6. BIOCHEMICAL, PHYTOCHEMICAL, PIGMENT CONTENT, ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF SEAWEEDS

1. Introduction

Algae have become very useful in many different industries. Aside from its uses in the food manufacturing industry, it is also being studied for its components which are believed to have medicinal effects. According to Abou-Elela et al., 2009 marine organisms are rich source of structurally, biologically active metabolites. Studies suggested that, some bioactive compounds isolated from marine organisms had shown to exhibit anti-cancer, anti-microbial, anti-fungal or anti-inflammatory and other pharmacological activities. Several algae have been found to have secondary metabolites and most of which are phenolic compounds, which have medicinal potentials (Aliyu et al., 2009) and are important in developing new pharmaceutics (Menelo et al., 2012).

Seaweeds have been used as food, particularly, in far Eastern countries, due to their high content of polysaccharides, minerals and certain vitamins. Seaweeds contain more minerals than any other food due to the surface cell wall polysaccharides that freely and selectively absorb inorganic nutrient from the sea (Jacobsen, 2010). Porphyra umbilicalis (purple laver) is among the most nutritious seaweeds (Indergaard and Minsaas, 1991). Furthermore, seaweeds are also used as animal fodder.

Reactive oxygen species such as hydroxyl, superoxide and peroxyl radicals are formed in human cells by endogenous factors and exogenously result in extensive oxidative damage that in turn leads to geriatric degenerative conditions, cancer and wide range of other human diseases (Aruoma, 1999; Borek, 1993; Reaven and Witzum, 1996).
Carotenoids, the natural pigments from plant origin react rapidly with these free radicals and retard or alleviate the extent of oxidative deterioration (Akoh and Min, 1997). Furthermore, antioxidants from natural sources increase the shelf-life of foods (Schwarz et al., 2001). Therefore, consumption of antioxidant and/or addition of antioxidant in food materials protect the body as well as fights against these events (Chandini et al., 2008).

Many researchers have reported various types of antioxidants in different kinds of higher plants (Larson, 1988; Shon et al., 2003). More recent reports revealed seaweeds to be a rich source of antioxidant compounds (Duan et al., 2006; Kuda et al., 2005; Lim et al., 2002; Park et al., 2004).

Having the advantages of low cost raw material, high amounts of secondary metabolites, and no secondary pollution, this alga may be used as a natural source of antibacterial and antioxidant agents. This study aimed to document qualitatively, the phytochemical, pigment and biochemical composition of eight seaweeds collected from Puducherry coast in order to have baseline on its medicinally active compounds, particularly, in its antibacterial and antioxidant activities.

2. Materials and Methods

2.1. Sample collection and preparation

Fresh thallus of Enteromorpha compressa, Enteromorpha intestinalis, Ulva fasciata, Ulva lactuca, Chaetomorpha antennina, Padina gymnoспора, Grateloupia lithophila, and Hypnea valentiaе were collected from the intertidal regions of Puducherry coast, India. The seaweeds were washed thoroughly with water to remove
extraneous materials. Washed samples were shade dried and grounded with the help of electric mixer. The powdered seaweed samples were then stored in refrigerator for further use.

2.2. Preparation of crude extract

The seaweed powders were extracted with acetone in soxhlet extractor for 12 h. The extracts were then concentrated under reduced pressure using a rotary flash evaporator. The crude extracts obtained were stored in dark at 4°C for further use.

2.3. Phytochemical analysis

2.3.1. Detection of alkaloids

To 2 g of seaweed powder, 1 g of calcium hydroxide and 5 ml of water was added and made into smooth paste and set aside for 5 min. It was then evaporated to dryness in a porcelain dish on a water bath. Alcohol (20 ml of 90%) was added and mixed well, then refluxed for half an hour on a water bath. Then it was filtered and the alcohol was evaporated in a desiccator. To this, dilute sulphuric acid was added and tested with various alkaloidal reagents such as Dragendorff’s reagent, wherein, reddish orange precipitation indicates the presence of alkaloids.

2.3.2. Detection of Sugar

Fehling’s test: The aqueous extract of the powdered material was treated with Fehling’s solution I and II and heated on a boiling water bath. Reddish brown precipitate was obtained indicating the presence of free reducing sugars.
2.3.3. Detection of Proteins and Amino Acids

Five ml of each extract was dissolved in 5 ml of water and were subjected to the following tests,

a) Biuret test: One ml of each of the various extract was warmed gently with 10% NaOH solution and a drop of dilute CuSO₄ solution. Formation of reddish violet showed the presence of proteins and free amino acids.

b) Ninhydrin test: One ml of each of the various extract was treated with a few drops of ninhydrin solution. Change in colour indicated the presence of protein and free amino acids.

2.3.4. Detection of Sterols

The powdered seaweed sample was extracted with petroleum ether and evaporated to a residue. Then the residue was dissolved in chloroform and tested for sterols

a) Salkowski’s test: To the chloroform solution few drops of conc. H₂SO₄ was added, shaken well and set aside. The chloroform layer turned red colour indicating the presence of sterols.

b) Libermann-Burchard’s test: To the chloroform solution, a few drops of acetic anhydride and 1 ml of conc. H₂SO₄ were added through the sides of the test tube and set aside for a while. At the junction of two layers a brown ring was formed and the upper layer turned to green colour indicating the presence of sterols.
2.3.5. Detection of Saponins:

About 0.5 g of each powdered material was boiled gently for 3 min with 20 ml of water. Filtered while hot and allowed to cool. Filtrate (5 ml) was diluted with water and shaken vigorously. Production of frothing indicated the presence of saponin.

2.3.6. Detection of Coumarins:

One ml of each alcoholic extract was treated with alcoholic NaOH solution. Production of dark yellow colour indicated the presence of coumarins.

2.3.7. Detection of Flavanoids:

Five ml of each extract was dissolved in 1 ml of alcohol (Stock solution) and then subjected to the following test.

a) **Ferric chloride test**: To one ml of stock alcoholic solution add a few drops of neutral FeCl₃ solution. Formation of blackish red colour indicates the presence of flavanoids.

2.3.8. Detection of Phenols:

One ml of each extract was dissolved in 5 ml of alcohol with a few drops of neutral FeCl₃ solution. Change in colour indicated the presence of phenolic compounds.

2.3.9. Detection of Tannins:

Each seaweed powdered sample (5 g) was dissolved in minimum amount of water, filtered and the filtrate was then subjected to the following tests.

a) **Ferric chloride test**: To the above filtrate, a few drops of ferric chloride solution was added. The colour change indicates the presence of tannins.
b) **Basic lead acetate test:** To the filtrate, a few drops of aqueous basic lead acetate solution was added. Formation of reddish brown precipitate indicates the presence of tannins.

**2.3.10. Detection of Carboxylic Acids:**

Each seaweed extract (1 ml) was treated with a few ml of saturated solutions of sodium bicarbonate. Observation of effervescence (due to liberation of CO₂) indicated the presence of carboxylic acids.

**2.3.11. Detection of Quinone:**

Each seaweed extract (1 ml) was treated with alcoholic KOH solution. Quinones give colourations ranging from red to blue.

**2.3.12. Detection of Xanthoprotein:**

Seaweed extract (1 ml) was treated separately with a few drops of conc. HNO₃ and NH₃ solution. Formation of reddish orange precipitation indicated the presence of xanthoproteins.

**2.4. Pigment Composition**

**2.4.1. Estimation of Chlorophyll**

Amount of chlorophyll present was estimated by the method of Arnon, (1949). 500 mg of seaweed sample was kept in a pestle and mortar with 10 ml of 80% acetone and it was ground well and the homogenate was centrifuged at 3000 rpm for 15 min and the supernatant was stored. The pellet was re-extracted with 5 ml of 80% acetone each time till the pellet became colourless. All the extracts were pooled and utilized for
chlorophyll determination. Absorbance was measured at 645 nm and 663 nm in a spectrophotometer. The chlorophyll content was determined using the following formula

\[
\text{Total Chlorophyll (mg/g.fr.wt.)} = 22.2 \times A_{645} - 8.02 \times A_{663} \times V
\]

\[
A \times 1000 \times W
\]

A= Absorbance at respective wave length

V= Volume of extract (ml)

W= Fresh weight of the sample (g)

2.4.2. Estimation of Carotenoids (Kirk and Allen, 1965)

The same chlorophyll extract was measured at 480 nm in spectrophotometer to estimate the carotenoids content.

\[
\text{Carotenoid : (\mu g/g.fr.wt.)} = \Delta A_{480} + (0.114 \times \Delta A_{663}) - (0.638 \times \Delta A_{645})
\]

\(\Delta A\) = Absorbance at respective wave length

2.5. Biochemical Composition

2.5.1. Estimation of Protein

The protein content was estimated by Biuret method (Raymont et al., 1964). To 5 mg of dried powdered sample, 1 ml of distilled water followed by 4 ml of biuret reagent were added and incubated for 30 min in room temperature. Then the mixture was centrifuged for 10 min at 4000 rpm. The supernatant was collected and the optical density was measured in a Spectrophotometer at 540 nm. The protein content was calculated using BSA as standard and expressed as mgg\(^{-1}\) protein.
2.5.2. Estimation of Lipid

Lipid content was estimated by chloroform-methanol mixture as described by Folch et al. (1957). To 10 mg of dried powder sample, 5 ml of chloroform-methanol (2:1) mixture was added. The mixture was incubated at room temperature for 24 h after closing the mouth of the test tube with aluminium foil. After incubation, the mixture was filtered using a filter paper. The filtrate was collected in a 10 ml pre-weighed beaker, which was kept on a hot plate. The chloroform-methonal mixture was evaporated leaving a residue at the bottom of the beaker. The beaker with the residue and the weight of the empty beaker was calculated to know the weight of the lipid present in the sample. The percentage of lipid present in the sample was calculated by using the following formula.

\[
\% \text{ of Lipid} = \frac{\text{Amount of Lipid in the sample}}{\text{Weight of the sample taken}} \times 100
\]

2.5.3. Estimation of Carbohydrates

The carbohydrate content was estimated by Dubois method (Dubois, 1956). 20 mg of dried seaweeds powder was taken and to this 1 ml of 4% phenol solution and 5 ml of concentrated sulphuric acid were added. After that, they kept in a dark room for 30 min. The colour intensity developed was read in a spectrophotometer at 490 nm. Sugar content was calculated by referring to a standard D- Glucose and the results have been expressed as mg g\(^{-1}\) sugar.

2.5.4. Statistical analysis

Analyses of variance for the difference in biochemical composition among the eight seaweeds wereanalyzed by Two-Factor ANOVA without replication.
2.6. Antibacterial activity

2.6.1. Bacterial strains

Human pathogens namely *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Vibrio parahaemolyticus* and *Klebsiella pneumoniae* were obtained from the Department of Medical Microbiology, Rajah Muthaiah Medical College, Annamalai University, Tamilnadu, India. The bacterial stock cultures were maintained in nutrient agar slant at 4°C.

2.6.2. Disc Diffusion assay

Whatmann filter paper (No.1) discs of 6 mm diameter were impregnated with 10 μl of the solution containing crude extracts obtained from the seaweeds (at a concentration of 100 mg/ml) and these discs were evaporated at 37°C for 24 h. Reference standard discs were prepared with streptomycin (50 μg/ml) to compare the antibacterial activity of seaweed extracts. After drying, the discs with seaweed extract and standard streptomycin were placed on Muller Hinton agar (MHA) already swabbed by bacterial stock cultures and incubated at 37°C for 24 h. After incubation, plates were examined for clear zone around the discs. A clear zone with diameter more than 2 mm was taken as a antibacterial activity. All the experiments were carried out in triplicate and the mean values were recorded (Kartmig *et al.*, 1991).

2.7. Antioxidant assay

2.7.1. Evaluation of antioxidant activity

The lyophilized seaweed extracts were dissolved in distilled water at a concentration of 10 mg ml⁻¹. The free radial scavenging activity of the seaweed extracts
was evaluated using standard procedures and Gallic acid was used as the reference compound. All analysis were run in triplicates and averaged.

I. Total phenolic content

Phenolic contents of crude extracts were estimated by the method of Taga et al., (1984). Briefly 100 µl of aliquot sample was mixed with 2.0 ml of 2% Na₂CO₃ and allowed to stand for 2 min at room temperature. After incubation, 100 µl of 50% FolinCiocalteau’s phenol reagent was added and the reaction mixture was mixed thoroughly and allowed to stand for 30 min at room temperature in the dark. Absorbance of all the sample solutions was measured at 720 nm using spectrophotometer (Phenolic content are expressed as GAE g⁻¹).

II. Total antioxidant activity

TAA was measured following the method of Prieto et al., (1999). To 7.45 ml of sulphuric acid (0.6 mM solution), 0.9942 g of sodium sulphate (28 mM solution) and 1.2359 g of ammonium molybdate (4 mM solution) were mixed together in 250 ml distilled water and labeled as TAC reagent. 300 µl of extract was dissolved in 3 ml of TAC reagent. Blank was maintained with distilled water replacing the TAC reagent. Absorbance of all sample mixtures was measured at 695 nm. TAA is expressed as the number of equivalents of AscA.

III. Reducing power

RP of different crude extract was determined by the method prescribed by Oyaizu (1986). 1.0 ml of different solvent extract containing different concentration of samples was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferric cyanide (1%). Reaction mixture was kept in a water bath at 50°C for 20 min. After
incubation, 2.5 ml of Trichloroacetic acid (10% of TCA) was added and centrifuged at 650 rpm for 10 min. From the layer, 2.5 ml solution was mixed with 2.5 ml of distilled water at 0.5 ml of ferric chloride (0.1%). Absorbance of all the solution was measured at 700 nm. Increased absorbance indicates increased reducing power.

IV. Hydrogen peroxide radical scavenging assay

The ability of seaweed extract to scavenge hydrogen peroxide was determined by following the standard procedure (Gulçin et al., 2004). Hydrogen peroxide 10 mM solution was prepared in the phosphate buffer saline of 0.1 M, pH 7.4. 1 ml (0.25 mg) of the extract was rapidly mixed with 2 ml of hydrogen peroxide solution. The absorbance was measured at 230 nm in the UV spectrophotometer against a blank (without hydrogen peroxide) after 10 min of incubation at 37°C. The percentage of scavenging of hydrogen peroxide was calculated using the following formula

\[
\% \text{ scavenging (H}_2\text{O}_2) = (A_0 - A_1/A_0) \times 100
\]

\(A_0\) - absorbance of control

\(A_1\) - Absorbance of sample.

V. DPPH radical scavenging activity

The Scavenging effects of crude methanol extract and fractions were determined by the method of Yen and Chen (1995). Briefly, 2.0 ml of 0.16 mM DPPH solution (in methanol) was added to the test tube containing 2.0 ml aliquot of sample. The mixture was vortexed for 1 min and kept at room temperature for 30 min in the dark. The absorbance of all the sample solutions was measured at 517 nm. The Scavenging effect (%) was calculated by using the formulae given by Duan et al., (2006).
Scavenging effect (%) = \[1 - (A \text{ sample} - A \text{ blank}) / A \text{ control}\] \times 100

A control - absorbance of the control (DPPH solution without sample)

A sample - absorbance of the test sample (DPPH solution + Test sample)

A blank - absorbance of the sample only (sample without DPPH solution).

3. Results

3.1. Phytochemical screening

The presence of important phytochemicals such as alkaloids, sugars, proteins and amino acids, sterols, saponins, coumarins, flavonoids, phenols, tannins, carboxylic acids, quinones, and xanthoproteins were screened and represented in the Table 1. Sugars, flavanoids and proteins and amino acids were present in all the eight seaweeds screened. Coumarins are present in all seaweeds analysed except Grateloupia lithophila. Saponins are present only in Padina gymnospora, and Hypnea valentiae. Padina gymnospora showed the presence of all the 12 phytochemicals screened. Ulvalactuca also showed the presence of almost all the phytochemicals screened except saponins and carboxylic acids.

3.2. Pigment composition

Photosynthetic pigments were estimated and the results were presented in Fig. 2. The total chlorophyll ranged from \(0.40 \pm 0.01\) to \(0.75 \pm 0.01\) mgg\(^{-1}\) with minimum in red seaweed Hypnea valentiae and maximum in green seaweed Ulva fasciata. The carotenoid content ranged from \(0.37 \pm 0.01\) to \(0.85 \pm 0.01\) mgg\(^{-1}\) with minimum in green seaweeds
*Enteromorpha compressa, Ulva lactuca, Chaetomorpha antennina* and maximum in brown seaweed *Padina gymnospora*.

### 3.3. Biochemical composition

#### 3.3.1. Protein

Quantitative analysis of protein content ranged from 10.6% to 26.3%. Maximum protein content was found in *Padina gymnospora* (26.3%) followed by *Grateloupia lithophila* (23.3%), *Hypnea valentiae* (22.3%). Minimum content was found in *Chaetomorpha antennina* (10.6%), *Enteromorpha compressa* (11%), *Enteromorpha intestinalis* (12%). The results are shown in Fig. 3. All the seaweeds analysed exhibited significant (p<0.05) value of protein content which is shown in Table 2.

#### 3.3.2. Lipid

Lipid content of seaweeds ranged from 1.5% to 2.9% as shown in Fig. 4. Maximum lipid content was found in *Ulva lactuca* (2.9%), followed by *Ulva fasciata* (2.5%), *Enteromorpha intestinalis* (2.2%) and *Chaetomorpha antennina* (2.2%). Minimum content was found in *Padina gymnospora* (1.5%), *Hypnea valentiae* (1.6%), and *Grateloupia lithophila* (1.7%) respectively. All the seaweeds analysed exhibited significant (p<0.05) value of lipid content which is shown in Table 2.

#### 3.3.3. Carbohydrates
Carbohydrate content of seaweeds ranged from 18.6% to 50%. Maximum carbohydrate content was found in Padina gymnospora (50%), followed by Grateloupia lithophila (41.3%) and Hypnea valentia (40.6%). Minimum content was found in Enteromorpha intestinalis (18.6%), Enteromorpha compressa (21.3%), and Chaetomorpha antennina (23.6%) respectively as presented in Fig. 5. All the seaweeds analysed exhibited significant (p<0.05) value of carbohydrate content which is shown in Table 2.

3.4. Antimicrobial activity

Eight seaweed extracts were examined for antibacterial activity against five bacterial pathogens (Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Vibrio parahaemolyticus and Klebsiella pneumoniae). Brown seaweed Padina gymnospora showed maximum zone of inhibition against all the pathogens examined with maximum activity against Klebsiella pneumoniae (14.7 ± 0.5 mm) and minimum activity against Escherichia coli (10.7 ± 0.6 mm). Pseudomonas aeruginosa strain showed resistance and thus minimum activity was recorded to extracts of Enteromorpha compressa, Enteromorpha intestinalis, Ulva fasciata, Ulva lactuca, and Chaetomorpha antennina while maximum zone of inhibition was recorded as 13.8 ± 0.9 mm against P. gymnospora which was higher than the value of positive control streptomycin (10.0 ± 1.0 mm). E. coli also showed resistance against Ulva fasciata, Ulva lactuca, and Chaetomorpha antennina while maximum zone of inhibition was recorded as 10.7 ± 0.6 mm against P. gymnospora. The results are shown in Table 2.

3.5. Antioxidant activity
3.5.1. Total phenolic content:

Phenolic compounds are commonly found in plants and have been reported to have several biological activities including antioxidant activity. Acetone extract of *Padina gymnospora* followed by *Grateloupia lithophila* exhibited higher phenolic content of 0.89 ± 0.02 and 0.61 ± 0.02 mg GAE/g of seaweed extract respectively (Fig. 6), as compared to other seaweeds analysed. Minimum phenolic content of 0.28 ± 0.03 mg GAE/g was recorded in *Chaetomorpha antennina*.

3.5.2. Total antioxidant activity:

Total antioxidant activities of eight seaweeds are presented in Fig. 7. Higher activity of 1.92 ± 0.05, 1.54 ± 0.07 and 1.27 ± 0.05 mg ascorbic acid equivalent/g of seaweed was observed in *Padina gymnospora*, *Grateloupia lithophila*, and *Hypnea valentinae* respectively. Minimum activity of 0.64 ± 0.03 mg ascorbic acid equivalent/g of seaweed was observed in *Chaetomorpha antennina*.

3.5.3. Reducing power:

Reducing power of the seaweeds analysed are presented in the Fig. 8. The maximum (2.678 ± 0.03) reducing power value was observed in 1 ml concentration of *Padina gymnospora* and minimum (0.69 ± 0.02) was obtained in *Enteromorpha compressa*.

3.5.4. Hydrogen peroxide radical scavenging assay:

Hydrogen peroxide radical scavenging activity of *Padina gymnospora* was recorded in highest percentage (91%) followed by *Grateloupia lithophila* (54%) and *Hypnea valentinae* (54%) respectively. The lowest scavenging activity was
recorded in *Chaetomorpha antennina* (28%), *Enteromorpha compressa* (31%) and *Enteromorpha intestinalis* (31%) respectively which was shown in Fig. 9.

3.5.5. DPPH radical scavenging activity:

Free radical scavenging ability of seaweeds were expressed in percentage (%) and shown in Fig. 10. Maximum activity was found in *Padina gymnospora* (90%) followed by *Grateloupia lithophila* (75%) and *Hypnea valentiae* (60%) respectively and the lowest activity was recorded in *Chaetomorpha antennina* (25%), *Ulva lactuca* (41%) and *Enteromorpha intestinalis* (41%).

4. Discussion

The results of the phytochemical analysis revealed the presence of various secondary metabolites with varied degree. Phenolic compounds are widely distributed in the plant kingdom and have been reported to have several biological activities including antioxidant properties (Johnson Marimuthu *et al.*, 2012). Earlier reports revealed that marine seaweed extracts, especially polyphenols have antioxidant activity (Chandini *et al.*, 2008; Ganesan *et al.*, 2008). Alkaloids are commonly found to have antimicrobial properties against both Gram-positive and Gram-negative bacteria (Cowan, 1999). Flavonoids are known as nature’s tender drug which possesses numerous biological and pharmacological activities. Recent reports of antiviral, anti-fungal, antioxidant, anti-inflammatory, antiallergenic, antithrombic, anticarcenogenic, hepatoprotective and cytotoxic activities of flavonoids have generated interest in studies of flavonoid containing plants (Veitch, 2007; Jiang *et al.*, 2008). Steroids may serve as an intermediate for the biosynthesis of downstream secondary natural products and it is
believed to be a biosynthetic precursor for cardenolides in plants. Marine algae have shown to be good source of unsaponifiable, non-toxic sterols that have medicinal value (Rajasulochana et al., 2009; Sanchez-Machado et al., 2004). Saponins possess numerous biological properties which include antimicrobial, anti-inflammatory, anti-feedent and hemolytic effects (Xu et al., 2000). The presence of alkaloids, sugars, proteins and amino acids, sterols, saponins, coumarins, flavonoids, phenols, tannins, carboxylic acids, quinones, and xanthoproteins in *P. gymnospora* suggest that seaweeds can be used as antimicrobial (anti-viral, anti-fungal and anti-bacterial), anti-parasitic, anti-inflammatory, anti-feedent, antioxidant, antiallergenic, anti-thrombic, anti-carcenogenic and anti-ulcer agents in the near future.

The overall observation of the pigment data revealed that maximum amount was recorded in green algae. The maximum total chlorophyll content was recorded in *Ulva fasciata* of $0.6 \pm 0.017$ mg/g and minimum in *Hypnea valentiae* of $0.40 \pm 0.01$ mg/g. The highest carotenoids content was observed in *P. gymnospora* of $0.85 \pm 0.01$ mg/g and lowest in *E. compressa, U. lactuca* and *C. antennina* of $0.37 \pm 0.01$ mg/g. The maximum total chlorophyll and carotenoids content were found in green and brown algae respectively. Saranya et al. (2013) also reported on better chlorophyll content observed in green algae and carotenoids in brown algae. Similarly, Sukran et al. (2003) also reported on better pigment content observed in green and brown algae.

Carotenoid detected on seaweedextract could speculate on its antibacterial effect. These compounds are liposolubletertaterpenes with series of conjugated olefinic bonds constituents (Menelo et al., 2012). The chromophoric group of a carotenoids have been
described to give color on food products from yellow to red (El-Refai et al., 2010). Extracted carotenoid potency have been documented in the studies of El-Badrwy and El-Fadaly, 2000, Nannapaneni et al., 2008 and Tao et al., 2010 which showed variable degree of antimicrobial effect. The study of Cucco et al., 2007, showed that β-carotene an antibacterial immune enzyme that digest bacterial cell walls which could lead to the accumulation of lysozyme, in effect it generates the antibacterial activity. In addition, neoxanthin, fucoxanthin, and violaxanthin have been detected on Padina australis extract. These are xanthophylls, a subclass of carotenoids consisting of the oxygenated carotenes (Moss et al., 1994). Specifically, violaxanthin is a natural constituent found in variety of plants with an orange color. Neoxanthinin plants is an intermediate in the biosynthesis of the plant hormone abscisic acid and produced from violaxanthinby the action of neoxanthin synthase (Bouvier et al., 2000). While fucoxanthin is found as an accessory pigment in the chloroplasts of brown algae like P. australis which gave its distinct olive-green color and including other heterokonts (Maeda et al., 2005). These xanthophylls have been associated with antibacterial activity and could consider on antibacterial property of P. gymnospora extract. Similar results have been documented on Toddalia asiatica dyes where most of its pigment are xanthophylls which showed its antimicrobial activity on Bacillus cereus, Escherichia coli, Klebsiella pneumoniae and Vibrio cholera (Das et al., 2011). The photosynthetic pigment fucoxanthin on Fucus vesiculosus, a brown alga appeared to be ecologically relevant as a surface-associated antimicrobial agent, acted against the settlement of bacteria on the surface of the F. vesiculosus as documented by Saha et al., 2011.
Proteins have crucial functions in all the biological processes. Their activities can be described by enzymatic catalysis, transport and storage, mechanical sustentation, growth and cellular differentiation control (Dera et al., 2003). Similarly the brown seaweeds have higher protein content than the other two groups of seaweed and the result is very much similar to earlier observation of Wong and Cheung, (2000). Marinho-Soriano et al. (2006) have studied tropical seaweed for their chemical composition and showed that brown algae contain more protein when compared to red algae. In this present study, protein content ranged from 10.6% to 26.3% and maximum protein content was found in Padina gymnospora as 26.3%. Among the different groups, brown seaweeds exhibited higher values than the other two groups. Dhamotharan, (2002) investigated on protein contents and found that the highest protein contents were observed in brown algae Stoechospermum marginatum (10.6 ± 0.162%). Protein content varied among different genera and also in different species of the same genus (Dhargalkar et al., 1980). Protein content in the same species but collected from different localities and different seasons also showed fluctuating values (Dave and Parekh, 1975). Variations in the protein content of seaweeds can be due to different species and seasonal periods (Fleurence, 1999; Galland-Irmouli et al., 1999).

Lipid content in the present study varied from 1.5 to 2.9%. Lipids are rich in –C=O– bonds, providing much more energy in oxidation processes than other biological compounds. They constitute a convenient storage material for living organisms. The maximum lipid content was found in Ulva lactuca of 2.9% and minimum in Padina gymnospora of 1.5%. Murugaiyan et al. (2012) recorded that lipid content varied from 0.9 ± 0.38 % to 3.58 ± 0.45% with maximum lipid content in green algae. Similarly,
in this present study also maximum result were observed in green algae. In seaweeds, the lipids are widely distributed, especially in several resistance stages (Norziah and Ching, 2000). Seaweeds are relatively low in lipid (1–5% of dry weight) (Burtin, 2003; Polat and Ozogul, 2008).

Total sugar is one of the important components for metabolism and it supplies the energy needed for respiration and other most important processes. Present study showed that the carbohydrate content of seaweeds ranged from 18.6% to 50% with maximum in Padina gymnospora (50%) and minimum in Enteromorpha intestinalis (18.6%). Ganga Devi et al. (1996) found that carbohydrate content of seaweeds was 1.36 to 9.66% and Ganesan and Kannan, (1994) also reported that carbohydrate content varied from 0.02 to 1.94% for seaweeds of Gulf of Mannar. These reports showed low carbohydrate content than the present study. McDermid and Stuercke, (2003) reported on carbohydrate of 11.8%, 15.2%, and 16.0% in Caulerpa, Gracilaria and Laurencia, respectively. Similar range was also noticed in the earlier reports (Reeta et al., 1990; Reeta and Kulandaivelu, 1999; Manivannan et al., 2008). As per the earlier reports, seaweeds contain large amount of carbohydrates but less amount of protein and amino acids (Burtin, 2003).

Antimicrobial activities found in seaweeds were considered to be an indication of synthesis of bioactive secondary metabolites. The seaweeds have an effective antibacterial activity against most of the human bacterial pathogens. It was reported that 151 species of seaweeds crude extracts showed inhibitory activities against pathogenic bacteria (Hornsey and Hide, 1985). There have been a number of reports that demonstrate the antimicrobial activity of marine plants (Zampini et al., 2009) such as, seaweeds (Devi et al., 2008; Haliki et al., 2005; Nair et al., 2007; Sasidharan et al., 2010),
Mangroves (Chandrasekaran et al., 2009) and seagrass (Kumar et al., 2008; Kannan et al., 2010).

Kandhasamy and Arunachalam, (2008) had studied the Chlorophyceae members and it showed high antibacterial activity than other members. The current study inferred with Phaeophyceae members showed higher antibacterial activity than Rhodophyceae and Chlorophyceae. The brown algae have naturally high secondary metabolites compared to red and green. The results shows that the brown algae P. gymnospora a strong antimicrobial activity against with streptomycin standard. Extracts of marine brown algae have been reported to exhibit antibacterial activity (Kim et al., 2007; Kamenarska et al., 2009) and antimicrobial activity (Tringali, 1997; Funahashik et al., 2001).

In the present study, brown seaweed Padina gymnospora showed maximum zone of inhibition against all the pathogens examined with maximum activity against Klebsiella pneumoniae (14.7 ± 0.5 mm) and minimum activity against Escherichia coli (10.7 ± 0.6 mm). The present results agreed with the findings of Rao and Parekh, (1981) and Padma Kumar and Ayyakkannu, (1997) that organic extract of Indian seaweed exhibit antimicrobial activity against gram negative and gram positive biomedical pathogens. In the present findings we have immense potential on the control of clinical pathogens, since the strains used in the study were collected from hospital sources and most of the strains appeared as multi drug resistant and cannot be controlled with commercially prescribed antibiotics (Adwan and Abu-Hasan, 1998).

Previous studies reported the screening of seaweeds on human and plant pathogenic virus, bacteria and fungi (Robles-Centeno et al., 1996; Arun Kumar and
Rengaswamy, 2000). Hence, more studies pertaining to the use of seaweed as therapeutic agent should be emphasized, especially those related to the control of multi drug resistant microbes. Margret et al., 2008 reported that methanol extract of *Acanthophora spicifera* was active against gram negative bacterial pathogens *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli*.

The antimicrobial activity of seaweeds may be influenced by some factors such as the habitat and the season of algal collection, different growth stages of plant, experimental methods, etc., although a variety of solvents have been employed in screening seaweeds for antimicrobial activity, it is still uncertain the kinds of solvents is the most effective and suitable for extraction of seaweeds. A few researchers tried using different solvents for screening the antimicrobial activity of seaweeds and made comparisons (Selvi et al., 1999; Rangaiah et al., 2010; Rajauria et al., 2012; Sridharan and Dhamotharan, 2012; Rhimou et al., 2010; Jeyaseelan et al., 2012; Uma Maheswari et al., 2006; Bansemir et al., 2006; Chiheb et al., 2009). Several researchers make on effort using diverse pathogens for selecting the antimicrobial assay (Del-Val et al., 2001; Tuney et al., 2007).

Natural antioxidants are not limited to terrestrial sources and reports have revealed seaweeds are also rich in natural antioxidant compounds (Lim et al., 2002; Duan et al., 2006; Kuda et al., 2007). The presence of phytoconsituents, such as phenols, flavanoids and tannins in seaweeds and seagrasses indicated a possible role that its extracts may have antioxidant activity. This activity was believed to help in preventing a number of diseases through free-radical scavenging activity (Anggadiredja et al., 1997; Ruberto et al., 2001). Due to the presence of different antioxidant components in the
crude extracts of biological tissue samples, it is relatively difficult to measure each antioxidant component separately. Therefore, several assay methods have been developed and applied in recent years to screen and evaluate the total antioxidant activity of such samples (Prabhakar et al., 2006; Wangensteen et al., 2004). In this study, antioxidant activities were tested using five different assays, total phenolic content, total antioxidant activity, reducing power, Hydrogen peroxide radical scavenging assay, DPPH radical scavenging activity.

In the present study, the total phenolic content ranged from 0.28 ± 0.03 to 0.89 ± 0.02 mg GAE g⁻¹ with minimum in the green seaweed *Chaetomorpha antennina* and maximum in the brown seaweed *P. gymnospora*. Similarly, Jimenez et al., (2001) reported that brown seaweeds contain higher phenolic content than the red seaweeds. Reports have revealed that phenolic compounds are one of the most effective antioxidants in brown algae (Nagai and Yukimoto, 2003) and also known to contain phlorotannins and fucoxanthin as major active compounds (Yan et al., 1996; 1999). Higher total antioxidant activity of 1.92 ± 0.05 mg ascorbic acid equivalent/g of seaweed was observed in *Padina gymnospora*, and minimum activity of 0.64 ± 0.03 mg ascorbic acid equivalent/g in *Chaetomorpha antennina*.

The effect of antioxidants on DPPH radical scavenging is thought to be due to hydrogen donating ability. The DPPH assay method is based on the reduction of DPPH, a stable free radical. With the odd electron, the free radical DPPH gives a maximum absorption at 517 nm by visible spectroscopy (purple color). As the odd electron of the free radical comes paired off in the presence of a hydrogen donor, e.g. a free radical scavenging antioxidant, the absorption strength is decreased, and the resulting
decolourization (yellow colour) is stoichiometric with respect to the number of electrons captured (Blois, 1958). In the present study, the maximum percentage of radical scavenging activity was observed in brown seaweed *P. gymnospora* (90%) and minimum in *Chaetomorpha antennina* (25%). Similarly, Ganesan *et al.*, (2008) noticed higher percentage DPPH radical scavenging activity in methanol extract of brown seaweed *Sargassum polycystum*. Hydrogen peroxide radical scavenging activity of *Padina gymnospora* was recorded in highest percentage (91%) and lowest in *Chaetomorpha antennina* (28%).

In the reducing power assay, antioxidants in the sample reduce ferric (III) to ferrous (II) in a redox linked colourimetric reaction (Li *et al.*, 2006) that involves single electron transfer, the reducing power indicates that the antioxidant compounds are electron donors and reduce the oxidized intermediate of the lipid peroxidation process, so that they can act as primary and secondary antioxidants (Yen and Chen, 1995). Concentration dependency of antioxidant activity was investigated as a function of reducing power as this gave a general view of reductones present in the sample. Reducing power increased with increasing concentrations in all the samples. In the present study also reducing power increased with increasing concentration, maximum reducing power value was observed in 1 ml concentration of *Padina gymnospora* (2.678 ± 0.03). The present results are in agreement with Matsukawa *et al.*, (1997), who found that the antioxidant activity of brown algae was superior to that of red or green groups.

Overall results showed that brown algae *P. gymnospora* exhibits maximum activity and shows remarkable phytochemical, biochemical and pigment composition compared to other seaweeds analyzed. The antibacterial and antioxidant activities could
be attributed to the presence of different secondary metabolites such as phenolic compounds, and carotenoids and the mechanism of action might be due to their individual or collective participation. The experimental findings envisaged *P. gymnospora* extract as a good candidate in developing new antibacterial and antioxidant agents.
<table>
<thead>
<tr>
<th>S.No</th>
<th>Seaweeds</th>
<th>Alk</th>
<th>Sug</th>
<th>Pro</th>
<th>Ste</th>
<th>Sap</th>
<th>Cou</th>
<th>Flv</th>
<th>Phe</th>
<th>Tan</th>
<th>Carb</th>
<th>Qui</th>
<th>Xan</th>
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<tbody>
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<td>+</td>
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<td>Chaetomorpha antennina</td>
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Table 2: Analysis of variance for the difference in biochemical composition by ANOVA

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<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
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<td><strong>Protein</strong></td>
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<tr>
<td>Between Groups</td>
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<td>115.994</td>
<td>74.09506</td>
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<td>2.764199</td>
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<tr>
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<td>2.041667</td>
<td>1.304183</td>
<td>0.302418</td>
<td>3.738892</td>
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<td></td>
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<tr>
<td><strong>Total sugar</strong></td>
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<td>Between Groups</td>
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<td>384.9464</td>
<td>45.00418</td>
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<td>Total</td>
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<td><strong>Lipids</strong></td>
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<tr>
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</table>
**Table 3:** Antibacterial activity of seaweeds from Puducherry coast

<table>
<thead>
<tr>
<th>S. No</th>
<th>Bacterial Pathogen</th>
<th>Zone of Inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>E. compressa</em></td>
</tr>
<tr>
<td>1</td>
<td><em>E. coli</em></td>
<td>3.0 ± 0.5</td>
</tr>
<tr>
<td>2</td>
<td><em>S. aureus</em></td>
<td>2.9 ± 0.3</td>
</tr>
<tr>
<td>3</td>
<td><em>P. aeruginosa</em></td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td><em>V. parahaemolyticus</em></td>
<td>4.7 ± 0.2</td>
</tr>
<tr>
<td>5</td>
<td><em>K. pneumoniae</em></td>
<td>3.2 ± 0.3</td>
</tr>
</tbody>
</table>
Fig. 2: Pigment content of seaweeds

Fig. 3: Protein content of seaweeds

Fig. 4: Lipid content of seaweeds
Fig. 5: Carbohydrate content of seaweeds

Fig. 6: Total phenolic content of seaweeds

Fig. 7: Total antioxidant activity of seaweeds
Fig. 8: Reducing power of seaweeds

Fig. 9: Hydrogen peroxide radical scavenging assay of seaweeds

Fig. 10: DPPH radical scavenging activity of seaweeds