CHAPTER I

Evaluation of nutritional profile of the marine brown macroalga Padina gymnospora
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3.1. INTRODUCTION

Seaweeds or macroalgae are noticeable seashore resources which are consumed by humans in several countries. Edible macroalgae were commonly consumed by seaside people, particularly in Asian countries as fresh, dried out, or as additive food ingredients in prepared foodstuffs. Compared to terrestrial flora, the detailed analysis of biochemical compositions of marine macroalgae is not available. The biological composition of marine macroalgae differs with types, location, ripeness and ecological conditions (Ratana-arporn & Chirapart, 2006). Currently seaweeds are given more attentiveness in pharmacological and the daily dietary requirements, because they hold a countless number of bioactive molecules including sulfated polysaccharides, dietary fiber, protein, carotenoids, fatty acids, vitamins and minerals. The nutritional profiles of marine macroalgae are not fully identified yet, and they are generally evaluated from their bioactive potential (Peng et al., 2012). In addition seaweeds produce a massive range of chemical constituents characterized by an enormous range of biological activities.

Though the brown macro alga Padina gymnospora is known to possess a variety of valuable medicinal properties, the detailed investigation of its nutritional profile is not available. Hence in the current study, the physicochemical properties, proximate composition, mineral content, vitamins, fatty acid and amino acid composition of P. gymnospora was investigated.
3.2. MATERIALS AND METHODS

3.2.1. Collection and processing of seaweed samples

*Padina gymnospora* (Kützing) Sonder was collected from the intertidal region of the Gulf of Mannar and identified by Dr M. Ganesan, Scientist, CSMCRI, Mandapam Camp, Tamil Nadu, India. A voucher specimen was deposited at the Department of Biotechnology, Alagappa University, under the accession number AUDBTPG20110704.

The seaweed was cleaned of large epiphytes, then washed with tap water, distilled water and 70% alcohol to remove adherent microflora. The processed seaweed was dried under shade and stored in an airtight zip-lock container. The air-dried *P. gymnospora* was powdered and successively extracted with different solvents (ranging from non-polar to polar) such as petroleum ether (PET), benzene (BEN), dichloromethane (DCM), chloroform (CLFM), ethyl acetate (EA), acetone (ACT), methanol (MET) and water (WAT) in a Soxhlet apparatus. The extracts were dried under reduced pressure in a vacuum desiccator. The dried extract was dissolved in distilled water containing less than 0.02% of methanol or Tween 20 as solvents and used for further analysis. The extraction procedures were carried out at <40°C to avoid thermal degradation of the compounds.

3.2.2. Qualitative phytochemical screening of *P. gymnospora*

To assess the presence of alkaloids, tannins, terpenoids, cardiac glycosides and flavonoids, qualitative phytochemical analysis was carried out on various solvent extracts of *P. gymnospora* using the following standard procedures (*Harborne*, 1980; *Trease & Evans*, 1989; *Sofowora*, 1993).
<table>
<thead>
<tr>
<th>S.no</th>
<th>Phytochemicals</th>
<th>Standard procedures</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Tannins</td>
<td>A few drops of 0.1% ferric chloride was added to the sample and observed for brownish green or a blue-black coloration</td>
</tr>
<tr>
<td>2.</td>
<td>Flavonoids</td>
<td>About five volumes of dilute ammonia solution were added to a portion of the sample followed by addition of concentrated H₂SO₄. A yellow coloration that was observed indicated the presence of flavonoids. The yellow coloration disappeared on standing</td>
</tr>
<tr>
<td>3.</td>
<td>Terpenoids (Salkowski test)</td>
<td>Five ml of crude extract was mixed in 2 ml of chloroform, and concentrated H₂SO₄ (3 ml) was carefully added to form a layer. A reddish brown coloration at the interface show positive results for the presence of terpenoids</td>
</tr>
<tr>
<td>4.</td>
<td>Cardiac glycosides (Keller-Killanitest)</td>
<td>Five ml of crude extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated H₂SO₄. A brown ring at the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer</td>
</tr>
<tr>
<td>5.</td>
<td>Alkaloids (Dragendorff’s reagent)</td>
<td>1.5 ml of 10% HCl was added to about 5 ml of the crude extract and the mixture was heated for 20 min. It was cooled, filtered and added 1ml of Dragendorff’s reagent. Formation of a reddish or orange colored precipitate indicates the presence of alkaloids</td>
</tr>
</tbody>
</table>

3.2.3. Physicochemical properties of *P. gymnospora*

Physicochemical properties such as swelling, water retention and oil-holding capacity were assessed in *P. gymnospora* using standard procedures (Robertson et al., 2000; Shad et al., 2011).

3.2.3.1. Swelling capacity (SWC)

SWC of leaf sample was analyzed by the bed volume technique after equilibrating in excess solvent. 0.5 g of sample was suspended in 40 ml deionized water and the mixtures were then vigorously stirred. Samples were incubated at 25°C and 37°C for
24 h. Swelling volume was measured and expressed as milliliter of swollen sample per g of sample (DW).

3.2.3.2. Water Holding Capacity (WHC)

WHC was analyzed by modified Centrifugation method. 200 mg of seaweed sample was placed in 20 ml of distilled water in a centrifugation tube and were kept in a shaker for 24 h. To determine the effect of temperature on WHC, the samples were kept at 25°C and 37°C. WHC was expressed as weight of gram of water held by 1 g of dry weight of sample.

3.2.3.3. Oil Holding Capacity (OHC)

The seaweed sample (3 g) was taken in 10.5 g of corn oil in a centrifugation tube. The tubes were left for 30 min at room temperature with constant agitation. The mixture was centrifuged at 2500 g for 30 min at room temperature. The oil supernatant was removed and used for measurement. The OHC of seaweed was measured as the number of grams of oil held by 1 g of dry weight of sample.

3.2.4. Proximate composition of *P. gymnospora*

The recommended methods of the Association of Official Analytical Chemists were used for the determination of ash, moisture, crude protein, crude lipid and crude fibre content (AOAC, 2005).

3.2.4.1. Ash content

The ash content was estimated by heating 5 g of sample overnight in a furnace at 525°C (AOAC, 2005). The ash content was expressed as g/g of dry mass.
3.2.4.2. Moisture content

Moisture content was determined using Karl Fischer moisture analyzer at 120°C and the results were expressed as percentage by weight of sample.

3.2.4.3. Protein content

Approximately 1 g of *P. gymnospora* powder was taken in a 50 ml centrifuge tube containing Diethyl ether and Water (1:4). The tube was kept in a shaker for 3 h. The supernatant was discarded, 1 N NaOH was added to the pellet and kept in a shaker for 3 h. The mixture was centrifuged at 7000 rpm for 10 min and the supernatant collected was precipitated with 10% TCA at pH 4.0. The sample was kept in ice for 30 min until visible precipitate appears, which was then centrifuged at 7000 rpm for 20 min. The precipitated protein was washed and dried.

3.2.4.3.1. Estimation of total protein content

According to *Lowry et al., method (1951)* the protein from seaweed sample was estimated using bovine serum albumin (BSA) as the standard.

**Reagents**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine serum albumin</td>
<td>50 mg in 50 ml of d.H₂O</td>
</tr>
<tr>
<td>Solution A – 2% Na₂CO₃ in 0.1 N NaOH</td>
<td>2 g of Na₂CO₃ was dissolved in 100 ml of 0.1 N NaOH</td>
</tr>
<tr>
<td>Solution B – 0.5% CuSO₄ in 1% sodium potassium tartrate</td>
<td>0.5 g of CuSO₄ was dissolved in 100 ml of 1% sodium potassium tartrate</td>
</tr>
<tr>
<td>Solution C</td>
<td>50 ml of Solution A + 1 ml of Solution B</td>
</tr>
<tr>
<td>Solution D</td>
<td>Folin-Ciocalteu : Water (1:2)</td>
</tr>
</tbody>
</table>
The standard and test samples (0.5 ml) was taken in a test tube and made up to 2 ml by adding water. BSA was used as standard solution (1mg/1 ml). 5 ml of solution C (2% Na₂CO₃ in 1N NaOH and 0.5% CuSO₄ in 1% sodium potassium tartarate) was added to the tubes and incubated at room temperature for 10 min. Then, 0.5 ml of solution D (Folin’sCiocalteu reagent 1:2) was added and incubated in dark for 45 min. The samples were then read at 660 nm in a UV-Vis spectrophotometer.

3.2.4.4. Extraction of crude lipid

Crude lipids were extracted from the powdered seaweed sample using Soxhlet apparatus (Wong & Cheung, 2000). The solvent mixture used for extraction is chloroform and methanol in the ratio of 2:1 (v/v). The contents of the crude lipids were determined gravimetrically after oven-drying (80°C) the extract overnight.

3.2.4.5. Total fiber content analysis

The content of total dietary fiber (TDF) in seaweeds was determined according to the AOAC enzymatic gravimetric method (AOAC, 2005).

3.2.4.6. Total carbohydrate content

Approximately 500 mg of seaweed powder were weighed and subjected to hydrolysis with 5 ml of 2.5 N HCl by keeping the tubes in a boiling water bath for 3 h. Tubes were cooled to RT and then neutralized with solid sodium carbonate until the effervescence ceases. The volume of the sample was made up to 100 ml and centrifuged. The supernatant was collected for carbohydrate estimation by phenol sulfuric acid method (Fournier, 2005). Glucose was used as standard solution. The 0.1 ml of sample was taken in a tube and the volume was made up to 1 ml with distilled water. One ml of phenol solution was added followed by 5 ml of 96% H₂SO₄. Tubes were shaken well and
then incubated for 10 min at 25°C for 20 min. The absorbance was read at 490 nm. Water was used as blank solution. The concentration of total carbohydrate present in the sample was calculated using standard graph prepared with glucose (100 mg/ml).

3.2.5. Proline content

Reagents

- 6 M Phosphoric acid: 19.77 ml of 15.17 M Phosphoric acid was added to d.H₂O and made to 50 ml
- 3% aqueous sulphosalicylic acid: 3 ml in 100 ml of d.H₂O
- Ninhydrin: 1.25 g in 30 ml of glacial acetic acid
- 1 M proline: 0.575 g in 5 ml of d. H₂O

Procedure

Proline content of the *P. gymnospora* was determined by Bates et al., 1972. About 1.25 g of ninhydrin was added to 30 ml of glacial acetic acid in a test tube containing 20 ml of 6 M phosphoric acid. The mixture was agitated until it was dissolved and the solution was kept at 4°C, which is stable for 24 h. 0.5 g of seaweeds were placed in 10 ml of 3% aqueous sulphosalicylic acid and filtered with Whatman no. 1 filter paper. Two ml of filtrate and 2 ml of acid ninhydrin was mixed with 2 ml of glacial acetic acid. The mixture was kept at 100°C for 60 min. The reaction was terminated by placing the mixture in ice bath. After that, the mixture was extracted with toluene, mixed vigorously with the test tube stirrer. Chromophore containing toluene was collected from the aqueous phase and warmed at room temperature and the absorbance was measured at 520 nm using UV-Vis spectrophotometer. Proline was used as standard and the experiments were done in triplicates.
Calculation

The amount of proline present in the sample can be calculated from the formula mentioned below:

\[
\frac{[(\mu g \text{ proline per ml} \times \text{Vol. of toluene}) / 115.5 \mu g \text{ per } \mu M]}{A/[(\text{g of sample})/5]} = \mu M \text{ proline/g of fresh weight material.}
\]

3.2.6. Estimation of Chlorophyll content (Dere et al., 1998)

The seaweed sample of *P. gymnospora* (1 g) was homogenized with 96% methanol (50 ml for each g) and centrifuged at 1000 rpm for 1 min. The homogenate was then filtered and centrifuged at 2500 rpm for 10 min. The supernatant was collected and the absorbance was measured at 400-700 nm in UV-Vis spectrophotometer. The chlorophyll A and B content was calculated according to the method of Lichtentaler and Wellburn (1983) as given below, using the specific absorption coefficient of methanol.

Chlorophyll A = 15.65 (A_{666}) - 7.340 (A_{653})

Chlorophyll B = 27.05 (A_{653}) - 11.21 (A_{666})

The amount of chlorophyll obtained was expressed as µg/g of fresh weight.

3.2.7. Estimation of mineral content by Atomic Absorption Spectrophotometer

Mineral content of *P. gymnospora* were estimated according to the method of Santoso et al. (2006). Approximately 2 g of powdered seaweed sample was dissolved in 10 ml of perchloric acid and incubated at 100°C for 5 min. Processed samples were then treated with 10 ml of conc. HNO₃ followed by 10 ml of conc. H₂SO₄. Acid treatments were carried out at 100°C for 5 min each in hot plate. Finally the sample was evaporated
to dryness and diluted with 10% HCl. Diluted samples were then filtered through an 
as-free, acid washed filter paper. Major mineral elements (Na, K, Ca, Mg) and trace 
elements (Cu, Fe, Zn and Pb) were determined in Atomic Absorption Spectrophotometer 
(Varian Model Spectra 220, Agilent Technologies) equipped with single hollow cathode 
lamps for each element and an air-acetylene burner.

3.2.8. Determination of Fatty acid composition in P. gymnospora 

The powdered seaweed (75 mg) was dissolved in toluene (1 ml) in a test tube 
fitted with a condenser, and to this 1% H$_2$SO$_4$ in methanol was added. The mixtures were 
left overnight in a stoppered tube at 50°C followed by the addition of sodium chloride 
solution (5 ml, 5%). The required esters were extracted with 5 ml of 2x hexane, and the 
organic layer was separated using Pasteur pipettes. The hexane layers were washed with 
4 ml of 2 % potassium bicarbonate solution and dried over anhydrous sodium thiosulfate 
(Na$_2$SO$_4$) and filtered. The organic solvent was removed under reduced pressure on a 
rotary evaporator to give fatty acid methyl esters (FAMEs).The FAME thus obtained was 
subjected to Gas Chromatography (Yayli et al., 2001). GC was performed in 6890N 
system for GC Agilent Technologies, USA. HP-5 capillary column was used which was 
equipped with Electron impact ionization. Initial temperature was 70°C which was 
increased to 250°C (10°C/min) and the injection temperature employed was 220°C. 
Helium was used as carrier gas at a flow rate of 1 µl/min. FAME peaks were identified 
by comparison of their retention times with those of standard FAME mix (Supelco; 
Sigma Aldrich).
3.2.9. **Amino acid analysis**

Amino acid composition of seaweed sample was determined according to Gratzfeld-Huesgen (1999). The powdered seaweed samples (2 g) were mixed with PO$_4$ buffer (pH 7.0) and centrifuged at 3000 rpm for 20 min at 4°C. The proteins present in the supernatant were precipitated separately using 10% TCA. The pellet was resuspended in 1N NaOH and subjected to acid hydrolysis by incubating with 6N HCl in boiling water bath for 24 h. The samples were then centrifuged at 3500 rpm for 15 min. The supernatant obtained was filtered and neutralized with 1N NaOH. The filtered solution was diluted to 1:100 of the volume with milli-Q water and subjected to HPLC analysis (HP-1101 Agilent technologies with UV and fluorescent detectors).

3.2.10. **Vitamin analysis**

The fat-soluble vitamins A, D and E were analysed using the standards retinyl acetate, cholecalciferol and alpha tocopherol, respectively. HPLC analysis of the seaweed sample used n-hexane and ethyl acetate (99.7:0.3) and 3N methanolic sulphuric acid solution as the mobile phase for vitamin A, D and E. The liquid chromatography apparatus was equipped with a 325 nm detector and 4.6 mm × 25 cm column that contained 5 μm packing L24. The flow rate was about 1.5 ml per min. The water-soluble vitamins analysed were vitamins B1, B2, B3, B6, B12 and C using the standards thiamine (10 μg/ml), riboflavin (12 μg/ml), niacin (50 μg/ml), pyridoxine hydrochloride (40 μg/ml), cyanocobalamine (10 μg/ml) and ascorbic acid (1 mg/ml). Vitamin C was analyzed by HPLC using acetonitrile and water (50:50) as the mobile phase, and vitamins B1 to B12 were analyzed spectrophotometrically according to the method of Bradbury & Singh (1986).
3.3. RESULTS AND DISCUSSION

3.3.1. Qualitative phytochemical screening of *Padina gymnospora*

Qualitative phytochemical analysis of the brown seaweed *P. gymnospora* showed the presence of flavonoids and cardiac glycosides, while alkaloids, tannins and terpenoids were not detectable. Flavonoids have gained attention in AD therapy, since they act as potent ROS scavengers and metal chelators (Porat et al., 2006). Recent reports show that cardiac glycoside derivatives also possess anti-AD properties (Addington & Newman, 2013). The preliminary phytochemical analysis of *P. gymnospora* results were tabulated in Table 3.1.

Table 3.1: Preliminary phytochemical screening of *Padina gymnospora*

<table>
<thead>
<tr>
<th>S.No</th>
<th>Compounds</th>
<th>P. gymnospora</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Cardiac glycosides</td>
<td>++</td>
</tr>
<tr>
<td>3</td>
<td>Flavonoids</td>
<td>++</td>
</tr>
<tr>
<td>4</td>
<td>Tannins</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Terpenoids</td>
<td>-</td>
</tr>
</tbody>
</table>

- No response; ++ high content

3.3.2. Determination of the nutritional properties of *P. gymnospora*

Physicochemical properties including swelling capacity (SWC), water holding capacity (WHC) and oil-holding capacity (OHC) differed according to temperature: at 25°C, SWC was 6.6 ± 1.4 ml/g of dry mass (DM) and a slight increase (10.8 ± 1.4 ml/g) was observed at 37°C. WHC showed a significant increase with increase in temperature from 4.5 ± 0.2 (at 25°C) to 4.8 ± 0.04 g/g of DM (at 37°C). This increase can be attributed to the increased solubility of fibre and proteins. OHC, which represents the emulsifying
properties of *P. gymnospora*, was 2.5 ± 0.219 g/g of DM. OHC is an important property of food ingredients, which is evaluated to assess the hydrophobic nature of the molecules which make up the fiber (Lopez-Cervantes et al., 2011). Physicochemical properties determine the physiological effects of dietary fibre. The biological effects of dietary fibre in the intestine and colon include modulation of absorption in the foregut, modification of sterol metabolism, inducement of faecal fermentation, and increase in stool weight (Eastwood & Morris, 1992).

The proximate composition of *P. gymnospora* is reported in Table 3.2, in which the ash content represents the total mineral content of the seaweed. Earlier report has suggested that algal dietary fibre promotes beneficial physiological effects, including laxation and blood cholesterol or glucose attenuation (Anonymous, 2001). The crude protein, lipid and carbohydrate content of *P. gymnospora* are also reported in Table 3.2. As moisture plays a key role in the preservation and stability of food products, it is often specified as compositional standard. The nutritional composition of the alga *P. gymnospora* in terms of fibre, proteins and minerals makes it a good nutritive supplement which represents it an important food alternative.

Table 3.2: Proximate composition, proline content and chlorophyll content of *Padina gymnospora*
(The values represent Mean ± SD of the triplicate determinations)

<table>
<thead>
<tr>
<th>Composition</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ash content</td>
<td>0.253 ± 0.025 g/g DM</td>
</tr>
<tr>
<td>Total dietary fibre content</td>
<td>2.98 ± 0.15g/100g DM</td>
</tr>
<tr>
<td>Crude protein content</td>
<td>5.704 ± 0.038 mg/g DM</td>
</tr>
<tr>
<td>Crude lipid content</td>
<td>0.020 ± 0.002 g/g DM</td>
</tr>
<tr>
<td>Total carbohydrate content</td>
<td>118.14 ± 6.88 mg/g DM</td>
</tr>
<tr>
<td>Moisture content</td>
<td>3.13 ± 0.16 % DM</td>
</tr>
<tr>
<td>Proline content</td>
<td>2.35 ± 0.12 μM DM</td>
</tr>
<tr>
<td>Chlorophyll A</td>
<td>0.277 ± 0.014μg/g FW</td>
</tr>
<tr>
<td>Chlorophyll B</td>
<td>0.186 ± 0.009 μg/g FW</td>
</tr>
</tbody>
</table>

DM-Dry Matter Content; FW- Fresh weight
In addition to the major nutrient elements, the evaluation of proline and chlorophyll content has become an important aspect of nutritional profiling. High values of proline and chlorophyll were observed in *P. gymnospora*. Proline elicits stress-stimulated phenolic biosynthesis and stimulation of antioxidant enzyme response pathways (*Shetty, 2004*), and polypeptides rich in proline have a beneficial effect in the treatment of AD (*Gladkevich et al., 2007*). Chlorophyll, at high concentrations, has excellent antioxidant activity and the possible mechanism of action might be either the protective effect of linoleic acid against oxidation or prevention of the decomposition of hydroperoxides. Traditionally chlorophyll and its various derivatives have been widely used for wound healing (*Singh et al., 2012*) due to their anti-inflammatory properties, control of calcium crystals and internal deodorization (*Subramoniam et al., 2012*).

*P. gymnospora* has significant amounts of essential elements (Table 3.3). Calcium was the most abundant element in the analyzed seaweed sample followed by the elements sodium and potassium. Sodium plays an indispensable role in the electrical conductivity of the brain and facilitates the improvement of brain functions (*Fiocco et al., 2012*). Magnesium is an essential cofactor for many enzymatic reactions, especially those involved in energy metabolism (*Morris, 1992*). Recent reports suggest that low levels of magnesium contribute to the heavy metal deposition in the brain that precedes Parkinson’s, multiple sclerosis and Alzheimer’s disease. Thus magnesium is essential in regulating central nervous system excitability and normal function (*Murck, 2002*). Apart from the major elements, *P. gymnospora* was found to have trace elements also such as iron, copper, zinc and lead.
Seaweeds have a low lipid content compared with vegetables such as soy and sunflower (Darcy-Vrillon, 1993), thus are a poor source of nutritional energy. Nevertheless, the lipid fraction might contain higher levels of essential polyunsaturated fatty acids compared with traditional vegetables. The results of fatty acid analysis of *P. gymnospora* (Table 3.4) illustrate that this brown seaweed is rich in monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA). A mixture of saturated (palmitic acid, margaric acid and stearic acid) and unsaturated (oleic acid, linolenic acid, alpha linolenic acid and morotic acid) fatty acids was found. Supplementation with PUFA might improve cognitive impairment in AD patients, since reports show that PUFA administration to AD patients considerably improves cognitive decline (Tully et al., 2003).

Table 3.3: Mineral composition of *Padina gymnospora*  
(The values represent Mean ± SD of the triplicate determinations)

<table>
<thead>
<tr>
<th>Element</th>
<th>Concentration (mg/100g of DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron</td>
<td>6.78 ± 0.54</td>
</tr>
<tr>
<td>Copper</td>
<td>0.93 ± 0.074</td>
</tr>
<tr>
<td>Sodium</td>
<td>145.6 ± 11.6</td>
</tr>
<tr>
<td>Potassium</td>
<td>122.3 ± 9.8</td>
</tr>
<tr>
<td>Zinc</td>
<td>3.56 ± 0.28</td>
</tr>
<tr>
<td>Calcium</td>
<td>156.2 ± 12.5</td>
</tr>
<tr>
<td>Magnesium</td>
<td>32.4 ± 2.6</td>
</tr>
<tr>
<td>Lead</td>
<td>2.33 ± 0.19 ppm</td>
</tr>
</tbody>
</table>

ppm -parts per million; DM-Dry Matter Content
Table 3.4: Fatty acid composition of *Padina gymnospora*

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Content (g/100g of DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmic acid 16:0</td>
<td>0.445</td>
</tr>
<tr>
<td>Margaric acid 17:0</td>
<td>0.509</td>
</tr>
<tr>
<td>Stearic acid 18:0</td>
<td>1.221</td>
</tr>
<tr>
<td>Oleic acid 18:1</td>
<td>0.893</td>
</tr>
<tr>
<td>Linolenic acid 18:2</td>
<td>0.135</td>
</tr>
<tr>
<td>Alpha linolenic acid 18:3</td>
<td>0.506</td>
</tr>
<tr>
<td>Morotic acid 18:4</td>
<td>0.112</td>
</tr>
</tbody>
</table>

DM-Dry Matter Content

Twenty amino acids that are common components of proteins were detected in *P. gymnospora* by HPLC (Table 3.5). There were large amounts of glycine and tyrosine and measurable amounts of the essential amino acids methionine, leucine, lysine, phenylalanine, arginine, isoleucine, threonine, tryptophan, histidine, and valine. Non-essential aminoacids like aspartic acid, glutamic acid, serine, alanine, asparagine, glutamine, proline and cysteine were also found.

Table 3.5: Amino acid composition of *Padina gymnospora*.  
(The values represent Mean ± SD of the triplicate determinations)
Table 3.6: Vitamin composition of *Padina gymnospora*  
(The values represent Mean ± SD of the triplicate determinations)

<table>
<thead>
<tr>
<th>Vitamins</th>
<th>Content (100 g of DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Water soluble vitamins</strong></td>
<td></td>
</tr>
<tr>
<td>Vitamin B1</td>
<td>0.293 ± 0.023mg</td>
</tr>
<tr>
<td>Vitamin B2</td>
<td>0.055 ± 0.004mg</td>
</tr>
<tr>
<td>Vitamin B3</td>
<td>1.45 ± 0.12mg</td>
</tr>
<tr>
<td>Vitamin B6</td>
<td>2.34 ± 0.19 mg</td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>0.33 ± 0.026µg</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>39.07 ± 3.13mg</td>
</tr>
<tr>
<td><strong>Fat soluble vitamins</strong></td>
<td></td>
</tr>
<tr>
<td>Vitamin A</td>
<td>8.01 ± 0.64µg</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>0.014 ± 0.001µg</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>145.6 ± 11.6 mg</td>
</tr>
</tbody>
</table>

DM-Dry Matter Content

Vitamin analysis of *P. gymnospora* showed high levels of vitamins E and C *(Table 3.6).* Earlier reports suggest that the use of higher-dose of vitamin E and vitamin C supplements may lower the risk of AD *(Fonteh et al., 2014).* Water-soluble vitamins like vitamin B1, B2, B3 and B6 were found in *P. gymnospora* but vitamin B12 was present only in a trace amount 0.33±0.0264 µg/100 g of DM. In the case of fat-soluble vitamins, vitamins A and D were found in trace amounts.
3.4. Conclusion

The physico-chemical properties, proximate composition and nutritional profile of the marine brown macro alga, *P. gymnospora* was assessed. The results of physiochemical properties publicized that the seaweed hold excessive fiber content. The investigation of proximate composition of *P. gymnospora* proposes that the seaweed hold high amount of proline, which plays a key role in purine metabolism and oxidative phosphorylation. Also the seaweed consists of high chlorophyll content, which has antioxidant action. Assessment of nutritional profile of *P. gymnospora* shows that it is rich in minerals, fatty acids, vitamins and amino acids. The minerals are energetic players in all the metabolic reactions. The existence of PUFA and MUFA recommends that the seaweed may display protective effect against cardiovascular diseases. Furthermore the seaweed holds most of the essential amino acids that are mandatory for the regular functions of the body. The presence of vitamin C and vitamin E suggests that *P. gymnospora* is rich in antioxidants, which are essential to avert oxidative stress mediated disorders. Hence, the result of the study verified that *P. gymnospora* has more nutritional value and could be used as an admirable nutritive supplement.
3.5. Summary

**Nutritional Profile of the marine macroalga *Padina gymnospora***

- High proline content
- High fiber content
- Rich in MUFA and PUFA
- High Vitamin content
- Rich in Essential amino acids
- High Mineral content