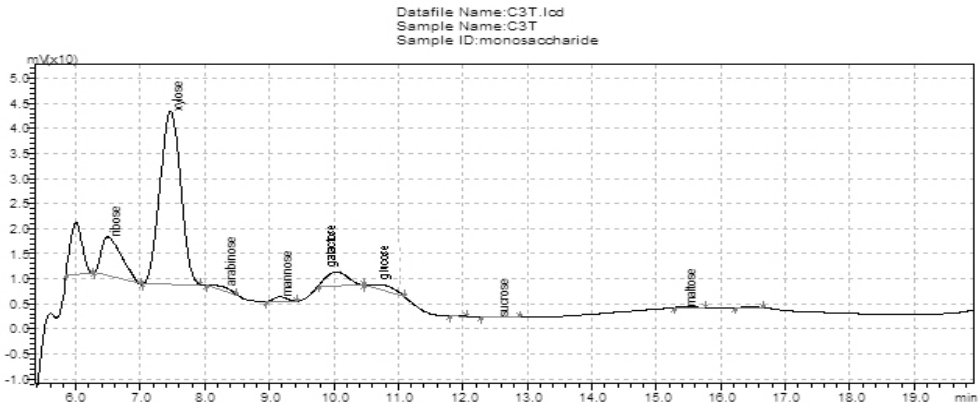


Amino acids chromatogram of *T. hemprichii* whole from Farm Pond

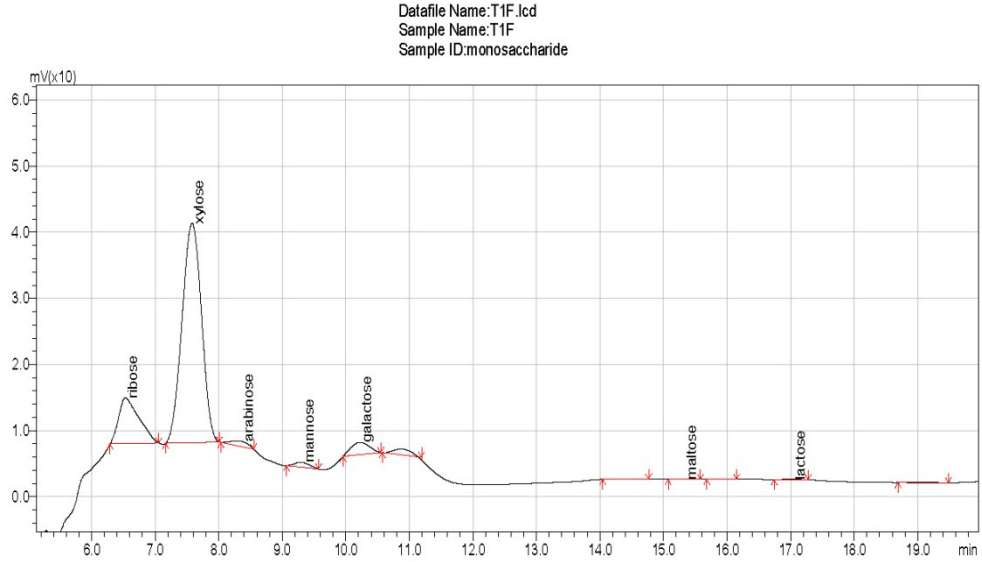


Appendix 4

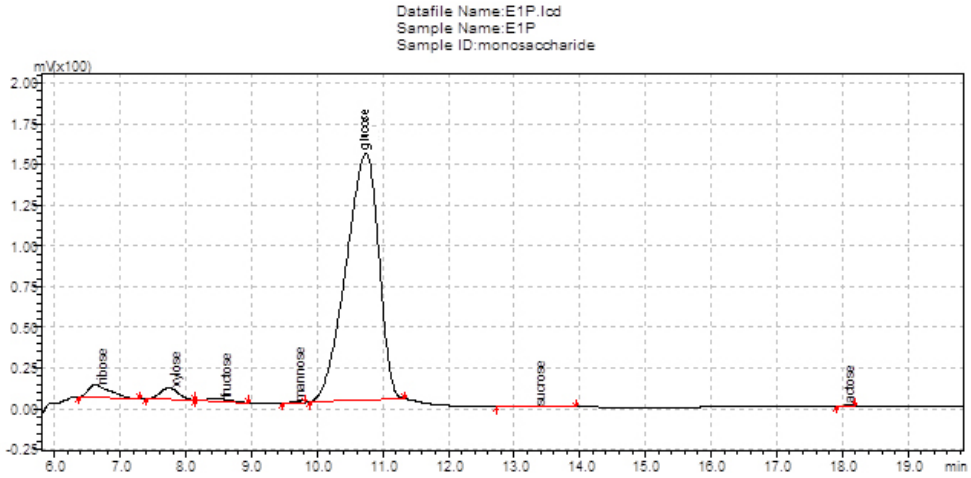
Carbohydrates chromatogram of *C. serrulata* of Farm Pond



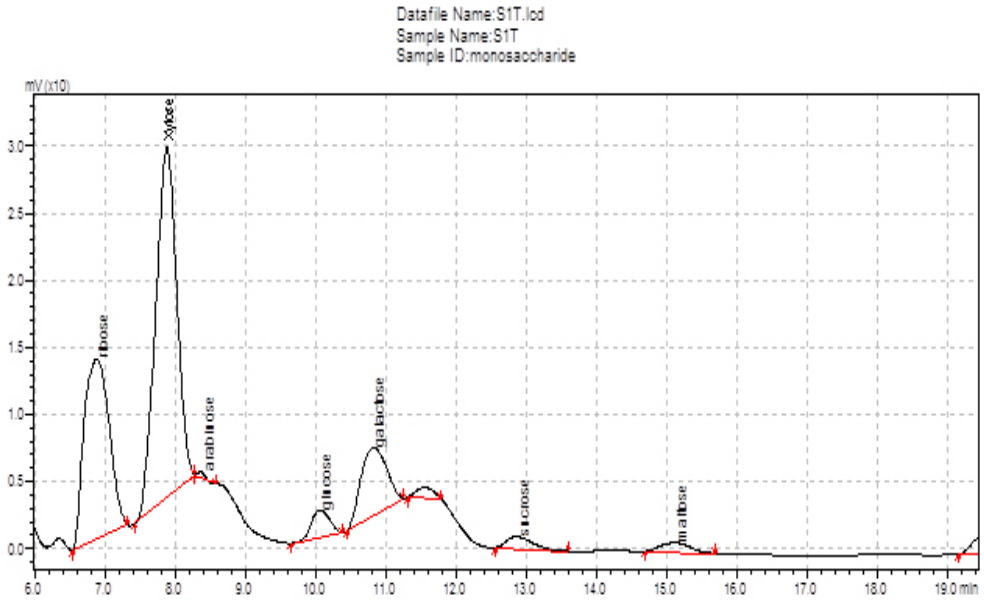
Carbohydrates chromatogram of *T. hemprichii* of Farm Pond



Carbohydrates chromatogram of *E. acoroides* of Chinnappalam

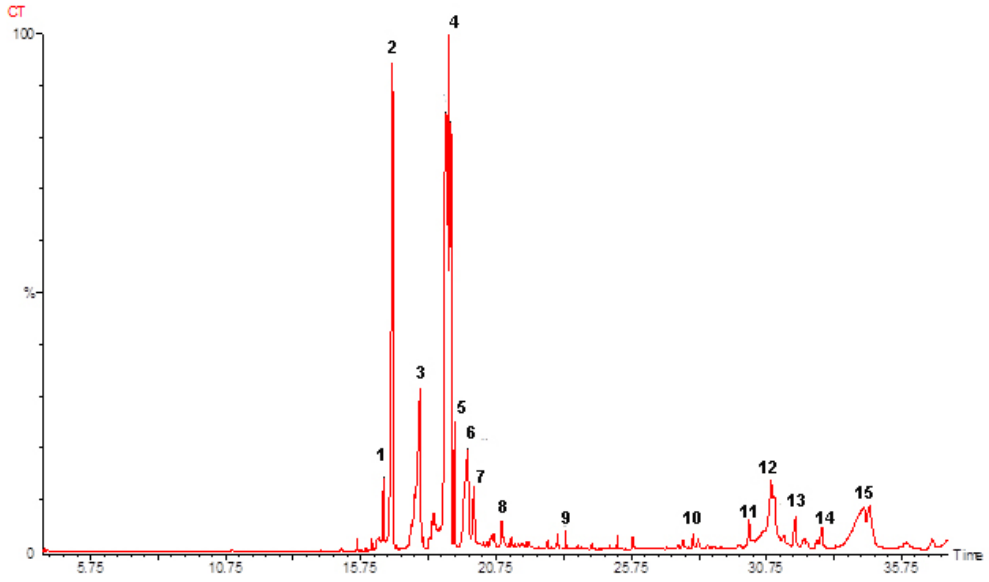


Carbohydrates chromatogram of *S. isoetifolium* of Thonithurai



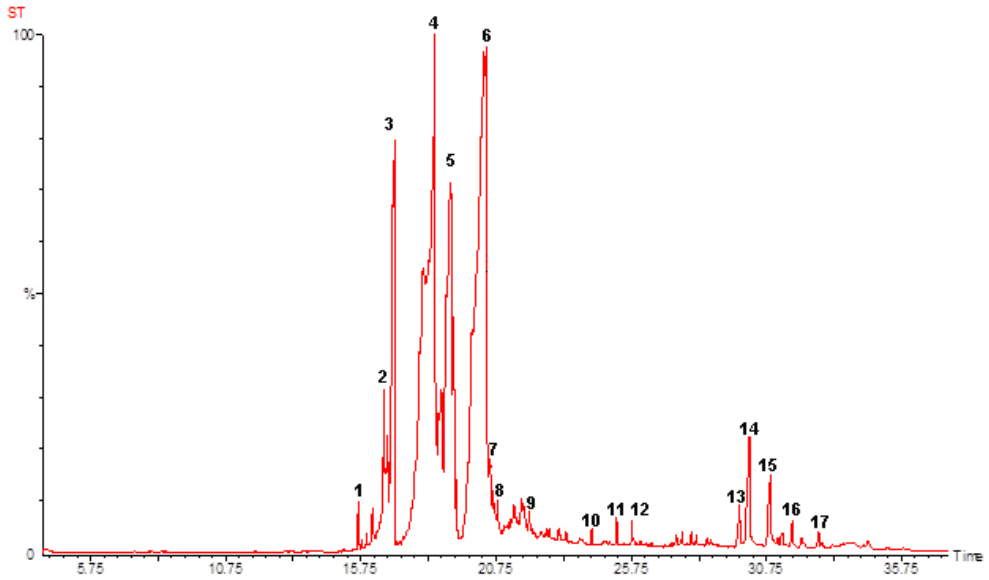
Appendix 4

Total ion chromatograms of crude methanol extract of *C. serrulata* whole



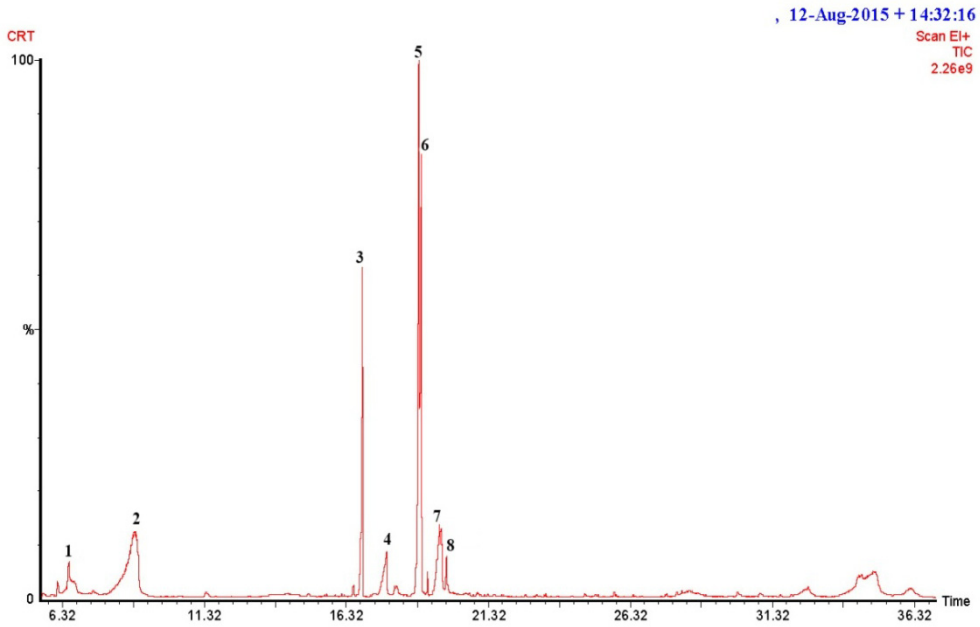
1. 3,7,11,15-Tertamethyl-2-hexadecen-1-ol
2. Tetradecanoic acid-10,13-dimethyl methyl ester
3. n-Hexadecanoic acid
4. Methyl 12,15-Octadiecanoate
5. Methyl 16- methyl Heptadecanoate
6. 9,12- Octadecanoic acid (Z,Z)
7. 1-Propyl-9,12- Octadecadienoate
8. Octadecanoic acid
9. Trans-2-methyl-4-pentythiane
10. Hop-22(29)-en-3-beta-ol
11. Stigma sterol
12. Gama sterol
13. 4,22-Stigmastadiene-3-one
14. Stigmast-4-en-3-one
15. Tetrapenta contane (less than 0.20%)

Total ion chromatograms of crude methanol extract of *S. isoetifolium* whole



1. Methyl 11-Hexadecanoate
2. 3,7,11,15-Tertamethyl-2-hexadecen-1-ol
3. Tetradecanoic acid-10,13-dimethyl methyl ester
4. Octadecanoic acid
5. Methyl 11,14,17-Eicosatrienoate
6. Phytol
7. Methyl 7, 11, 14-Eicosatrienoate (less than 0.20%)
8. 9, 12, 15-Octadecatrienoic acid (less than 0.20%)
9. Eicosanoic acid
10. Heptacosanoic acid-25-methyl methyl ester
11. Methyl octasanoate
12. 13-Docosenamide
13. Ergost-5-en-3 ol (3 beta)
14. Ergost-5, 22-en-3 ol-(3 beta, 22E)
15. Gama sterol
16. 4,22-Stigmastadiene-3-one
17. Stigmast-4-en-3-one (less than 0.20%)

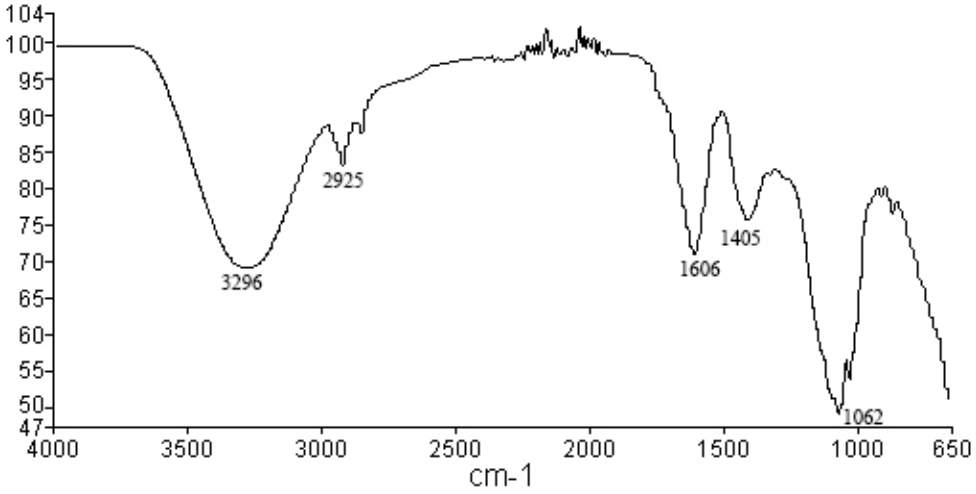
Total ion chromatograms of crude methanol extract of *C. serrulata* roots and rhizomes



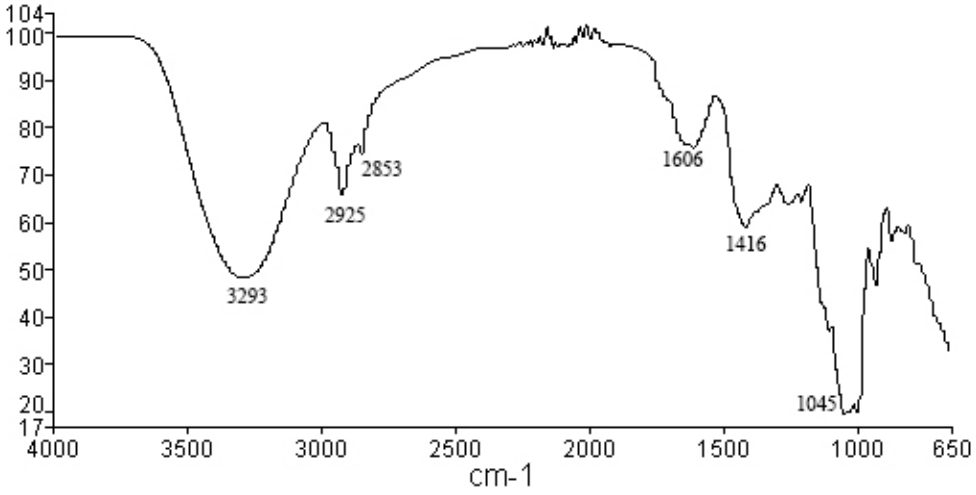
1. Hepta-2,4-dienoic acid methyl ester (less than 0.20%)
2. Furancarboxylaldehyde,5-(hydroxyl methyl)
3. Tetradecanoic acid-10,13-dimethyl methyl ester
4. n-Hexadecanoic acid
5. Methyl-9-cis,11-trans Octadecadienoate
6. Methyl 11,14,17-Eicosatrienoate
7. Ethyl 9,12-Hexadienoate
8. n-Propyl-11- Octadecenoate

Appendix 5

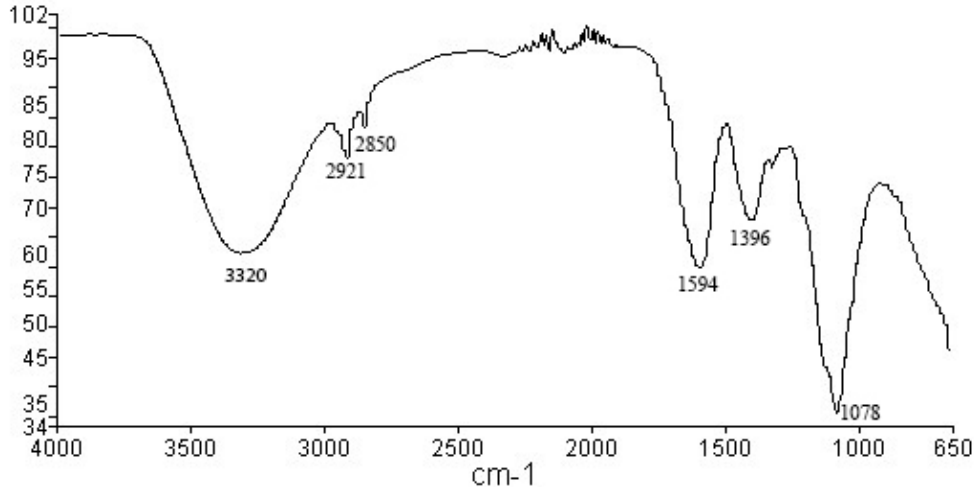
FTIR spectrum of crude methanol extracts of *C. serrulata* whole.



FTIR spectrum of crude methanol extracts of *C. serrulata* roots and rhizomes



FTIR spectrum of crude methanol extracts of *S. isoetifolium* whole



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List of Publications

- Libin Baby, Sankar T. V. and Chandramohanakumar N. (2017). Changes in phenolic compounds in seagrasses against changes in the ecosystem. J. Pharmacog. & Phytochem., 6(3), 742-747.
- Libin Baby, Gireesh Kumar T. R., Remyakumari K.R., Johns Varkey, Sankar T. V. and Chandramohanakumar N. (2017). Comparison of hydrographic and sediment characteristics of seagrass meadows of Gulf of Mannar and Palk Bay, South West Coast of India. Int. J. Fisheries and Aquatic Studies, 5(2), 80-84.
- T. V. Sankar, Libin Baby and R. Anandan (2016). Organochlorine pesticides, Polychlorinated biphenyls and Heavy metals residues in myctophids off South West coast of India. Fishery Technology, 53, 250 – 256.
- Libin Baby, Thazhakot Vasunambisan Sankar and Rangasamy Anandan (2014). Comparison of Lipid Profile in Three Species of Myctophids from the South West Coast of Kerala, India. Nat. Acad. Sci. Lett., 37(1), 33–37.
- K. Rajamoorthy, K. Pradeep, R. Anandan, Libin Baby, T. V. Sankar and P.T. Lakshmanan (2013). Biochemical Composition of Myctophid Species *Diaphus watasei* and *Myctophum obtusirostre* Caught from Arabian Sea. Fishery Technology, 50, 41–44.

Published Abstracts

- Pradip Kumar Mahato, **Libin Baby**, T.V. Sankar, R. Anandan P.K. Vijayan, George Ninan and A.A. Zynudheen. Myctophids: An Alternate Protein Source from Deep Sea. Abstract of papers, National Seminar on Conservation and Sustainability of Coastal Living Resources of India, 1-3 December 2009, Cochin, India, p 731.