CHAPTER V
RESULTS AND DISCUSSION

Food and Agricultural organization’s most recent estimates indicate that 12.5 per cent of the world’s population (868 million people) are undernourished, 26 per cent of the world children are stunted, 2 billion people suffer from one or more micronutrient deficiencies and 1.4 billion people are overweight, 500 billion are obese. The social burden due to child and maternal malnutrition has declined almost half during the last two decades, while that due to overweight and obesity has almost doubled, yet the former remains by far the greater problem, especially in low – income countries. Economic and social development lead to gradual transformation of agriculture, characterized by rising labour productivity, declining shares of population working in agriculture and rising urbanization. New modes of transportation, leisure employment and work within the home cause people to lead more sedentary life styles and to demand more convenient food. These changes in activity and dietary patterns are part of a “nutrition transition”. Agricultural productivity growth contributes to better nutrition through raising income, and by reducing the cost of food for all consumers. It is however, important to realize that the impact of agricultural growth is slow and may not be sufficient to cause a rapid reduction in malnutrition. Beyond staple foods, healthy diets are diverse, containing a balanced and adequate combination of energy, fat and protein, as well as micronutrients. Therefore research and development priorities must be made with a stronger focus on nutrient dense food. (The state of Food and Agriculture, 2013).

Sea vegetables, which are commonly referred to as marine algae or seaweeds, have been a staple food since ancient times in countries located by the sea, viz., U.K,
Ireland, Norway, the Pacific Islands, African countries and American countries. In modern days, they have become primarily associated with Asian cuisine. Japan which has the world’s largest seaweed consumption per capita with 10 – 15%, is also accorded with a significantly lower rate of cancer, thyroid diseases, heart diseases and dementia (Fitz Gerald et al., 2011). Today China, Japan and the republic of Korea are the largest consumer of seaweeds as food. Seaweeds are considered to be the food supplement for 21st century and as source for proteins, lipids, polysaccharides, minerals, vitamins and enzymes. In India, seaweeds are generally being used as raw materials for the production of agar, alginates and seaweed liquid fertilizer, in spite of their great potential as therapeutic health booster, beauty enhancer and the source of nutrition. Hence it becomes essential to popularise seaweeds as health food which will help to feed undernourished people in India. For this purpose it requires a thorough study on the occurrence, availability status and diversity of seaweeds both locally and regionally, their neutraceautical and therapeutical value which provide a baseline data for further exploration. India has water on three sides but like a landlocked nation it has been oblivious to seaweeds. In the last decade kappaphycus alvarezii has been commercialized in India which is one of the most important sources of carrageenan, a gel forming material widely used in frozen desserts, cottage cheese, sauces, jellies etc. So in the present study an attempt has been made to picturise the diversity and availability status of seaweeds, found along the coast of Thoothkudi, Gulf of Mannar, a Marine Biosphere Reserve Park for deployment of seaweeds in food industry.

5.1 Diversity of seaweeds

Seaweeds were surveyed during three seasons viz., pre monsoon (June – September), monsoon (October – January) and post monsoon (February – May) at
unique and distinct stations during low-tide in the year 2011 – 2012 by random sampling method. The study indicated that, Hare Island was endowed with numerous taxa belonging to Chlorophyceae (30 species) Phaeophyceae (28 species) and Rhodophyceae (32 species). Seventeen taxa were found as most abundant in this area. In Hare Island the seaweeds were abundant during post monsoon followed by monsoon season and were less in pre monsoon season (Table 1 to 3). Among three seasons, species diversity was rich in post-monsoon season followed by monsoon and very less in pre-monsoon, which was in agreement with earlier reports (Kalimuthu et al., 1992 and Darsis and Arunkumar, 2008). In Hare Island red seaweeds like *Gracilaria corticata*, *G. Verrucosa* and *G. pygmaea* (agarophytes) and brown seaweeds such as *Padina tetrastromatica* and *P. pavonia* (alginophytes) were found most abundant in all the seasons, which exhibited their good adaptability and reproducibility in the prevailing environmental conditions. These species would serve as ideal raw material for phycocolloid and food industries. Some of the species like *Ulva lactuca*, *Ulva reticulata*, *Enteromorpha compressa* (Chlorophyceae), *Spathoglossum asperum*, *Stoechospermum marginatum*, *Turbinaria ornata*, *Turbinaria conoides* (Phaeophyceae) and *Hypnea musciformis*, *Acanthophora spicifera*, *Corynophora prismatica* and *Kappaphycus alvarezii* were most abundant in post monsoon season than other periods. Species such as *Turbinaria ornata*, *Turbinaria conoides*, *Rosenvingea intricata*, *Pocokiella variegata* and *Scinaia furcellata* were completely absent in pre monsoon season. The study revealed that the occurrence of seaweeds varied seasonally within the same locality (Sree Kala Devi et al., 2004). In Thoothukudi region post monsoon season was noted for proliferous growth of different members of Chlorophyceae, Phaeophyceae and Rhodophyceae which deviated from the Tirunelveli region (John Peter Paul and Sri Devi, 2013).
observed pattern of seasonal distribution in Hare Island, Tuticorin coast is likely to be related to the life cycle pattern of the seaweeds (Worm et al., 2001). The supply of nutrients from inland after the short rainy season, and the associated climatic conditions would probably favoured the growth and establishment of these seaweed species during post - monsoon season. Distributional diversity and abundance of species were fairly high in the coast of Hare Island agreeing with data reported from nearby regions (Stella et al., 1997; Edwin et al., 2004; Krishnamurthy, 2006; Kaliaperumal, 2007; Mary Josephine et al., 2013) leading to suggest that Hare Island is highly suitable for seaweed harvest and cultivation.

5.2 Analysis on the nutritive components in seaweeds

Seaweeds grow in the intertidal as well as in the sub tidal region up to a certain depth where 0.1% photosynthetic light is available. They are one of the ecologically and economically important living resources of the world oceans. Seaweeds are flexible, tenacious and prolific, being continuously bathed in nutrient rich seawater, absorb high level of nutrients and thus form important source of food, feed, fertilizer and chemicals. Certain seaweeds were reported to contain significant quantities of proteins, lipids, carbohydrates / polysaccharides, minerals, amino acids and vitamins (Reeta and kuladaivelu, 1999; Vinoj kumar and kaladharan, 2007; Manivannan et al., 2008; Nirmal Kumar et al., 2010; Anantharaman et al., 2011; Van Ginneken et al., 2011; Ramesh kumar et al., 2012; Thinakan and Sivakumar, 2012; Usha et al., 2013). The seaweeds are used in human or animal food for their mineral contents or for the functional properties of their mineral contents or for the functional properties of their polysaccharides (Joel Fleurence, 1999). In Asia, seaweeds have been used in human nutrition since ancient times. The most common seaweeds used
as food include green algae such as *Enteromorpha intestinalis*, *Ulva lactuca*, *Caulerpa racemosa*, brown algae like *Laminaria digitata*, *Undaria pinnatifida*, *Sargassum fusiformis*, *Ascophyllum nodosum* and red algae *Porphyra umbilicalis*, *Palmaria palmata*, *Gracilaria corticata*, *Hypnea musciformis* (Morrisey *et al*., 2001; Dhargalkar and Neelam Pereira, 2005; Paul Mac Artain *et al*., 2007; Gour Gopal Satpati and Ruma Pal, 2011). A wide variety of seaweeds growing along the Hare Island, Gulf of Mannar viz., green algae *Ulva lactuca*, *Ulva reticulata*, *Enteromorpha compressa*, *Caulerpa scalpelliformis*, brown algae *Padina tetrastromatica*, *Padina Pavonia*, *Spathoglossum asperum*, *Sargassum sp*, *Stoechospermum marginatum* and red algae *Hypnea musciformis*, *Gracilaria corticata*, *G. verrucosa*, *G. pygmaea*, *Kappaphycus alvarezii* were found most abundant particularly in post monsoon season and abundant in other seasons and the nutritive properties of these seaweeds and their seasonal oscillations are poorly known. Variability in chemical compositions may be inter specific, intra-annual or inter-seasonal. The present study discusses the variability in the nutritive composition of three groups of algae (seaweeds), widely distributed in Hare Island.

5.2.1 Carbohydrate

Carbohydrate is the most important component for metabolism as it supplies the substrate and energy needed for respiration and other metabolic processes. Changes in carbohydrate content in different seaweeds in different seasons were observed during the present study (Table 4). The carbohydrate level of seaweeds ranged from $14.25 \pm 1.21$ mg/g DW to $291.11 \pm 4.34$ mg/g DW. Among Chlorophycean members the highest amount of carbohydrate was found in *Valoniopsis pachynema* during post monsoon ($175.55 \pm 7.3$ mg/g DW), monsoon ($169.8 \pm 0.48$ mg/g DW) and
pre- monsoon (160.50 ± 0.43 mg/g DW). Significant seasonal differences (p<0.01) were observed among green seaweeds. There was greater variation in the amount of carbohydrate among brown seaweeds (Table 4). Highest amount was noted in *Sargassum ilicifolium* during post monsoon season and the minimum was observed in *Colpomenia sinuosa* during monsoon. In brown seaweeds except *Sargassum polycystum* and *Sargassum ilicifolium* all other seaweeds showed significant seasonal variation (p<0.01). Carbohydrate content in Rhodophycean members presented in table (4) indicated a greater inter-specific difference and the value were ranged between 14.25 to 291.11 mg/g DW. Among Rhodophyceae highest value was found in *Rhodymenia palmata* (291.11± 4.34 mg/g DW) and lowest value was found in *Amphiroa aniceps* (14.25± 1.21 mg/g DW). Significant (p<0.01) seasonal differences could be found in members of Rhodophyceae except *Gracilaria corticata*, *Corynomorpha prismatica* and *Kappaphycus alvarezii*.

In the present study it was found that the carbohydrate content was more in post monsoon in 97% of the species. Among the three groups of seaweeds Rhodophycean members contained the maximum amount of carbohydrate, followed by members of Phaeophyceae and Chlorophyceae. Similar results were reported by Dhargalkar *et al.*, (1980) and Shoba *et al.*, (2001) who had analyzed seaweeds from Maharashtra coast and Kovalam coast respectively. The high content of carbohydrate in red algae might be due to higher phycocolloid content in their cell walls (Dharagalkar *et al*; 1980). Variable level of carbohydrate were recorded during July in *U. reticulata* 50.24% of DW by Shanmugam and Palpandi, (2008), *Gracilaria sp* 48.4% of DW by Reeta and Kulandaivelu (1999) and *Enteromorpha sp* 54.71% of DW by Haroon, (2000). Furthermore, Sasikumar (2000) recorded respective carbohydrate
contents of 20.4 and 54.6% in the green algae Enteromorpha, Codium linum, but Hossain et al., (2003) found 19.93% in the brown alga Sargassum horneri. Thus the previous reports remained in conformity with the variation observed among different species in the present investigation. Marked changes in carbohydrate level among species with seasons may possibly be due to surface water temperature, prevailing environmental conditions, light penetration, in flow of nutrients from inlands as well as the phases of seaweeds growth. Comparatively, many of the Phaeophycean members possessed considerable amount of carbohydrate as revealed in the present study could be accounted to the alginate present in them. Alginates of seaweeds have a soothing and cleansing effect on the digestive tract in human beings (Dhagalkar and Neelam Pereira, 2005). Sulphated polysaccharides from seaweeds have been used in the films that are placed between bones to be grafted in order to accelerate the growth of the connective tissue (Jens and Von Linden, 2002). Seaweed polysaccharides are also used to treat arthritis as they are active in promoting and aiding the healing process of the body (Skjak- Brack et al., 1990). Alginates reduce the absorption of cholesterol in the gut, promote wound healing and inhibit the growth of cancer cells (Laurienzo, 2010; Kraan, 2012). Alginates can be easily blended into food items such as yogurts, creamy desserts, pasta, bread, pizza dough and meat patties without any adverse effect on the taste or texture. (Rajapakse and Kim, 2011; Venugopal, 2011). Therefore, the results advocated the utilization of seaweeds such as Rhodymenia palmata, Sargassum sp, Caulerpa racemosa, Enteromorpha sp, Ulva sp, Kappaphycus alvarezii, Gracilaria edulis, G. corticata and Hypnea musciformis in food preparations and also as food supplement with admissible quantity in place of high calorific food products.
5.2.2 Dietary fiber

The main components of dietary fiber are alginates, carrageenans and agar, depending on the types of seaweeds. These fibers are not digested to any great extent in the gut, but show some fermentative capacity in the lower intestine. In addition, dietary fibre can increase feelings of satiety and aid digestive transit through their bulking capacity (Goni et al., 2001; Brownlee et al., 2005). *Porphyra umbilicalis* which is normally processed into ‘nori’ sheets contains slightly more fiber (3.8g / 100g wet wt) than bananas (3.1 g/100g wet wt) in direct weight comparison. On comparing *Laminaria digitata* (Kombu) with brown rice (3.8%), the seaweed shows a higher (6.2%) level of total fiber (Paul Mac Artain et al., 2007). Seaweeds can provide up to 12.5% of a person’s daily fiber need in an 8 g serving. The main beneficial action of fiber in the digestive system is linked to the increase in fecal bulk associated with the water holding and binding capacity of the polysaccharide. This helps decrease colon transit times which are a positive factor in preventing colon cancer (Sanderberg et al., 1994; Goni et al., 2001). The results of the present study clearly indicated that *Rhodymenia palmata*, *Sargassum ilicifolium*, *Padina tetrastromatica* and *Hypnea musciformis* contained comparatively higher amount of dietary fiber (Table 5). Therefore *R. palmata*, *S. ilicifolium* *P. tetrastromatica* and *H. musciformis* could be substituted with terrestrial vegetables for human consumption with proper processing. However, palatability, acceptability and incompatible problems must be analyzed before consumption. The study placed a record of resources which could be tapped and scientifically tested for commercial utility.
5.2.3 Protein

Development of novel food could be a new possibility for the use of seaweeds especially the protein-rich species in human nutrition. The use of algae with high protein levels in the production of feeds for fish farmed by aquaculture could be another application. The analysis on protein content among members of Chlorophyceae, Phaeophyceae, and Rhodophyceae revealed that most of the species were rich in protein ranging from 2.11 mg/g DW to 105.87 mg/g DW. Among the Chlorophyceae members, Ulva lactuca (69.30 ± 4.54 mg/g DW) contained highest amount of protein. However, very low amount of protein could be noted in Halimeda tuna (3.13 ± 0.63 mg/g DW). There was seasonal variation in protein level among Chlorophycean members, maximum being observed in U. lactuca during post monsoon. Data represented in Table (6) shows the protein content found among Phaeophyceae members. The maximum level of protein was estimated in Colpomenia sinuosa (105.87 mg/g DW) collected during post monsoon season. Turbinaria conoides seen only during monsoon and post monsoon seasons also contained appreciable amount of protein. Among Rhodophyceae members the highest amount was noted in Acanthophora spicifera (85.14 ± 0.49 mg/g DW) and lowest in Corynomorpha prismatica (2.11 ± 0.77 mg/g DW). Among three groups of seaweeds Phaeophycean members were found to be enriched with protein. Analysis of variance also revealed significant difference in protein among members of Chlorophyceae (Ulva lactuca, Ulva reticulata, Enteromorpha intestinalis, Enteromorpha compressa, Codium tomentosum, Codium bursa, Caulerpa racemosa, Caulerpa sertularioides, Caulerpa scalpelliformis and Valoniopsis pachynema), Phaeophyceae (Colpomenia sinuosa, Padina tetrastromatica, Stoechospermum marginatum, Spathoglossum asperum, Sargassum polycystum, Sargassum wightii,
Sargassum ilicifolium and Sargassum tenerrimum) and Rhodophyceae (Amphiroa aniceps, Hypnea musciformis, Hypnea valentiae, Gracilaria corticata, Gracilaria verrucosa, Gracilaria pygmaea, Acanthophora spicifera, Acanthophora delilei, Corynomorpha prismatica and Kappaphycus alvarezii) during pre-monsoon, monsoon and post-monsoon (Table 6). Insignificant variation was noticed between seasons in species such as Halimeda tuna, Padina pavonia, Sargassum cinereum, Sargassum duplicatum and Rhodymenia palmata. The observed difference in protein content among species is in corroboration with earlier reports (Dharagalkar et al., 1980). Generally the protein fraction of green seaweed is low in comparison with red or brown seaweeds which is in accordance with others (Selvi et al., 1999). Ulva pertusa, which is frequently consumed under the name of ‘ao–nori’ by the Japanese people, has a high protein level between 20 and 26% in dry product (Fujisawa–Arasaki et al., 1984; Joel Fleurence, 1999). Higher protein levels were recorded for the red seaweeds such as Porphyra tenera (47% of dry mass) Palmaria palmata (35% of dry mass) (Morgan, 1980; Arasaki and Arasaki, 1983) and these algae known under the names of nori and dulse respectively have protein levels higher than that of pulse and soyabeans. In the present study species such as Colpomenia sinuosa, Turbinaria conoides, Acanthophora spicifera and Hypnea musciformis contained protein comparable to terrestrial pulse crops, which suggest the possible contribution of seaweeds for balanced diet. However the bioavailability of seaweed protein can sometimes be inhibited by the entrapped nature of the proteins in cellular matrix. Increasing the bioavailability by using physical processes or fermentation to break down the fibres and to liberate more protein has to be studied. The level of digestibility of these proteins seems to be related to the amount of soluble fiber in the
seaweed species, preventing bioavailability of the protein (Paul Mac-Artain et al., 2007).

5.2.4 Protein digestibility:

The digestibility of seaweed protein was carried out in selected seaweed species, with the enzyme trypsin, α chymotrypsin and peptidase. The relative digestibility of seaweed protein is expressed as percentage of casein digestibility base (100%). The relative digestibility of alkali-soluble proteins from *Sargassum tenerrimum* is higher (69.7 %) than other seaweed proteins. The results indicated a varied digestibility pattern among species i.e., *Colpomenia sinuosa*, *Padina pavonia*, *Gracilaria verrucosa* and *U. lactuca* which could be comparable to other reports (Joel Fleurence, 1999). *Sargassum tenerrimum* appeared to be more sensitive to the action of human intestinal juice than to the action of trypsin or chymotrypsin (Joel Fleurence, 1999). In conclusion, the *in vitro* digestibility of seaweed proteins can differ according to the species. The compounds which limit the digestibility of seaweed proteins are either phenolic molecules or polysaccharides. Studies performed on brown seaweeds showed the strong inhibitory action of soluble fibres on *in vitro* pepsin activity and their negative effects on protein digestibility (Horie *et al*., 1995). However an enzymatic pre- treatment of seaweeds allowing the removal of polysaccharides could be an alternative way to limit the influence of algal fibres as anti- nutritional factors. The seaweeds, especially the Chlorophyceaeen and Rhodophyceaeen members could be a complementary source of food protein for human beings and animal nutrition. A biotechnological treatment of seaweeds by enzymatic degradation of algal fibers and antinutritional factors could be attempted to improve protein digestibility and thus increase the nutritional value of these proteins.
The use of high level proteinaceous seaweeds seem to be promising way for the utilization of this marine resource in partial substitution of animal meal and plant meal in fish feed.

5.2.5 Total free amino acids

Total free amino acids was estimated in selected members of three groups of seaweeds and recorded in Table (8). The data indicated that among green seaweeds, *U. lactuca* (25.9 mg/g DW) contained the maximum amount while most of the other green seaweeds possessed very low amount of total free amino acids. There was insignificant variation in amino acid value found in seaweeds between seasons and also between species. In brown seaweeds the amino acid level varied, with the highest value found in *Colpomenia sinuosa* (8.99 mg/g DW) which was comparatively lesser than green seaweeds. Furthermore, insignificant species specific variations and seasonal variations were noticed among brown seaweeds. Red seaweeds were noted for their higher level of total free amino acids with maximum amount being in *Gracilaria pygmaea* (29.20 mg/g DW). Except *Corynomorpha prismatica*, *Amphiroa anceps* and *Hypnea valentiae* all the other Rhodophycean members contained considerable amount of total free amino acids which could be comparable to land plants. Among red seaweeds also seasonal variation was noticed but it was insignificant. Among the three groups studied, red seaweeds were found to have more total free amino acids. Similar study was reported by others in different seaweed species. (Reeta, 1993; Reeta and kulandaivelu, 1999; Sasikumar, 2000; Gour Gopal Satpati and Ruma Pal, 2011; Ommee Benjama and Payap masniyom, 2012; Rameshkumar et al., 2012; Thinakaran and Sivakumar, 2012).
5.2.6 Amino acid composition

The nutritive evaluation of seaweeds on the basis of amino acid composition was carried out in five seaweeds namely *Ulva reticulata*, *Enteromorpha intestinalis*, *Sargassum cinereum*, *Gracilaria verrucosa* and *Gracilaria pygmaea* using high performance liquid chromatography. Data of essential amino acids and non essential amino acids of investigated seaweeds were shown in Table (9). The quantity of non-essential amino acids such as asparatic acid, glutamic acid, asparagine, serine, glutamine, glycine, alanine, cysteine and proline were ranged from 0.948 to 2.713 mg/100g. In *U. reticulata* aspartic acid and glycine were found higher and proline was found to be absent. In *E. intestinalis* alanine and aspartic acid were found considerably higher than other non-essential amino acids. Proline was negligible and cysteine was not detected except *U. reticulata*. In *S. cinereum* all the non-essential amino acids were seemed to be comparatively lesser than other species investigated. In red seaweed *G. verrucosa*, similar to *E. intestinalis* alanine and aspartic acid were higher. However *G. pygmaea* contained all the non-essential amino acids except cysteine in considerable amount and it ranked first in the composition of non-essential amino acids among investigated species. Also it was noteworthy to find that stress response amino acid proline was not found in *U. reticulata* and present in very low amount in all other species. This result reflected that the habitat of these seaweeds was well suited for their growth and establishment. However Chakraborty and Bhattacharrya, (2012) reported higher proline content in *G. corticata* which indicated that amino acid composition dependent upon the physico chemical condition of the growing environment. In the present study the species analysed also contained aspartic and glutamic acids, which are responsible for the special flavour and taste of
seaweeds. Similar results have been obtained in previous studies (Wong and Cheung, 2000; Gressler et al., 2010).

Essential amino acids like threonine, arginine, alanine, tyrosine, histidine, methionine, isoleucine, phenylalanine, leucine, lysine, were found in all the seaweeds investigated (Table 9). In *U. reticulata* tyrosine and phenylalanine were found considerably in higher level. Isoleucine was present in significant amount in *E. intestinalis* however all the other essential amino acids were reported to be low. In *S. cinereum* except methionine all other essential amino acids were present in lesser quantity. Threonine was more than all other amino acids in *G. verrucosa*. Similar to non-essential amino acids, essential amino acids were also found to be more in *G. pygmaea*. The higher amount of some amino acids like isoleucine (0.9914 mg/g DW) methionine (0.5439 mg/g DW) leucine (0.5093 mg/g DW) recorded during the study is in conformity with earlier studies (Hanan Hafer omar et al., 2013). Tabarsa et al., (2012) reported that there were some pronounced differences between amino acid profiles of various seaweeds. In the present study of cysteine was noted only in *Ulva reticulata* and tryptophan in *Sargassum cinereum*. Seaweeds contain 1000 times as much iodine as cod as average iodine containing fish. Seaweeds provide di-iodotyrosin which is the precursor of thyroid hormones; thyrosin and tri-iodo thyrosin (Davis, 1991). As *Ulva reticulata* and *G. pygmaea* were enriched with tyrosine these seaweeds could be effectively utilized to regulate thyroid hormones level. The ratios of essential amino acid to total amino acids of tested species were almost high (above 0.5) reflecting the distribution of more essential amino acids among all the species. Presence of 45-49% of essential amino acid in *K. alvarezii* and *H. musciformis* and more than 40% in *C. lentillifera* and *U. reticulata* (Ratana-arporn
and Chirapart, 2006; Vinoj Kumar and Kaladharan, 2007) occurred in various regions of the sea, corroborate with the present study. The results of the study suggested that these species could be used as an alternative nutrient sources of protein and amino acids for human and animal consumption.

5.2.7 Lipid

Lipid provides more energy in oxidation processes than any other biological compounds. They constitute a convenient storage material for living organisms. Marine algae produce a variety of glycerides or lipids having biocidal and antimicrobial properties. (Padmini Sreenivasa Rao, 1991; Venkataraman Kumar, 1993). The lipid content of seaweeds varied from 0.5±0.03 mg/g DW to 34.4 ± 0.11 mg/g DW (Table 10). In fact, in comparison to other chemical constituents, lipid was observed to be very low in all the species studied. The present investigation exhibited the maximum lipid level in *Kappaphycus alvarezii* 34.4 mg/g DW which is in confirmity with Fayaz et al., (2005) suggesting the utility of *K. alvarezii* in various nutritional products including antioxidants, for use as health food or nutraceutical supplement. Among the members of Phaeophyceae lipid content was more in *Stoechospermum marginatum* (5.3 ± 1.56 mg/g DW) in post - monsoon and less in *Turbinaria ornata* (0.5 ± 0.001 mg/g DW) and *Sargassum duplicatum* (0.6 ± 0.01 mg/g DW) in monsoon season. Among Chlorophyceae, the highest lipid content was recorded in *Caulerpa racemosa* (9.1 ± 0.01 mg/g DW) and lowest lipid content in *Enteromorpha intestinalis* (1.1 ± 0.01 mg/g DW). Lipid content present in *E. intestinalis* was closely related to the results of Manivanan et al., (2008) establishing that this biochemical component did not vary much with their habitat. The lipid content of *Ulva reticulata* (3.2 ± 1.04 mg/g DW) recorded in the present
study resembled Muthuraman and Ranganathan, (2004). Moreover very low lipid level found in many seaweed species is comparable to previous reports (Kumar, 1993; Mercer et al., 1993; Ratana-arporn and Chirapart, 2006; Shanmugam and Palpandi, 2008). Variation in the amount of lipid could be noted among different seasons. However, more amount was estimated in many of the species during post-monsoon season. So post-monsoon season is considered to be more suitable for seaweed collection and seaweed exploration in food industries. Significant variation among seasons was noticed among members of Chlorophyceae (p<0.01) except *Ulva reticulata* and *Caulerpa scalpelliformis*. Among phaeophycean members significant (p<0.01) variation was found between seasons only in certain members (Table10). Insignificant variation between seasons was noticed in *Colpomenia sinuosa*, *Padina pavonia*, *Spathoglossum asperum*, *Sargassum cinereum* and *S.tenerrimum*. Similar trend was noticed among members of Rhodophyceae (Table10).

**5.2.8 Fatty acid composition:**

Seaweeds contain many essential fatty acids, which may add to their efficacy as a dietary supplement or as part of a balanced diet. Lipids are major sources of metabolic energy during the embryonic and pre-feeding fish larval stages (Evans et al., 2000). The chromatograms of fatty acid profile of seaweeds namely *Ulva reticulata*, *Caulerpa racemosa* (Chlorophyceae) *Padina tetrastromatica*, *Spathoglossum asperum* (Phaeophyceae) *Gracilaria verrucosa* and *Kappaphycus alvarezii* (Rhodophyceae) are shown in Fig. 2 (a to g). Fatty acid composition of six seaweeds and their relative percentage are presented in Table (11). The fatty acids detected commonly in all seaweeds are palmitic acid (C16:0), margaric acid (C17:0), stearic acid (C18:0), oleic acid (C18:1 n-9), linolenic acid (C18:2n-6), α linolenic acid
(C18:3, n-3) and morotic acid (C18:4 n-3) which totally ranged between 0.1279 and 14.1816 mg/100g DW. Highest amount of total fatty acids was present in S. asperum (14.1816 mg/100g DW) and K. alvarezii (13.743 mg/100 g DW) and lowest level of total fatty acids was noticed in G. verrucosa (0.6236 mg/100g DW) and C. racemosa (0.1279 mg/100g DW). The unsaturated fatty acids of all five seaweeds except K. alvarezii was higher than saturated fatty acids which is in agreement with previous reports (Khotimchenko and Svetashev, 1987; Khotimchenko and Kulikova, 2000). Relatively higher amount of saturated fatty acids recorded in K. alvarezii in the present study was in confirmatory with Muralidhar et al., (2010). Fatty acid compositions of algal lipids vary widely with species, habitat, light, salinity, pollution and environmental conditions (Kim et al., 1996; Ratana-arporn and Chirapart, 2006). The quantity of palmitic acid (C16:0) ranged between 0.0034 to 2.45 mg/100g DW. Of all the seaweeds, S. asperum contained high amount of palmitic acid (2.93) and G. verrucosa contained the least (0.0034). Heptadecanoic acid or margaric acid, a saturated fatty acid, was found to be more in K. alvarezii (0.345 mg/100g) and the order tend to be decreased as S. asperum (0.2034 mg/100g) > U. reticulata (0.1131 mg/100g) > P. tetrastrumatica (0.0191 mg/100g) > C. racemosa (0.0011 mg/100g). It was completely absent in G. verrucosa. Stearic acid, (C18:0) another saturated fatty acid ranged between 0.0545 mg/100g (K. alvarezii) to 3.912 mg/100g (C. racemosa). Oleic acid (C18:1) is one of the mono unsaturated fatty acids varied from 0.1023 mg/100g (C. racemosa) to 3.03 mg/100g (S. asperum). Unsaturated fatty acids were predominantly present in brown and green seaweeds investigated which concurred with earlier findings (Gour Gopal Sapati and Rupa pal, 2011). Kamenarska et al., (2002) have investigated lipid composition of Cystoseira crinita from Eastern Mediterranean region and found palmitic acid, myristic (C14:0) and oleic (C18:1n-9)
acids as main fatty acids in them. Marine algae are rich in polyunsaturated fatty acids (PUFAs) (Wood, 1988; Kayama et al., 1989) and are of potential value as sources of essential fatty acids, important in the nutrition of human beings and animals (Floreto et al., 1996; Newton, 1996). Although macro algae have been reported to have low lipid content (Mabeau and Fleurence, 1993; Ambreen et al., 2012; Parthiban et al., 2013) their polyunsaturated fatty acid composition is superior to those of terrestrial vegetables in regard to human diet (Goecke et al., 2010; Kumari et al., 2010). Linoleic acid (C18:2) and α-linolenic acid (C18:3) are the two PUFAs which cannot be synthesized by human beings and other vertebrates. When seaweeds are consumed, within the body both can be converted into other polyunsaturated fatty acids such as arachidonic acid (C20:4, n-6), eicosapentaenoic acid (EPA, C20:5, n-3) and docosahexaenoic acid (DHA, C22:6, n-3) (Ginneken et al., 2011). Among seaweeds investigated linolenic acid (C18:2 n-6) was ranged between 0.0019 to 2.225 mg/100g DW. Highest amount of linolenic acid (C18:2n-6) was found in K. alvarezii and S. asperum accounting 2.225 and 2.114 mg/100g DW respectively. In C. racemosa linolenic (0.0019 mg/100g DW) and α-linolenic (0.0214 mg/100g DW) were found to be very low. α- linolenic acid (C18:3, n-3) was recorded more in S. asperum, U. reticulata and K. alvarezii and was 3.09 mg/100g, 1.093 and 1.0134 mg/100g DW respectively. Morotic acid (C18:4n) was present only in U. reticulata (0.0134 mg/100g DW) and it was found in traces among other seaweeds and absent in C. racemosa. Lipids represent only 1–5% of algal dry matter and exhibited an interesting polyunsaturated fatty acid (PUFA) composition particularly omega 3 and omega 6 fatty acids which play an important role in the prevention of cardio vascular diseases, osteoarthritis and diabetes (Banerjee et al., 2009). In conclusion, the taxonomic differences in fatty acid composition of marine red, brown and green algae
observed in the present study invariably be due to habitat conditions, growth stages, nutrients and eco physiological status of seaweeds. Variation in the fatty acid content of seaweeds from different biogeographical regions have been reviewed, (Arao and Yamada, 1989; Heiba et al., 1997; Kamenarska et al., 2002; Orhan et al., 2003; Kumari et al., 2010; Ommee Benjama and Payap Mashiyom, 2012) which confirmed the present results. In spite of the variability of the fatty acid composition of algae under different habitat conditions, their specific features remain constant. Marine algae are rich in poly unsaturated fatty acids of the n-3 and n-6 series, which are considered as essential fatty acids for human beings and animals. Some of these fatty acids (20:3n-6, 20:4n-6, 20:5n-3) have high biological activity and are converted into eicosanoids. In addition, polyunsaturated fatty acids are of interest in cosmetics as components of sun lotions and as regenerating and anti-wrinkle products (Helme, 1990). It is concluded that seaweeds could be competently used as a potential source of fatty acids for cosmetics and as dietary source of essential fatty acids.

5.2.9 Minerals:

From nutritional point of view seaweeds are low calorie food with high concentration of minerals, vitamins, proteins, indigestible carbohydrates and low concentration of lipids. Minerals are very important for the biochemical reactions in the body as a co-factor of enzymes. The high amount of minerals found in them are due to their marine habitat, and the wide diversity of the minerals they absorb. Seaweeds generally contain 8 to 40% of minerals, including essential minerals and trace elements needed for human nutrition, and the mineral content is higher than that of land plants and animals. (Mabeau and Fleurence, 1993; Ortega – Calvo et al., 1993; Matanjun et al., 2009; Polat and Ozogul, 2009). Totally eight seaweeds, belonging to
Chlorophyceae (Ulva reticulata, Caulerpa racemosa, and Enteromorpha intestinalis) Phaeophyceae (Padina tetrastromatica and Sargassum cinereum) and Rhodophyceae (Acanthophora spicifera and Gracilaria verrucosa) were analysed for their mineral value. Calcium concentration varied between the seaweeds investigated and was found to be the highest in *U. reticulata* (431.3 mg/100g dw) and lowest in *C. racemosa, A. spicifera* and *G. verrucosa* (134.5 mg/100g dw). Calcium is required for the formation of bones and teeth structure and also controls the functions of nerves and muscles. It acts as a cofactor for extra cellular enzymes and proteins. Calcium malnutrition causes abnormal bone formation, namely osteoporosis (Reinhold, 1988; Martinez – Navareete *et al.*, 2002).

Magnesium level varied from 67.6 to 10.34 mg/100g dw; the maximum being recorded in *S. cinereum* (67.6 mg /100g dw) and minimum level was attained in green seaweed *E. intestinalis* (10.34 mg/100g dw). Magnesium plays key roles as cofactor of many enzyme-linked biochemical reactions in different physiological processes; ATP dependant metabolic reactions; essential for brain and liver function; promotes cell growth; increases tissue elasticity; neuromuscular functions (Mc Dermid and Stuercke, 2003). Maximum level of zinc was noted in Phaeophycean member, *S. asperum* (2.8399 mg/100g dw) and the lowest level was observed in Rhodophycean member, *A. spicifera* (1.2344 mg/100g dw). Minerals such as iron and copper are present in seaweeds at higher levels than in many well-known terrestrial sources of minerals, such as meat (5%) and spinach (2%). Iron was observed more in green seaweed *E. intestinalis* (5.67 mg/100g dw) and the lowest amount was recorded in brown seaweed *P. tetrastromatica* (0.3343 mg/100g dw). Concentration of copper level was varied from 0.4254 mg/100g to 0.2034 mg/100g dw with the maximum in
E. intestinalis (0.4254mg/100g DW) and the minimum level in U. reticulata (0.2034 mg/100g DW). In P. tetrasstromatica and S. cinereum copper was found to be absent. Manivannan et al., (2009) estimated the mineral composition of different seaweeds from Mandapam coast, and reported P. gymnospora as a rich source of minerals. Sodium and potassium involve in acid – base balance and transfer of nutrients in and out of individual cells (Ensminger et al.; 1995). Sodium concentration varied from 34.65 to 156.7 mg/100g DW with maximum in E. intestinalis (156.7 mg/100g DW) and minimum level in U. reticulata (34.65 mg/100g DW). Sodium is stored in stomach walls, joints, and gallbladder; helps preventing blood clotting; important for membrane function, nerve impulses, and muscle contractions; major cation in body fluids; contributes to the alkalinity of the lymph and blood; works with the bicarbonate buffer system in the digestive tract to prevent hydrochloric acid from burning stomach walls; helps retain calcium and cholesterol level in the body (SubbaRao et al., 2007). Potassium was present in elevated level and the values ranged from 201.3 mg/100g DW in G. verrucosa to 41.35 mg/100g DW in U. reticulata (Table 12). It is an important nutrient for proper membrane function, nerve impulses, and muscle contractions; a major cation in cytoplasm; a primary electrolyte and alkalizer; attracts oxygen to tissues; helps to eliminate toxins from the body (Ruperez, 2002). Mercury and cadmium were found absent in these seaweeds. Mineral composition of seaweeds were reported to vary according to factors such as species, geographical region, seasons, environmental, physiological fluctuations and species specificity (Mabeau and Fleurence, 1993; Kaehler and Kennish, 1996) which concurred with the present study. It is suggested that seaweeds could be used as a food supplement to meet the recommended daily intake of some essential mineral nutrients.
5.3 Seaweeds as antioxidants

About 5% or more of the inhaled oxygen is converted into reactive oxygen species (ROS) such as $O_2^-$ (superoxide radical), $H_2O_2$ (hydrogen peroxide) •OH (hydroxyl radical) and •NO (nitric oxide radical) by univalent reduction of oxygen (Maxwell, 1995). The oxidative stress, defined as the “imbalance between oxidants and antioxidants in favour of the oxidants potentially leading to damage” has been suggested to be the cause for series of events that deregulate the cellular functions, destroying biological molecules such as lipids, proteins, enzymes and nucleic acids and leading to various pathological conditions viz., aging, arthritis, asthma, atherosclerosis, autoimmune disease, broncho pulmonary displasia, carcinogenesis, cardiovascular disjunction, cataract, diabetes, genetic disorder, inflammatory diseases, liver disorders, muscular dystrophy, neuro degenerative diseases, alzheimer’s disease, pulmonary fibrosis, etc., (Jose and Janardhan, 2000; Tiwari, 2001; Kuda et al., 2005; Vinayak et al., 2010). Usually balance between formation of reactive species and antioxidant defence is kept in the body, but oxidative stress may result when these system fail to cope up with the production of reactive oxygen species (ROS) / reactive nitrogen species (RNS) (Kim et al., 2008). Antioxidant chemicals found in nature inhibit or prevent oxidation of substrate leading to ROS and thus protect the biological systems (Hwang et al., 2010). Butylated hydroxyanisole, butylated hydroxytoluene and tertiary butyl hydroxyquinone are common synthetic antioxidants that are being used by several food industries for preventing lipid oxidation in food products. Moreover, these antioxidants have been suspected to be carcinogenic, hence their use as food ingredients has been prohibited (Huang and Wang, 2004). Also social awareness has increased almost half during the last few years, imposed searching into antioxidants containing balanced food. Natural antioxidants comprised of non-
detrimental chemical combination, are considered to be rather more safer for use in food products. Amongst the sources of natural antioxidants marine seaweeds are now being considered to be not only a rich source of antioxidants (Chandini et al., 2008, Kim et al., 2008) but also low calorific food which would be a better solution for protecting people from physiological diseases, malnutrition and also obesity. Therefore, dominant seaweed species inhabited in Hare Island were analysed for their antioxidant chemicals and antioxidant activities in aqueous preparation.

5.3.1 Total phenol

Total phenol was estimated by folin-ciocalteu method and was expressed as mg.gallic acid equivalent per gram. The total phenolic content of studied Chlorophycean seaweeds ranged from 0.14 to 2.14 mg GAE/g. *Caulerpa racemosa* and *Valoniopsis pachynema* contained comparatively higher amount of total phenol whereas *Ulva reticulata*, *Enteromorpha compressa*, and *Halimeda tuna* had very low quantity (Table 13). All the brown seaweeds were found to have significantly higher concentration of total phenolics (Table 14) which paralleled with other reports (Cox et al., 2010; Amna Tariq et al., 2011). In brown seaweeds the phenol level was 4-5 folds greater than green and red seaweeds. Similar to green seaweeds except *Acanthophora spicifera* (3.65 mg GAE/g) total phenol was low in red seaweeds (Table 15). Phenolic compounds in plants and seaweeds have been reported to have a wide range of biological activities including antioxidant properties (Duan et al., 2006; kuda et al., 2007; Wang et al., 2009). Chandini et al., (2008) reported that brown seaweeds had phenolic content ranging from 24.61 to 49.16 mg GAE/g. Wang et al., (2009) recorded phenol level in different seaweeds of Iceland ranging from 4 to 242 mg GAE/g. Sanaa and Shanab, (2007) reported that phenol in red seaweed, *Jania*
_carniculata_ was more abundant than brown seaweed _Sargassum dentifolium_. Thillaikannu Thinakaran _et al._, (2012) brought out the presence of more amount of phenol in brown than green and red seaweeds from south coast of Gulf of Mannar. Thus the present study established that quantity of phenol varied with species and their area of occurrence. All the brown seaweeds analysed possessed significant amount of phenol, which was comparable to others. (Chandini _et al._, 2008; Thillaikannu Thinakaran _et al._, 2012).

### 5.3.2 Total Flavonoid

Data presented in Tables (13-15) show the total flavonoid content of three groups of seaweeds occurred in Hare Island. Similar to total phenol, flavonoid level was lesser in members of Chlorophyceae except _Ulva lactuca_ and the value ranged between 0.63 and 29.97 mg QE/g DW, thus revealing a wide variability even among species of Chlorophyceae. The study revealed that _Enteromorpha intestinalis_ and _Halimeda tuna_ possessed very low quantity of flavonoid. It was found that brown seaweeds in Hare Island were endowed with higher amount of flavonoid than other groups, with values ranging from 22.44 to 45.20 mg QE/g DW. _Spathoglossum asperum, Stoechospermum marginatum, Padina pavonia, Padina tetrastromatica_ and _Sargassum wightii_ were enriched with flavonoid, however other species like _Colpomenia sinuosa, Sargassum ilicifolium_ and _Turbinaria conoides_ also contained significant amount. Rhodophycean members exhibited wider variation in flavonoid level from 0.39 to 28.12 mg QE/g DW. _Acanthophora spicifera_ was noted for higher flavonoid level among Rhodophycean members. Further, _Amphiroa aniceps_ and _Rhodymenia palmata_ had negligible amount. Flavonoids are probably the most important natural phenolics with broad spectrum of chemical and biological activities.
including antioxidant and free radical scavenging properties (Kahkohen et al., 1999). Flavonoids include flavonols, flavones, catechins, proanthocyanidins, anthocyanidins and iso flavonoids (Ferreira and Pinho, 2012). Flavonoids have been reported as antioxidants, scavengers of a wide range of reactive oxygen species and inhibitors of lipid peroxidation, and also potential therapeutic agents against many diseases (Ross and Kasum, 2002; Williams et al., 2004). The study clearly indicated that brown seaweeds in Hare Island were more befitting for flavonoid extraction.

5.3.3 Tannin

Total condensed tannin content varied among three groups of seaweeds, (Tables 13-15). Except *Ulva reticulata* all the Chlorophycean members investigated, accounted considerable amount of tannin. However *Valoniopsis pachynema* which was noted for very poor protein and amino acids contained maximum amount of tannin in comparison with other members of Chlorophyceae. It was interesting to notice that all the members of Phaeophyceae analysed, showed higher level of tannin, and however with species variability. *Sargassum polycystum* and *Padina tetrastromatica* were found to have more tannin than others. The members of Rhodophyceae showed lower value in comparison with green and brown seaweeds. Among them, *Gracilaria corticata* ranked first, while *Acanthophora spicifera, Gracilaria pygmaea, Hypnea musciformis* possessed more or less same amount of tannin (Table 15). The present study exemplified that red and green seaweed species contained lower total condensed tannin than brown seaweeds. Many researchers have shown that phlorotannin are the only phenolic group detected in brown algae (Jormalarnen and Honkanen, 2004; kovivikko et al., 2007). Phlorotannin purified from several brown algae was reported to have strong antioxidant activity which may
be associated with their unique molecular skeleton (Ahn et al., 2007). Phlorotannin from brown algae has up to eight interconnected rings, and it is therefore more potent free radical scavenger than other polyphenols derived from terrestrial plants including green tea catechins having three to four rings (Hemat, 2007). Higher concentration of tannin found in brown seaweeds in the present study is in agreement with earlier reports (Cox et al., 2010) and varied greatly among different species of brown, red and green seaweeds, as well as among different geographical areas. (Pavia and Toth, 2000; Venkatesh et al., 2011; Cosman Domettila et al., 2013). Tannin was used therapeutically as anti-viral, antibacterial, anti-ulcer and antioxidative agent. Many tannin containing drugs are used in treatment of piles, inflammation burns and also as astringent (Kolodziej and Kiderlen, 2005) and therefore, these seaweeds could be effectively utilized in various drugs preparations.

5.3.4 Vitamins:

Seaweeds are a good source of water- (B₁, B₂, B₁₂, C) and fat-soluble (β-carotene with vitamin A activity, vitamin E) vitamins. The known B - complex vitamins are B₁ (thiamine), B₂ (riboflavin), B₃ (niacin), B₆ (pyridoxine, pyridoxal, pyridoxmine), B₁₂ (cobalamin), biotin and folic acid (folate, folacin, pteroylglutamin). The B-complex vitamins provide the body with energy by aiding in the conversion of carbohydrates to glucose, which the body "burns" to produce energy. They are also vital in the metabolism of fat and protein. In addition, the B vitamins are necessary for normal functioning of the nervous system (Skrovánkova, 2011). The study was aimed to determine different vitamins in eight seaweeds (Ulva reticulata, Caulerpa racemosa, Enteromorpha intestinalis, Padina tetrastromatica, Sargassum cinereum, Spathoglossum asperum, Gracilaria verrucosa, and Acanthophora spicifera). The
status of water soluble vitamins namely thiamine, riboflavin, niacin, and pyridoxine are shown in Table (16). Results indicated that A. spicifera is overall a good source of water soluble vitamins, containing thiamine (0.4034 mg/100g) riboflavin (1.223 mg/100g), niacin (0.3945 mg/100g) and pyridoxine (0.1934 mg/100g). Of the Chlorophycean members, U. reticulata was seemed to be a rich source of thiamine, riboflavin, niacin and pyridoxine by possessing 0.334 mg/100g, 0.437 mg/100g, 0.734 mg/100g and 0.117 mg/100g respectively. Even though C. racemosa was noticed to be a poor source of water soluble vitamins, it contained significant amount of riboflavin (1.34 mg/100g DW). Among the members of Phaeophyceae thiamine was high in Padina tetragastomatica (0.4934 mg/100g DW). Spathoglossum asperum was found to be high in pyridoxine (0.2231 mg /100g DW). Thiamine, riboflavin, and niacin are members of vit B complex series; act as coenzyme in various oxidative reactions. Thiamine is necessary for the normal metabolism of carbohydrate. It also functions in the utilization of pentoses and in the synthesis of certain amino acids. Its deficiency causes anorexia, fatigue, constipation and retarded growth. Riboflavin coenzyme acts as receptors for transfer of protons between NAD$^+$ and NADP$^+$, and cytochromes, which transport electrons in mitochondria. Similarly, niacin is an essential constituent of NAD$^+$ and NADP$^+$, the important coenzyme involved in biological oxidation and reduction. Niacin deficiency results in pellagra (rough skin). The deficiency of riboflavin in human produces lesions in the corners of mouth (cheilitis), inflammation of tongue (glossitis), and lesions on the lips, and around eyes and nose. Vitamin B$_6$ is essential for proper growth and maintenance of body functions.(Khalil and Saleemullah, 2004). These B vitamins were present in significant amount in all the seaweeds investigated.
Vitamin C (Ascorbic acid) is water-soluble vitamin required in high amount, as its loss is frequent from the body. It participates in reversible oxidation reduction system. Vitamin C prevents scurvy disease and also aids in the formation of folic acid derivatives, which are essential for DNA synthesis (Chatterjea and Shinde, 1998). Vitamin C was found in considerable amount in twenty nine species of seaweeds analysed. *Padina tetrastromatica, Spathoglossum asperum, Sargassum sp, Steochospermum marginatum* were noted for their high vitamin C level. Higher concentration was recorded in most of the species of Phaeophyceae followed by Rhodophyceae and Chlorophyceae. Maximum amount of vitamin C was estimated in *Steochospermum marginatum* member of Phaeophyceae (26.56 ± 1.56 mg/g DW), and less in Rhodophyceaeen member *Gracilaria verrucosa* (1.03 ± 0.12 mg/g DW). Among Rhodophyceae, the vit- C content was higher in *Amphiroa anceps* (19.14 ± 2.11 mg/g DW) and lower in *Hypnea musciformis* (8.2 ± 0.05 mg/g DW). Among Chlorophyceae, it was found to be more in *Enteromorpha compressa* (9.08 ± 0.11 mg/g DW) and less in *Codium bursa* (1.34 ± 0.01 mg/g DW). Appreciable amount of vitamin C was also estimated in some members of Phaeophyceae, Rhodophyceae and Chlorophyceae (Table 17). Formulation of these algal species into palatable products containing intrinsic antioxidants protect from oxidative stress, and forming the first line of defense (Sandhir and Gill, 1999). Seaweed varieties are rich sources of vitamin C, vitamin B-complex, e.g., folic acid and B12, and vitamin A precursors, such as β-carotene (Watanabe et al., 1999; Yamada et al., 1999; Takenaka et al., 2001; Watanabe et al., 2002; McDermid and Stuercke, 2003; Yon and Hyun, 2003). It is generally believed that the ability to synthesize ascorbic acid is absent in some animals including invertebrates and fishes due to the lack of L- gulono lactone oxidase that catalyses the terminal step in the conversion of glucose to ascorbic acid.
(Chatterjee et al., 1975). Human body requires vitamin C for normal physiological functions. It helps in the metabolism of tyrosine, folic acid and tryptophan, lowers blood cholesterol and contributes to the synthesis of amino acids needed for tissue growth and wound healing (Iqbal et al., 2004).

Vitamin E is one of the most important fat-soluble vitamins with a strong antioxidant activity. Its special function is protection of lipids from peroxidation. It exists in eight forms: α, β, γ, δ-tocopherols and α, β, γ, δ-tocotrienols. The α-forms showed the highest antioxidant effect (Yamamoto et al., 2000). In the present study, vitamin E level of seaweeds varied from 0.021 to 2.837 mg/g DW. Maximum amount was noted in Phaeophyceae members namely Sargassum wightii (2.837 ± 0.094 mg/g DW), Padina pavonia (2.007 ± 1.343 mg/g DW) and Stoechospermum marginatum (1.872 ± 0.331 mg/g DW) followed by Rhodophyceae member Hypnea musciformis (1.871 ± 0.235 mg/g DW) and Chlorophyceae members, Enteromorpha intestinalis (1.210 mg/g DW) and Caulerpa racemosa (1.21 mg/g DW). Present study revealed that brown seaweeds are rich source of vitamin E than green and red seaweeds and was corroborated with earlier observations (Sanchez-Machado et al., 2002). The lowest vitamin E content was found in Caulerpa serutularioides (0.035 ± 0.002 mg/g DW) followed by Valoniopsis pachynema (0.60 ± 0.002 mg/g DW). It was documented that brown algae contain alpha, beta and gamma tocopherol and green and red algae contain alpha tocopherol. Gamma and alpha tocopherols play an important role in the prevention of cardiovascular diseases (Solibami and Kamat, 1985). Therefore, it is suggested that intake of these seaweeds with significant amount of many vitamins would serve as a viable option for health benefits.
5.3.5 Antioxidant activity:

Exogenous chemicals and endogenous metabolic processes in human body produce free radicals especially oxygen derived radicals, which are capable of oxidizing biomolecules, resulting in cell death. DPPH (2, 2-diphenyl-1-picrylhydrazyl reagent) has been used extensively for investigating the free radical scavenging activities of compounds. The assay is based on the reduction of alcoholic DPPH (2, 2-diphenyl-1-picrylhydrazyl) in the presence of a hydrogen-donating antioxidant which resulted into the formation of non-radical form DPPH-H (diphenyl-1-picrylhydrazyl) (Shon *et al*., 2003). Dried seaweed extracts were potentially able to reduce stable DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical to yellow coloured diphenylpricrylhydrazine. The antioxidants present in soluble fraction of seaweeds functioned as proton source to reduce DPPH and their reducing ability was compared with ascorbic acid (standard). The antioxidant activities of all the seaweeds were invariably different. Among Chlorophyceae, *C. scalpelliformis* exemplified higher DPPH radical scavenging activity (27.58%). Every brown seaweed investigated, exerted more scavenging ability in comparison with members of Chlorophyceae and Rhodophyceae. Red seaweeds were noted for their lower radical scavenging ability, however all the species of *Gracilaria* assayed, reduced DPPH by 28 – 30%. Among all the seaweeds *S. marginatum* and *P. pavonia* showed maximum DPPH radical scavenging activity. The results of the present study are in line with *Yan et al.*, (1999) and *Wang et al.*, (2009) who also found that higher amounts of polyphenols and free radical scavenging activity in brown seaweeds than red and green algae. However, *Chandini et al.*, (2008) reported low levels of free radical scavenging activity in brown seaweeds, in the range of 17.79 to 23.16% at an extract concentration of 1000 µg/ml.
Superoxide anion radical is one of the strongest reactive oxygen species, playing an important role in the formation of other reactive oxygen species such as singlet oxygen, hydrogen peroxide, hydroxyl radical, which can cause damage to DNA, lipids and proteins leading to various diseases (Pietta, 2000). Superoxide anion radical assay is a rapid method to identify seaweed species which could be potentially explored in preparation of natural antioxidant sources. Superoxide anion radical activity of investigated seaweeds varied from 26.58 ± 6.03 to 64.16 ± 0.955 mg/ ml. Among the members of Phaeophyceae superoxide anion radical scavenging was more in *P. pavonia* and *S. Polycystum* (56.16 %) and less in *S. wightii* (51.65 %). Among Chlorophyceae, the highest activity was recorded in *C. scalpelliformis* (56.36%) and lowest in *U. reticulata* (26.58%). Maximum activity was noted in Rhodophyceaeen species, *G. corticata* (64.16%), followed by *A. aniceps* (57.95%) and minimum in *H. musciformis* (34.01%). Cells are equipped with different kinds of mechanism to fight against ROS and to maintain the redox homeostasis for cell protection. When the mechanism becomes imbalanced in human body, antioxidant supplements such as seaweeds may be used to reduce oxidative damage. Although superoxide anion is a weak oxidant, it leads to the generation of powerful and dangerous hydroxyl radicals as well as singlet oxygen, both of which contribute to oxidative stress. Reducing power assay may serve as a significant method in reflecting antioxidant activity (Oktay *et al.*, 2003). Compounds with reducing power are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes. In the reducing power assay, presence of antioxidants in the sample would result in the reduction of Fe$^{3+}$ to Fe$^{2+}$ by donating an electron (Olayinka *et al.*, 2010). Reducing capacity of compound is generally dependent upon reductones (Pin-Der Duh, 1998) which cause breaking of the free radical chain by donating a hydrogen atom and exhibited
antioxidant potential (Gordon, 1990). Reductones are also reported for preventing peroxide formation by reacting with certain precursors of peroxide (Sawant et al., 2009). The reducing power of seaweed extracts varied among different species (Table 18). Significant amount of reducing power was noted in green and brown seaweeds with the highest value in *C. scalpelliformis* (5.8 mg/g DW), *S. wightii* (5.33 mg/g DW) and *S. polycystum* (5.8 mg/g DW) which was in accordance with earlier reports (Rice-Evans et al., 1995 and Habila et al., 2010)

Oxidation of linoleic acid generates peroxyl free radicals due to the abstraction of hydrogen atoms from diallylic methylene groups of linoleic acid (Kumaran and Karunakaran, 2006). The free radicals then will oxidize the highly unsaturated beta carotene, consequently orange colour of carotene would be degraded and monitored spectrophotometrically. The highest total antioxidant activity value was reported in *S. tenerrimum* (3.50 ± 0.081 mg/g DW) followed by *S. marginatum* (3.23 ± 0.012 mg/g DW) and *C. scalpelliformis* (1.54 ± 0.02 mg/g DW). The lowest value was reported in *G. verrucosa* (0.35 ± 0.008 mg/g DW). In general, brown algae showed more total antioxidant activity than other groups. This corroborates with Meenakshi et al., (2009) who reported more total antioxidant activity in *S. wightii* (1.16 ± 0.11 mg GAE/g) than *U. reticulata* (0.91 ± 0.09 mg GAE/g). Aqueous extracts used in the present study showed promising antioxidant activity which could be comparable to studies with ethanol extracts (Amna Tariq et al., 2011). This study showed that different seaweeds possessed varying degrees of antioxidant activity. Although their antioxidant activities were lower than that of commercial antioxidants (ascorbic acid) they can contribute to human health when consumed regularly. Therefore these species could be processed suitably into acceptable form and used as natural antioxidants for prevention and
protection from degenerative diseases. The ability of seaweeds to reduce free radicals over a long period of time (Yuan et al., 2005) may also have benefits for extending the shelf life of processed foods during storage and distribution.

Karl Pearson’s correlation coefficient analysis between antioxidants and antioxidant activity revealed that phenolic content was negatively correlated with DPPH radical scavenging activity \( r = -0.5277 \) and superoxide radical scavenging activity \( r = -0.3218 \). Whereas positive correlation existed between reducing power \( r = 0.636 \) and total antioxidant activity \( r = 0.701 \) with phenol. Tannin content was significantly correlated with reducing power \( r = 0.652 \). Moreover insignificant positive correlation \( r = 0.1397 \) existed between tannin and total antioxidant activity. Flavonoid showed insignificant positive correlation with free radical scavenging activity \( r = 0.3934 \), superoxide radical scavenging activity \( r = 0.3151 \), reducing power \( r = 0.6521 \) and total antioxidant activity \( r = 0.1397 \). So, this study suggests that besides phenols, flavonoids and tannins, other seaweed components such as low molecular weight polysaccharides, pigments and proteins may have contributed and influenced antioxidant activity of seaweeds. Also the low incidence of chronic diseases among the Japanese as compared to people having low to zero seaweed intake is attributed to the antioxidant property of seaweeds (Yuan et al., 2005). So future of seaweeds as a natural antioxidant food supplement seems enormous.

5.4 Studies on antimicrobial activity of seaweeds

5.4.1 Antifungal activity against human pathogens:

A wide analysis of biochemical components in quiet a number of seaweeds belonging to three major groups of algae in the present study disclosed that these seaweeds are rich source of bioactive compounds such as protein, dietary fibre,
essential fatty acids, unsaturated fatty acids, vitamins, minerals, important polysaccharides such as agar, alginates and carrageenans. The study also highlighted the presence of secondary metabolites like phenols, flavonoids and tannin among various seaweeds, and they were also found to exhibit free radical scavenging ability. It is known that most of the secondary metabolites produced by seaweeds have bactericidal or fungicidal properties. Although most of the antibiotics used as therapeutic agents are derived from terrestrial sources, the oceans have enormous and rich biodiversity and potential to contribute to novel compounds production with commercial value (Hay, 1996; Smit, 2004). The use of antimicrobial drugs has certain limitations due to changing patterns of resistance in pathogens and the side effects they produce. These limitations necessitated the continued research for search of new antimicrobial compounds for the development of effective drugs. Hence the present investigation was carried out to bring out the efficacy of various seaweeds in controlling human pathogens by extracting the active components with different solvents and the results were compared with control, fluconazole. In these study human pathogens such as _Candida albicans_ (MTCC 227), _Trichophyton simii_ (110/02) and _Trichophyton rubrum_ (MTCC 296) were cultured individually on selective broth at 37°C for 72 h before inoculation. The anti-fungal activity was evaluated by agar diffusion method. The antifungal activity of different seaweed extracts were studied with different concentrations and found 1mg / ml was sufficient to elicit detectable inhibition. Data collected on antifungal activity of ten algal species against human pathogens are presented in Tables (19 to 21) and plates (5 to 9). The mean zone of inhibition ranged from 4 mm to 24.33 mm and the mean zone of (fluconazole), positive control ranged from 16.56 mm to 21.89 mm. The blind control (negative control) DMSO (dimethyl sulfoxide) recorded insignificant inhibition. The data
revealed that *Candida albicans* (MTCC227) was moderately sensitive to fluconazole, though *Candida albicans* NCW1W3074 (National collection of industrial microorganism) was reported to be resistant to a less toxic azole drug i.e., fluconazole (White *et al*., 2002). Similarly *Trichophyton simii* and *Trichophyton rubrum* were also exhibited sensitivity against the drug used.

Effect of hexane extracts of seaweeds (Table 19) illustrated that Chlorophycean members had no impact on *Candida albicans*, however inhibiting the growth and establishment of *Trichophyton simii* and *Trichophyton rubrum*. Of the four species of brown seaweeds tested, hexane extract of *S. tenerrimum* was most active against tested human pathogens. Furthermore, *P. pavonia* hexane extract had no inhibitory effect on any of the human pathogens analysed. All the red species exhibited antagonistic effect on tested organisms, however with lesser extent compared to brown seaweeds. It was noted that benzene extract of all the seaweeds exerted antifungal activity towards all the tested human pathogens. The extent of inhibitory effect varied with seaweed species with maximum being effected by *Spathoglossum asperum* (19.66 mm) and *Hypnea musciformis* (24.33 mm) against *Trichophyton rubrum*. Study also indicated that, *Caulerpa scapelliformis* extract of both hexane and benzene had proportionately less antagonistic effect on *Trichophyton rubrum*. More or less benzene extracts of other seaweeds inhibited the growth of tested human pathogens, but it was lesser extent than their respective control. Effect of chloroform extracts of different seaweeds on *Candida albicans*, *Trichophyton simii* and *Trichophyton rubrum* were recorded in Tables (19-21). It was found out that all the seaweeds in chloroform extract inhibited the test organisms however the extent of inhibition varied. It was remarkable to note that chloroform extract of
C. scalpelliformis inhibited the growth of Candida albicans more effectively than the synthetic drug fluconazole by exhibiting 19.66 mm inhibition zone. This may probably be attributed to caulerpein (Doty and Santos, 1966., Paul et al., 1987) or flexin and trifar in (Blackman and wills, 1978) or caulerpanyene (Amico et al., 1978) which might have been extracted better in chloroform rather than other solvents. Similar results were reported by Mtolera and Stemesi (1996). Similarly chloroform extract of S. tenerimum (brown), A. spicifera, and H. musciformis (red) showed significant inhibitory activity against Trichophyton rubrum and G. verrucosa, A. spicifera and S. tenerimum against Trichophyton simii. Nevertheless, all the seaweeds in chloroform extraction expelled conspicuous inhibitory activity against all the human pathogens.

Data represented in Tables (19-21) show the impact of ethanolic extract of different seaweeds on three human pathogens. The study revealed that except U. reticulata all the seaweed ethanolic extracts showed antifungal activity on Candida albicans, Trichophyton simii and Trichophyton rubrum. The detrimental impact was more pronounced with C. scalpelliformis, S. marginatum and A. spicifera. However, all the seaweed extracts had considerable antifungal effect on Candida albicans. U. lactuca extract was seemed to be more effective against Trichophyton simii. Except S. marginatum all the seaweeds with ethanol extraction had significant antagonist effect on Trichophyton simii and the negative role was more than control except P. pavonia and H. musciformis. In contrast, H. musciformis inhibited the establishment of Trichophyton rubrum more severely when compared to all the seaweed species and was 38% more than the control. It was notable that S. marginatum was highly inhibitory against Candida albicans but was ineffective.
against *Trichophyton simii* and *Trichophyton rubrum* showing the species specific action of seaweeds and the response of test pathogens. Antifungal activity of seaweeds extracted with petroleum ether (Table 19) brought to light that *Candida albicans* was more sensitive to all the seaweeds except *U. reticulata*. *C. scalpelliformis, S. tenerimum, S. marginatum* extract arrested the growth more severely than fluconazole. It was also found that *Trichophyton simii* was insensitive to *U. reticulata* extract whereas *S. marginatum* was detrimental to *Trichophyton simii* and *Trichophyton rubrum*. Among the seaweed extracts obtained by petroleum ether extraction *C. scalpelliformis* effectively retarded the growth of *Trichophyton rubrum*. The study also established that all the organic solvent extracts of seaweeds are found to be suitable for controlling the human pathogens in one way or other.

Impact of aqueous extract of different seaweeds on *Candida albicans*, *Trichophyton rubrum* and *Trichophyton simii* are presented in Tables (19-21). The inhibitory effect was comparatively lesser when water was used as solvent, however most of the seaweeds in aqueous extraction showed inhibitory activity. Of all aqueous extracts, *S. asperum* and *S. marginatum* controlled the growth of *Trichophyton rubrum* more prominently than the control. Antifungal property exhibited by different seaweeds in the present investigation could be variously discussed. All the investigated species of seaweeds inhabited in Hare Island, Gulf of Mannar showed antifungal activity against every human pathogenic fungal species with different degrees. Earlier reports from other countries viewed the absence of antifungal activity against *Candida albicans* (My 1055), *Saccharomyces cerevisiae* (W303) and *Aspergillus fumigatus* (MF5667) with extracts of 40 species of marine algae excluding *Cympolya barbata* (green alga) and *Aspargopsis laxiformin* (red alga) (Gonzalez
et al., 2001). However, methanolic extracts of *Cystoseira tamarisciflora* (brown), *Padina pavonia*, (brown) *Rhodomela confervoides* (red), *Ulva lactuca* (green) reported to have antifungal activities against *Candida albicans*, *Aspergillus niger*, *Mucor maniannu* (Saidani et al., 2011). Also many researchers have documented the antifungal potential of seaweeds with different solvent extraction in many parts of the country and outside (Gao et al., 2011; Zovko et al., 2012). There were also reports to substantiate the varied activity of different seaweed extracts against different human pathogens. Tuney et al., (2006) found that ethanolic extract of *P. pavonia* was active against *Candida albicans* whereas methanolic and acetone extracts of the same alga were inactive against *Candida albicans* and supporting the species specific activity of seaweeds extracted with different solvents and response of pathogens recorded in the present study. The differences between different seaweeds in their antifungal activity may be due to several factors. Inter specific variability in the production of secondary metabolities like tannins, phenolic compounds, flavonoids, certain fatty acid, caulerpin, sulphated poly saccharides etc (Moreau et al., 1988 ; Febles et al., 1995; Itoh and Shinya, 1994; Latusnus,1996; Amina kabir khanzada et al., 2007; Pandurangan Aruna et al., 2010; Kayalvizhi et al., 2012) are assumed to be responsible for varied antifungal property. These differences may also be due to extraction protocols to recover the active principles and the assay methods (Karthikadevi et al., 2009)

### 5.4.2 Antifungal activity of seaweed extracts on plant pathogens.

Finding of metabolites with biological activity from algae increased progressively in the last three decades. The algal primary and secondary metabolites exhibit an appreciable level of antiviral, antifungal and insecticidal action (kladi
et al., 2006; Machado, 2010, 2011) and majority of these compounds are terpenes and polyphenols (Blunt et al., 2006). Brown algae have shown effectiveness in controlling plant diseases. The laminarin, polysaccharides isolated from Laminaria digitata is able to elicit host defence responses in plants (Klarzynski, 2000). Ulva fasciata extract is able to reduce the number of colonies in powdery mildew (Stadnik and Maraschin, 2004). Mycotoxins, mainly aflatoxins produced by Aspergillus flavus and A. parasiticus found in contaminated grains when ingested may cause hepatitis, hemorrhage, edema, immunosuppression, hepatic carcinoma and death (Maracas and Nelson 1987, Baldissera, 1993). Curvularia lunata cause leaf spot of maize (Akinbode, 2010) and lotus (Cui and Sun, 2012). Aspergillus niger causes a disease called black mold on certain fruits and vegetables such as grape, onion and peanut and is a common contaminant of food (Ruchi Sharma, 2012). To protect crop plants and plants produce from fungal phytopathogens use of seaweeds become promising. Seaweeds are also found to stimulate growth and yield potential of many crop species, thus analyzing the antifungal property of seaweed extracts on plant pathogens become beneficial for both pharmaceutical and agro industries. The present research work aimed to bring out the antifungal effect of seaweeds extracted with different organic solvents and water under laboratory conditions. Phytopathogens, Aspergillus niger (MTCC1344) and Curvularia lunata (46/01) were used as test organisms and fluconazole was used as standard. The results summarized in Tables (22 and 23) indicated the significant antifungal activity of hexane extract of Spathoglossum asperum (brown) and Acanthophora spicifera (red) against Curvularia lunata which were greater than the synthetic chemical, fluconazole. Further, all the brown and red seaweeds in hexane exerted antagonistic effect on Curvularia lunata, however green algae in the same solvent were found ineffective. Hexane extract of Caulerpa scalpelliformis,
Sargassum tenerrimum and Gracilaria verrucosa were more inhibitory repressed the growth of *Aspergillus niger* more than others. Data recorded in Tables (22 and 23) expressed that benzene extract of all the seaweeds found to possess growth retarding effect on both *Aspergillus niger* and *Curvularia lunata* with considerable variations. Hexane extract of *C. scalpelliformis* and *P. pavonia* showed higher inhibitory effect on *Aspergillus niger* (14.67mm), however it was comparatively lesser than the control. Brown seaweeds except *P. pavonia* in hexane extract were seemed to be more antagonistic against *Curvularia lunata* showing 16 to 20 mm inhibition zone and resembled fluconazole in their antifungal activity. Effect of chloroform extract of green, brown and red seaweeds on controlling *Aspergillus niger* and *Curvularia lunata* are tabulated (Tables 22 and 23). The results showed that all the seaweed extracts in chloroform except *Ulva reticulata* restricted the growth of *Aspergillus niger* to different extent. *A. spicifera* was found to be more effective in controlling the growth and spread of *Aspergillus niger*. Also red algae were noticed to be comparatively more inhibitory than brown and green seaweeds in chloroform extract. In contrast green seaweeds in chloroform formulations were more inhibitory against *Curvularia lunata*, achieving same zone of inhibition by *U. reticulata* (17 mm) to that of fluconazole. Except *P. pavonia* all brown and red seaweeds in chloroform extraction inhibited the growth and establishment of *Curvularia lunata* with lesser differences. Analysis on ethanolic extract of seaweeds portrayed greater efficiency of brown and red seaweeds in eliciting growth inhibition on *Aspergillus niger* and *Curvularia lunata* which was visualized as inhibition zone ranging from average zone 8.33 to 21.89 mm and 6.33 to 22 mm respectively. The maximum zone of inhibition was produced by *S. tenerrimum* extract with *Aspergillus niger* which was similar to the standard (Control). *S. marginatum* ethanolic extract also enabled to reduce growth
of *Aspergillus niger* more sufficiently reaching equivalent to the control. Conversely, disc loaded with *S. tenerrimum* caused negligible inhibitory actively against *Curvularia lunata*. However except *P. pavonia* and *S. tenerrimum* all the other seaweed species in ethanolic preparations retarded the growth of *Curvularia lunata* even greater than the synthetic chemical, fluconazole. Petroleum ether extracts of all the sea weeds did not show appreciable level of antifungal activity. Green seaweeds such as *U. lactuca* and *U. reticulata* exhibited higher antifungal actively against *Aspergillus niger*, but it was comparatively lesser than the control (Table 22). *C. scalpelliformis* and *S. marginatum* had no inhibitory role on *Aspergillus niger* and *U. reticulata* and *S. marginatum* on *Curvularia lunata* in petroleum ether extract. Further *C. scalpelliformis* caused growth reduction in *Curvularia lunata* more conspicuously and it was more than the control. However all the other seaweeds in petroleum ether extraction did show comparatively lesser antagonistic effect towards *Curvularia lunata*. Aqueous extract of all the seaweeds had lesser antifungal actively except *S. asperum* against *Aspergillus niger*. The results of the present study established that all the sea weeds in different solvent extractions exhibited antifungal activity; however with variations. Similar variations with different phytopathogens and seaweed species were reported by many researchers (Pitchaimuthupandian *et al.*, 2011; Saidani *et al.*, 2012). Thus it is concluded that seaweeds such as *H. musciformis* and *S. tenerrimum* in ethanol extract were highly effective against all fungal species. Organic solvents always provide a higher efficiency in extracting anti-microbial active principles as compared to water extraction (KAYALVIZHI *et al.*, 2012). Along with other biological activities, green, brown and red seaweeds exhibited antifungal property concurring with earlier reports (Newman *et al.*, 2003; BHAKUNI AND RAWAT, 2005; BLUNT, 2006). Seaweeds with antiphyto-pathogenic property, could be explored
for biopesticides in agronomic applications. In India every year fungi are responsible for world wide great economic losses in agriculture. *Aspergillus niger* is found associated with rotten and spoiled fruits and vegetables, responsible for post-harvest losses of fruits during storage, as well as in transit and commercialization. Seaweeds are also known to aid and stimulate growth of vegetables, fruits and other crops (Blunden, 1991; Crouch and Van staden, 1994) and also protect them from different pathogens (Taskin et al., 2007; Flora and Maria Victorial Rani, 2012) and physiological hazards either in vivo or storage conditions (Washington et al., 1999). Application of seaweeds as antifungal agent against human and phytopathogens open a new dimension to be explored further.

### 5.4.3 In vitro antibacterial property of seaweeds

Life threatening diseases and high rate of mortality occur in animal and human population due to bacterial infection. Many bacteria both Gram positive and Gram negative contaminate food, water, air, soil, etc., and cause biological / microbial pollution. *Bacillus subtilis* is responsible for causing food borne gastroenteritis, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* cause diseases like mastitis, abortion and upper respiratory complication while *Salmonella typhi* causes diarrhoea and typhoid fever (Jawetz et al., 1995 and Leven, 1987). Now a days, use of antibiotics has increased significantly due to severe infection and resistance acquired by different pathogenic bacterial strains as a consequence of indiscriminate use of drugs, synthetic chemicals and antibiotics. Moreover the cost of drugs is becoming high and also they cause adverse side effect on hosts including hypersensitivity and depletion of beneficial microbes. Increased resistance of pathogens to antibiotics, soaring price of life protecting drugs has necessitated to
search for easily accessible, cost effective alternatives, where the seaweeds stand forefront. Many workers have revealed that crude extracts of Indian seaweeds are active against pathogenic bacteria (Hellio et al., 2000 and Lima-Filho et al., 2002). Seaweeds from South African coast, and Red sea extracted with different organic solvents exhibited antibacterial activity (Vlachos et al., 1997 and Salem et al., 2011). Hare Island, part of Gulf of Mannar endowed with rich seaweed diversity in abundance throughout the year has not been explored for antimicrobial drugs development. Hence, in this investigation bactericidal property of ten seaweeds, belonging to Chlorophyceae (Ulva lactuca, Ulva reticulata and Caulerpa scalpelliformis) Phaeophyceae (Padina Pavonia, Sargassum tenerrimum Stoechospermum marginatum and Spathoglossum asperum) and Rhodophyceae (Acanthophora spicifera, Gracilaria verrucosa and Hypnea musciformis) were extracted with various solvents and were tested against human pathogens: Escherichia coli, Bacillus subtilis, Salmonella typhi, Pseudomonas areuginosa and Klebsiella pneumoniae.

The bacterial strains were sub-cultured before a day of use to maintain log phase. Agar diffusion method was adopted on pre-seeded nutrient agar spread plates to determine in vitro bactericidal activity. The antibacterial activity of seaweed extracts were visualized as clear zone (inhibition zone) around the disc on agar plates. All the extracts were serially diluted and standardized with above said pathogens and found 2.5 mg/ml to be the effective concentration. Antibacterial activity of Ulva lactuca is represented in Table (24). The data collected by using different solvents exemplified that benzene, chloroform and methanol extracts of U. lactuca interacted with Salmonella typhi more negatively causing growth inhibition to some extent. The
inhibitory effect of *U. lactuca* was less in comparison with streptomycin irrespective of the solvent used for extraction. In contrast, *U. reticulata* was found to be moderate in reducing the growth of all the pathogens tested. More or less *Bacillus subtilis* was revealed to be more sensitive to *Ulva reticulata* extracts. Influence of *Caulerpa scalpelliformis* extracted with hexane, benzene, chloroform, methanol petroleum ether and water are recorded in Table (26). The results clearly indicated that like *U. recticulata*, *Caulerpa scalpelliformis* also exerted its antagonistic effect more on *Bacillus subtilis* than others. Hexane extract of *Caulerpa scalpelliformis* interacted with *Escherichia coli* and resulted into growth inhibition (9.46mm) more closed to streptomycin. Similarly *Bacillus subtilis* was arrested by *Caulerpa scalpelliformis* in hexane and methanol extracts. However *Salmonella typhi* was found to be resistant to *Caulerpa scalpelliformis* when compared to other organisms. Among three green seaweeds, *Caulerpa scalpelliformis* was found to be more effective in controlling the human pathogens studied. Aqueous extracts of all the three green seaweeds were observed to be less effective.

Bactericidal potential of brown seaweeds are presented in Tables (27-30). *P. pavonia* extracted with all solvents except water portrayed significant negative impact on *Escherichia coli, Bacillus subtilis, Salmonella typhi, Pseudomonas areuginosa* and *Klebsiella pneumoniae*. More or less all the solvents were proven to be suitable for isolation of bioactive, bactericidal compounds. Methanolic extract of *P. pavonia* was apparently more detrimental to *Escherichia coli*, where as chloroform and benzene extracts were very active against *B. subtilis* and *Salmonella typhi, Pseudomonas areuginosa*. All the solvents extracts of *P. pavonia* apart from water, produced inhibition zone more or less similar to the synthetic antibiotic, streptomycin.
Data presented in Table (28) reveal the antibacterial potential of *S. marginatum*. The zone of inhibition ranged from 9.6 mm to 13.6, 8.2 to 11.8, 5.3 to 13.6, 6.8 to 14.8 and 5.6 to 15.6 mm in *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhi*, *Pseudomonas areuginosa* and *Klebsiella pneumoniae* respectively. Methanol extract of *S. marginatum* was more active against *Escherichia coli*. Except chloroform and water other extracts displayed antibacterial activity similar to streptomycin against *Bacillus subtilis*. Methanol extract was found to be superior in restricting the growth of *Pseudomonas areuginosa* and *Klebsiella pneumoniae* than streptomycin. Comparatively methanol was more suitable in extraction and isolation of bactericidal components from *S. marginatum* for clinical testing. Effect of *S. tenerrimum* on five pathogenic bacteria are shown in Table (27). The results indicated that all the solvents including water were seemed to be appropriate for active principle extraction. Aqueous extract portrayed higher inhibition on *Escherichia coli* and *Pseudomonas areuginosa* than streptomycin. All the solvent extracts of *S. tenerrimum* were found to be more inhibitory against *Escherichia coli* than control. Apart from water and petroleum-ether, extract of all the other solvents retarded the growth of *Bacillus subtilis* and the effect was greater than streptomycin. Similarly *Pseudomonas areuginosa* and *Klebsiella pneumoniae* were also effectively controlled by *S. tenerrimum* extracts. However *Salmonella typhi* showed growth reduction with *Sargassum tenerrimum* extracts lesser than streptomycin. Antibacterial activity of *Spathoglossum asperum* extracted with different solvents against some human pathogens is shown in Table (30). It was found that benzene extract of *Spathoglossum asperum* was ineffective in controlling all the five pathogens tested. However chloroform, methane, petroleum ether extracts of *Spathoglossum asperum* exerted antagonistic effect similar to streptomycin. Methanol and petroleum ether extracts
retarded the growth of *Pseudomonas aeruginosa* more severely by showing 11.2 mm inhibition zone which was greater than streptomycin (10.1 mm). Methanolic extract controlled the growth of *Klebsiella pneumoniae* (12.5 mm) which remained similar to control (12.2 mm). Among all the brown seaweeds analysed *Padina pavonia* was found to have the highest antibacterial potential. Further more, all the brown seaweed extracts were substantially more reactive against all the pathogens tested in the study.

Effect of red seaweeds *A. spicifera, G. verrucosa* and *H. musciformis* on five human pathogens were analysed and the data are presented (Tables 31 to 33). It was observed that both organic and aqueous extracts of *A. spicifera* produced inhibition zone more than synthetic drug streptomycin, ranging from 12.5 mm to 15.3 mm, 10.6 mm to 16.8 mm and 10.2 mm to 14.5 mm with respect to *Escherichia coli, Bacillus subtilis* and *Pseudomonas aeruginosa*. Petroleum ether and hexane extracts of *A. spicifera* were assessed to be more antagonistic against *Salmonella typhi* and *Klebsiella pneumoniae* respectively. Perusal of data indicated that methanolic extract of *A. spicifera* was more detrimental to all the pathogens studied. Study also viewed that aqueous extract of *A. spicifera* displayed bactericidal role approximately equal to other extracts. Among red seaweeds tested, *G. verrucosa* and *H. musciformis* had insignificant impact over all the human pathogens irrespective of the solvents used for extraction. Methanolic extract of all the seaweeds tested in the present study brought out a wide range of antibacterial activity, which is in conformity with earlier reports (Manilal *et al.*, 2009; Rangaiah *et al.*, 2010). Based on the results accounted, it is suggested that brown seaweeds are more antagonistic towards all the pathogens. *A. spicifera*, red seaweed was also as effective as brown seaweeds in controlling and inhibiting the growth of all pathogens. Among green seaweeds *C. scalpelliformis* was
found to be effective, but incomparable with *S. marginatum, P. pavonia, S. tenerimum, A. spicifera*. Similar results were also obtained by others (kandasamy and Arunachalam, 2008; Karthikaidevi *et al.*, 2009 and Salem *et al.*, 2011). The strong antagonistic activities of brown seaweeds could be related to their phenolic compounds such as pholorotannin, amines and antioxidants (Tables 27 to 30) that have strong bactericidal activity (Nagayama *et al.*, 2002). Many researchers (Kumar *et al.*, 2008; Rajasulochana *et al.*, 2009; Seenivasan *et al.*, 2012; Ramalingam and Amuitha, 2013) have reported the activity of *Padina gymnospora, Sargassum wightii* and *Caulerpa sp, Gracilaria sp*, against Gram positive and Gram negative bacteria. Rangaiah *et al.*, (2010) investigated the antimicrobial potentiality of *Ulva lactuca, Caulerpa taxifolia*, against bacterial and fungal pathogens that cause diseases and disorders in man, animals and plants. The observed variation in antibacterial activity between solvent extracts and between species pointed out that species specific difference in chemical composition, bioactive compounds, their dependence on time (season) and place/location of sample collection, environmental fluctuations are responsible for antimicrobial function of seaweeds. Also differences in the capabilities of the extraction protocols to recover active principles, individual differences in assay methods would account for the results achieved in the present investigation. Results of the present study confirmed the potential use of brown, red and green seaweeds as a source for the development of antibacterial drugs.

### 5.5 Study on the utilization of seaweeds in biosynthesis of silver nanoparticles

Silver nanoparticles of size between 1 nm and 100 nm have attracted intensive interest on research. Biological method of synthesis of nanoparticles has paved way for the “greener synthesis” of nanoparticles and it has been proven to be a better
method due to slower kinetics. This method also offers better manipulation and control over crystal growth and their stabilization. This has motivated an upsurge in research on the biosynthesis of silver nanoparticles utilizing different seaweeds present in Hare Island. Use of seaweed extract for the synthesis of silver nanoparticles offers eco-friendliness and compatibility in pharmaceutical and other biomedical applications. Green synthesis is a viable option over chemical and physical methods as it is cost effective, environmentally healthy, and easily scaled up for large scale synthesis (Singh et al., 2010). In this method there is no need to use high pressure, energy, temperature and toxic chemicals. It is generally recognized that silver nanoparticles attached to the cell wall of microorganisms, thus disturbing cell wall permeability and cellular metabolism. The nanoparticles may also penetrate inside the cell causing damage by interacting with phosphorus and sulphur containing compounds such as DNA and protein (Elumalai et al., 2010). Hence exploitation of naturally growing seaweeds for the biosynthesis of nanoparticles develops a new insight in the field of pharmacology. Reduction of silver ions into silver nanoparticles was confirmed by the colour change from colourless to pale yellow, yellow, brown and dark brown. (Ahmad et al., 2003; Jain et al., 2009; Jae and Beom, 2009). The extracts of seaweeds belonging to Chlorophyceae, (Ulva lactuca and Caulerpa scalpelliformis) Phaeophyceae (Padina pavonia, Sargassum tennerrimum, Stoechospernum marginatum and Spathoglossum asperum) and Rhodophyceae (Acanthophora spicifera, Gracilaria verrucosa and Hypnea musciformis) were mixed with silver nitrate at ambient temperature and the colour change started from pale yellow to yellow and became brown within 24 hours. It is well known that silver nanoparticles exhibit yellow brown colour in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles. In the present study, all seaweeds
mediated the production of silver nanoparticles, however with different incubation periods. Among the various seaweeds *P. pavonia* and *A. spicifera* were found to be more effective in mediating the formation of silver nanoparticles. Synthesis of silver nanoparticles is assumed to be assisted by the phytochemicals present in the seaweed extract. The important phytochemicals involved in this process are terpenoid, flavones, ketones, aldehydes, amides and carboxylic acids. Flavones, organic acids and quinones are water soluble phytochemicals that are responsible for the immediate reduction of the silver ions. In the present study appearance of brown colour after mixing with seaweed extracts was assumed to be due to antioxidants like phenol, flavonoid, tannin (Tables 13 to 15) hydroxyl, carboxyl, primary and secondary amines, esters, alkanes and other functional groups (Tables 36 to 40) present in seaweeds which can serve as capping agents to provide a robust coating on the metal nanoparticles in a single step (Mahdavi *et al.*, 2013). Importance of polyphenolic compounds is that they have amphiphilic properties which facilitate their antioxidant mechanism in both water and lipid phase (Sies and Stahl, 2004). Antioxidant property of polyphenolic compounds (includes phenols, flavonoids, tannins etc.) is due to the presence of hydroxyl group attached to aromatic ring as electron donating group (Ng *et al.*, 2000). More electron donating groups available in the antioxidants is associated with the stronger reduction of silver ions. Hence it is suggested that these seaweeds could be effectively explored for synthesis of silver nanoparticles.

5.5.1 Characterization of silver nanoparticles

5.5.2 UV-visible spectral analysis

Silver nanoparticles synthesized using seaweed extracts were further characterized by UV-visible spectrophotometric analysis. The peak occurred at 420 nm
corresponds to the absorbance by silver nanoparticles. Strong interaction of the silver nanoparticles with light occurs because the conduction electrons on the metal surface undergoes a collective oscillation when excited by light of specific wavelengths known as surface plasmon resonance (SPR) (Steven and Oldenberg, 2002); and this oscillation results in usually strong scattering and absorption properties. The spectral response of silver nanoparticles is a function of its diameter. As the diameter increases, the peak plasmon resonance shifts to longer wavelength and broadens. In the present study the peak was observed at 420nm for all seaweeds mediated AgNPs revealing that silver nanoparticles synthesized using these species were smaller in size. Therefore, seaweeds can be effectively explored for production of nanoparticles of medicinal value as smaller particles penetrate the cell membrane of bacteria (pathogens) more readily and causing cell death (Kim et al., 2007). The peak plasmon resonance shifted to longer wavelengths and broadened at 580 and 640 nm in P. Pavonia and A. spicifera (Fig: 14 to 15) mediated silver nanoparticles indicating the formation of silver nanoparticles greater than 80 nm also. There was no secondary peak formation in any of the seaweed extracts mediated silver nanoparticles except P. Pavonia and S. tenerrum, revealing the absence of quadropole resonance and confirming primary dipole resonance.

5.5.3 Scanning and Transmission electron microscopic analysis of silver nanoparticle

As the size of the nanoparticles influence antimicrobial properties (Panacek et al., 2006), the size and morphology of silver nanoparticles synthesized by using Padina pavonia extract were analysed by transmission and scanning electron microscopes. The scanning electron microscope image (Fig 6) shows the high density AgNPs, which confirmed the development of silver nanostructures. The scanning
electron microscope micrograph of silver nanoparticles found in the *Padina pavonia* assisted synthesis of AgNPs were spherical shaped, size 100 nm and were well distributed without aggregation in solution. Transmission electron micrograph of silver nanoparticles synthesized by the reaction of 1 mM silver nitrate with *Padina pavonia* extract revealed that the average mean size of the silver nanoparticles was 50 nm and confirmed to be spherical in morphology as shown in Fig 7. The study leads to account that seaweeds can be used as an ecofriendly option for developing silver nanoparticles.

### 5.5.4 Antibacterial assay

Silver nanoparticles are reported to have antibacterial (Guzman *et al*., 2009; Yamini sudha Lakshmi *et al*., 2011), anti-inflammatory (Nadworny *et al*., 2008) and antiviral activities (Rogers *et al*., 2008). Silver has long been recognized as having inhibitory effect towards many microorganisms. However silver nanoparticles seemed to have the ability to anchor on to the cell wall of bacteria and subsequently penetrate into the bacterial cell more efficiently causing death (Danil Cauk *et al*., 2006). Therefore assessing antimicrobial property of silver nanoparticles synthesized using seaweeds make these nanoparticles to be a candidate for use in the medicinal field. Antibacterial study revealed that silver nanoparticles synthesized by seaweed extracts considerably inhibited the growth of both Gram positive and Gram negative human pathogens (Tables 34 and 35). The diameter of the inhibition zone against tested pathogens ranged from 4 to 14 mm. The study revealed that AgNPs synthesized by seaweeds were highly effective against bacterial pathogens rather than their respective seaweed extracts (Morones *et al*., 2005). The bactericidal activity of synthesized silver nanoparticles was high against Gram negative bacteria, *E. coli* and the
inhibition zone ranged from 5 to 14 mm at the concentration of 50 µg/ml. In Gram positive bacteria *Bacillus subtilis* action of silver nanoparticles was comparatively less by producing inhibition zone ranging from 6 to 12 mm. The inhibition variation occurred in the present study may invariably be due to the differences in cell wall composition of Gram positive and Gram negative bacteria. Still the exact mechanism of inhibitory action of nanoparticles against bacteria was not well known. However the results of present study indicated that the silver nanoparticles synthesized by green seaweeds have comparatively less antibacterial effect on comparison with streptomycin. So further study is needed to increase the antibacterial effect of nanoparticles synthesized by these seaweed extracts. It has been predicted that the extract of *Padina pavonia*, *Sargassum tenerrimum*, *Spathoglossum asperum* and *Acanthophora spicifera* are capable of producing silver nanoparticles extracellularly and these nanoparticles are quite stable in solution due to capping likely by the protein present in the extract. The AgNPs exhibited potential antibacterial activity against Gram negative human pathogen and therefore, nanoparticles of silver in combination with commercially available antibiotics could be used as an effective antimicrobial agent after further trials on experimental animals.

5.5.5 Fourier Transform Infra- red spectroscopy analysis

Fourier Transform Infra-red spectroscopy measurement were carried out to identify the possible biomolecules responsible for the antifungal, antibacterial properties; reduction of silver ions, and capping of the bio-reduced silver nanoparticles synthesized by *Ulva lactuca*, *Caulerpa scalpelliformis*, *Sargassum tenerrimum*, *Padina pavonia* and *Gracilaria verrucosa*. The FT-IR spectra of seaweeds were depicted in Figs 8 to 12. The representative spectrum of *U. lactuca*
showed absorption peaks located at 3394.08, 2932.63, 1581.73, 1401.09, 1125.63, 710.04 and 627.86 cm\(^{-1}\). Strong peaks in the region of (3500-3200) cm\(^{-1}\) (O-H stretch) indicates phenolic active compounds, (1500-1400) cm\(^{-1}\) (C-C stretch) aromatics clusters, (1000-650) cm\(^{-1}\) (C-H bend-alkenes) and (900-675) cm\(^{-1}\) (C-H stretch-aromatic) corresponds to biomolecules such as primary and secondary amines, aromatic methane, vinyl terminal, aromatic methane, aliphatic aldehydes, and aromatic esters. The FTIR spectrum of \textit{C. scalpelliformis} showed peaks at the range of 3394.08, 3014.07, 2932.63, 1922.1407, 1833.6, 1581.73, 1394.52, 1265.25, 1153.13, 990.55, 922.77, 854.75 and 706.36 cm\(^{-1}\) showing the presence of aliphatic ketones C=O (strong), aromatic esters (very strong), cycloalkenes (medium), cycloalkenes (weak), meta disubstituted (very strong), meta disubstituted (medium to strong). The FTIR spectrum of the \textit{S. tenerimum}, samples also showed fifteen peaks lying between 3407.63 (Primary amines) 430.19 cm\(^{-1}\) (Quinones). FTIR spectrum of \textit{Padina pavonia} showed different peaks at 3935.20, 3420.35, 2926.62, 2368.85, 1567.35, 1403.98, 1258.81, 1117.62, 1036.19, 870.42, 765.22, 711.55, 618.68, 533.28, and 463.77 cm\(^{-1}\). 1250-1020 represented C-N stretch aromatic amines. 850-550 (m) C-Cl stretch showed alkyl halines. The analysis confirmed the presence of compounds belong to such as primary amines, alkanes, secondary amines, aliphatic aldehydes, tertiary butyl aromatic esters, cycloalkanes, heterocyclic monosubstituted benzenes, flavonoids (anthocyanins) and xanthophylls (cryptocanthin). FTIR analysis performed on \textit{G. verrucosa} revealed peaks at 3394.07, 2220.22, 1815.30, 1582.05, 1397.26, 1126.32 and 675.83 cm\(^{-1}\) confirmed the presence of intermolecular hydrogen bonding OH, C-H alkanes, quaternary compounds, C=O aliphatic aldehydes, aromatic esters and (N-H stretch) primary amines. The results of the present study lead to conclude that compounds other than phenols, flavonoids and tannins, other chemical
components like amines, aliphatic aldehydes, aromatic esters, cycloalkenes, heterocyclic, monosubstituted beneze etc., present in seaweeds were responsible for the antibacterial and antifungal properties exhibited by respective seaweeds. The results also revealed that the capping ligand of AgNPs may be aromatic compounds or alkanes or amines. It is advocated that Caulerpa scalpelliformis, Padina pavonia, Sargassum tenerrimum and Gracilaria verrucosa can be sustainably utilized for extraction and purification of bioactive compounds which would open a new door in the field of drug development.