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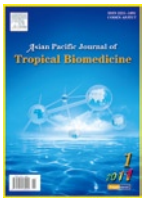

Document heading

In vitro antioxidant properties and FTIR analysis of two seaweeds of Gulf of Mannar

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ABSTRACT

Objective: To evaluate the *in vitro* antioxidant activity of *Sargassum wightii* (*S. wightii*) and *Ulva lactuca* (*U. lactuca*). **Methods:** Dried seaweeds of *S. wightii* and *U. lactuca* were tested for total phenolic content. *In vitro* antioxidant activity was determined by DPPH assay and ferric reducing antioxidant power (FRAP) assay. Functional groups of two seaweeds were analysed by fourier transform infrared spectroscopy (FTIR). **Results:** The highest total phenolic content was observed in *S. wightii* (0.65±0.02 mg GAE/g) when compared with *U. lactuca*. *In vitro* antioxidant activity of *S. wightii* showed higher activity in all assays than *U. lactuca* with the higher total antioxidant activity (123.40±4.00 mg ascorbic acid/g), DPPH radical scavenging activity (108.06±1.02)% and ferric reducing antioxidant power (153.40±1.41 mg GAE/g). FITR spectrum of standard gallic acid was compared with seaweeds and same number of peaks lying between 449.32 and 3 495.89 cm^{-1} and 462.89 and 3 407.05 cm^{-1} was recorded. **Conclusions:** These results show that *S. wightii* has higher antioxidant capacity than *U. lactuca*. Further study is necessary to exploit the multifunctional properties of seaweeds which will be usefull to treat many diseases.

1. Introduction

Seaweeds are nutritionally valuable as fresh or dried vegetables, or as ingredients in a wide variety of prepared foods. The total global seaweed production in the year 2004 was more than 15 million metric tones[1] of which nearly 15%-20% 100 000 metric tones is contributed by Indian Ocean (wet weight)[2]. About 150 species of seaweeds are used as a food world wide[3], but in India, seaweeds are exploited mainly for the industrial production (phycocolloids such as agar-agar, alginate and carrageenan) and not in health aspects. Seaweeds are the excellent source of bioactive compounds such as carotenoids, dietary fibre, protein, essential fatty acids, vitamins and minerals[4] and they contain many biologically active substances like lipids, proteins, polysaccharides and polyphenols[5].

Antioxidants in biological systems have multiple functions, including defending against oxidative damage in the major signaling pathways of cells. Several synthetic antioxidants, such as butylated hydroxyanisol (BHA), butylated hydroxytoluene (BHT), and tert-butylhydroquinone (TBHQ) are commercially available and are currently in use. However, their use is now restricted due to their side effects. It has been shown that they promote the development of cancerous cells in rats. These findings have reinforced the efforts for the development of alternative antioxidants from natural origin[6]. Over the past decades, seaweeds or their extracts have been reported that they possess biological activity of potential medicinal value[7]. Therefore, new interest has been developed to search natural and safe antioxidative agents from marine seaweeds which are considered as a source of bioactive compounds as they are able to produce a great variety of secondary metabolites and are characterized by a broad spectrum of biological activities. Compounds with cytostatic, antiviral, antihelmintic, antifungal and antibacterial activities have been detected in green, brown and red algae[8] and it should be exploited for their multifunctional properties in the form of food, energy, medicine and cosmetics and as biotechnological tools[5]. Recent reports revealed marine genus of sargassum and ulva are found to be rich source of antioxidant compounds with potential free

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radical scavenging activity[9,10]. In this regard this work was designed and executed to evaluate the antioxidant activity of two seeweeds of *Sargassum wightii* (*S. wightii*) and *Ulva lactuca* (*U. lactuca*).

2. Materials and methods

2.1. Sample collection and preparation

Fresh *S. wightii* and *U. lactuca* were collected from the intertidal region of the Mandapam coast (Lat. 09° 17.417′N; Long. 079° 08.558′E) and immediately brought to the laboratory in plastic bags containing water to prevent evaporation. Then the plants are washed with tap water to remove extraneous materials. The samples were shade-dried and grounded in an homogenizer. The powdered samples were then stored in refrigerator at 4 ℃ for future use.

2.2. Preparation of seaweed extracts

Powdered seaweeds (10 g) were extracted for 24 h in 200 mL aqueous methanol at room temperature under dark condition. The extraction was twice repeated. The extracts were filtered through glass funnel and Whatman No. 1 filter paper. Each filtrate was concentrated to dryness under reduced pressure using rotary evaporator.

2.3. Determination of total phenolic content

Total phenolic content was estimated as gallic acid equivalent (GAE) according to the Folin-Ciocalteu reagent[11]. 1.0 mL aliquot of each sample (previously diluted with 0.1 mg/mL ethanol) was added to 1.5 mL deionized water and 0.5 mL of 0.1 M Folin-Ciocalteu reagent and the contents were thoroughly mixed. After 1 min, 1.0 mL of 20% sodium carbonate solution was added and the mixture was mixed thoroughly. The control was maintained in all the reaction reagents except the sample. After 30 min of incubation at 37 ℃, the absorbance was measured at 750 nm, using the Shimadzu spectrophotometer. Aqueous methanol extract of the samples were evaluated at a final concentration of 0.1 mg/mL. Total phenolic content was expressed as mg/g GAE. A calibration curve of gallic acid (ranging from 0.1-1.0 mg/mL) was prepared and the total phenolic content was standardized against gallic acid and was expressed as mg GAE per gram of sample on a dry weight basis. y= 0.157x + 0.012, R^2=0.986.

2.4. Determination of antioxidant activity

2.4.1. Total antioxidant activity

Total antioxidant activity of the crude extracts was determined according to the method of Prieto *et al*[12]. Briefly, 0.3 mL of sample was mixed with 3.0 mL reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). Reaction mixture was incubated at 95 ℃ for 90 min under water bath. Absorbance of all the sample mixture was measured at 695 nm. Total antioxidant activity was expressed as the number of equivalence of ascorbic acid. A calibration curve of ascorbic acid (ranging from 0.1-1.0 mg/mL) was prepared and the total antioxidant activity was standardized against ascorbic acid and was expressed as mg ascorbic acid equivalents per gram of sample on a dry weight basis.

2.4.2. DPPH radical-scavenging activity

The scavenging effects of samples for DPPH radical were monitored according to the method of Yan and Chen[13]. Briefly, 2.0 mL of aliquot of test sample was added to 2.0 mL of 0.16 mM DPPH methanolic solution. The mixture was vortexed for 1 min and then left to stand at room temperature for 30 min in the dark and its absorbance was read at 517 nm. The ability of scavenging DPPH radical was calculated using the following equation:

$$\text{Scavenging effect } (\%) = [1-(A_{sample} - A_{sample\ blank}) / A_{control}] \times 100$$

Where the $A_{control}$ is the absorbance of the control (DPPH solution without sample), the A_{sample} is the absorbance of the test sample (DPPH solution plus test sample), and the $A_{sample\ blank}$ is the absorbance of the sample only (sample without DPPH solution). Synthetic antioxidants, gallic acid and ascorbic acid were used as positive controls.

2.4.3. Ferric reducing antioxidant power (FRAP) assay

Reducing power of crude extract was determined by the method prescribed by Oyaizu[14]. Briefly, 1.0 mL of two different concentrations of sample was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL potassium ferricyanide (1%). Reaction mixture was incubated at 50 ℃ for 20 min. After incubation, 2.5 mL of trichloroacetic acid (10%) was added and centrifuged at 650 g for 10 min. From the upper layer, 2.5 mL solution was mixed with 2.5 mL distilled water and 0.5 mL $FeCl_3$ (0.1%). Absorbance of all the sample solutions was measured at 700 nm. Ascorbic acid is used as a positive control. Reducing power is expressed as the number of equivalence of gallic acid. A calibration curve of gallic acid (ranging from 0.1-1.0 mg/mL) was prepared, and the FRAP was standardized against gallic acid and was expressed as mg GAE per gram of sample on a dry weight basis.

2.5. Fourier transform infrared spectroscopy (FTIR) analysis

The solid samples of *S. wightii* and *U. lactuca* (10 mg) were mixed with 100 mg of dried potassium bromide (Kbr) and compressed to prepare as a salt disc. The disc was then read spectrophotometrically (Bio-Rad FTIR-40-model, USA). The frequencies of different components present in each sample were analyzed. The same procedure was followed for the standard.

3. Results

The phenolic content of aqueous methanol of two

seaweeds was evaluated using the Folin-Ciocalteu method. In the present experiment, the total phenolic content was determined and results were presented in Figure 1. The variation in phenolic content was quite large. The brown seaweed *S. wightii* (0.65±0.02) showed higher phenolic content.

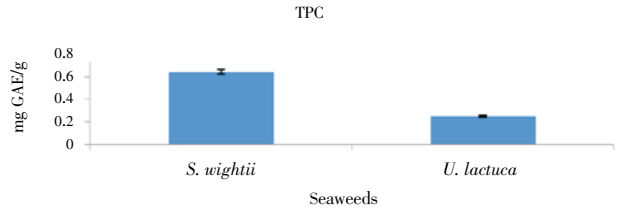

Figure 1. Total phenolic content present in seaweeds.

The total antioxidant activity of seaweeds was evaluated and the results were presented as mg ascorbic acid /g. In phosphomolybdenum method, molybdenum VI (Mo^{6+}) is reduced to form a green phosphate/Mo^{5+} complex. Higher activity of (123.40±4.00) mg ascorbic acid/g extract was observed in *S. wightii* (Figure 2). The effect of antioxidants on DPPH radical scavenging is due to hydrogen donating ability. In the present study, the maximum DPPH content was (108.06±1.02) obtained from *S. wightii* and minimum was (14.20±0.66) observed from *U. lactuca* (Figure 3).

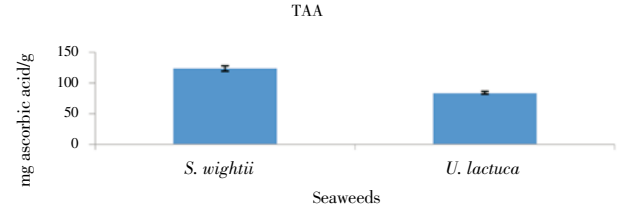

Figure 2. Total antioxidant activity in seaweeds.

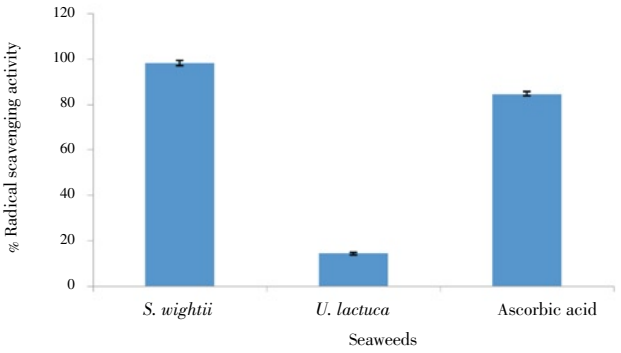

Figure 3. Amount of DPPH content present in seaweeds.

In the FRAP assay, antioxidants in the sample reduce ferric (III) to ferrous (II) in a redox-linked colourimetric reaction that involves single electron transfer. The reducing power indicates that the antioxidant compounds are electron donars which can reduce the oxidized intermediate of the lipid peroxidation process, so that they can act as primary and secondary antioxidants. In the present study, the FRAP was measured and the results were plotted in Figure 4. The maximum (153.40±1.41 mg GAE/g) FRAP value was observed in *S. wightii*. The positive control ascorbic acid showed higher antioxidant activity than all the seaweed samples.

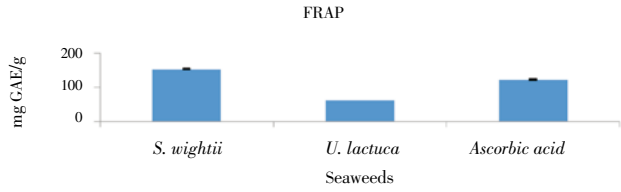

Figure 4. Amount of FRAP present in seaweeds.

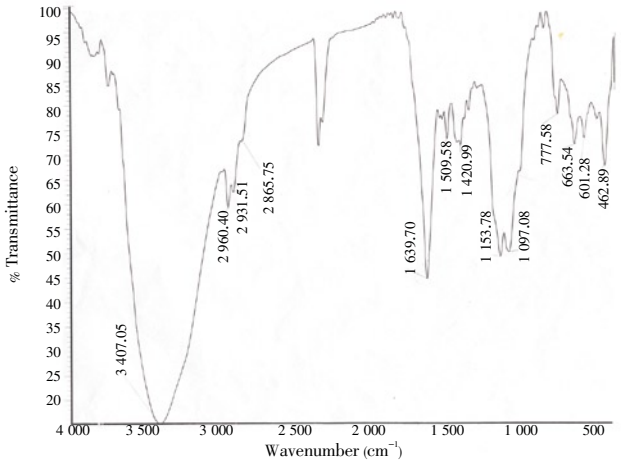

Figure 5. FTIR spectrum of gallic acid.

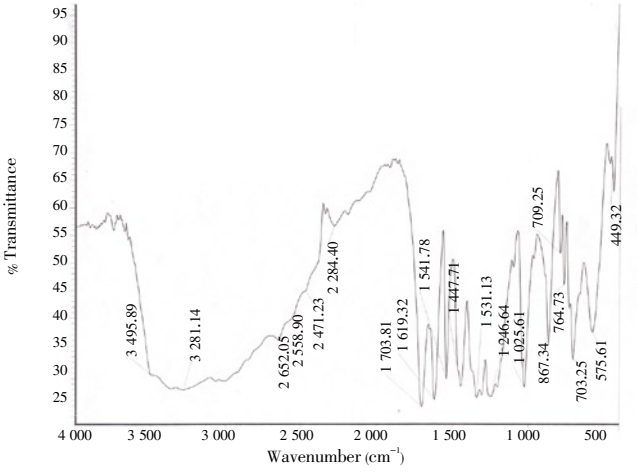

Figure 6. FTIR spectrum of *S. wightii*.

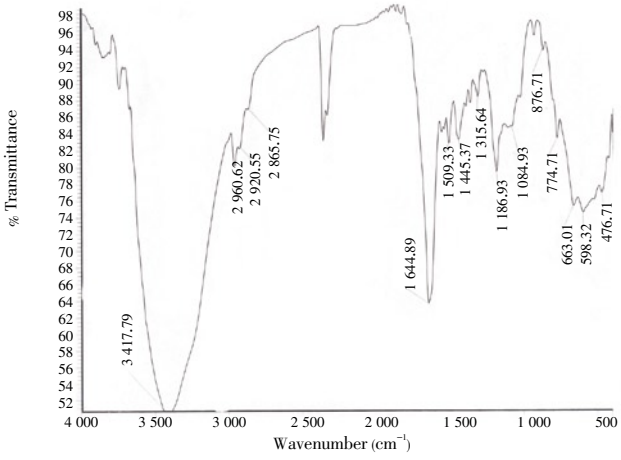

Figure 7. FTIR spectrum of *U. lactuca*.

The FTIR analysis of the samples was done and the associated functional groups were determined (Figure 5-7). In FTIR spectrum of both the samples were compared with that of the standard gallic acid. The FTIR spectrum of standard gallic acid showed thirteen major peaks at the range of 3 407.05, 2 960.40, 2 931.51, 2 865.75, 1 639.70, 1 509.58, 1 420.99, 1 153.78, 1 097.08, 777.58, 663.54, 601.28, 462.89 cm^{-1}, whereas the FTIR spectrum of the *S. wightii* and *U. lactuca* samples also showed the same number of peaks lying between 449.32 cm^{-1} and 3 495.89 cm^{-1} and 462.89 cm^{-1} and 3 407.05 cm^{-1}, respectively (Figure 5-7).

4. Discussion

The search for new metabolites from marine organisms has resulted in the isolation of more or less 10 000 metabolites, of which many are endowed with pharmacodynamic properties. Seaweeds are the economic potential resource in marine environment and it will be a great significance in drug development[15].

Phenolics are secondary metabolites that play a role in the maintenance of the human body[16]. The presence of phytoconstituents, such as phenols, flavonoids and tannin in seaweeds and seagrasses, indicates the possibility of antioxidant activity and this activity will help in preventing a number of diseases through free-radical scavenging activity[17]. Phenolic compounds are commonly found in plants and have been reported to have several biological activities including antioxidant properties. Many studies focused on the biological activities of phenolic compounds, which have potential antioxidants and free radical scavengers[18,19]. Earlier report states that seaweed extracts, especially polyphenols, have antioxidant activity[20]. Phlorotannins and fucoxanthin are the major compounds of seaweeds[21].

In the present study the phenolic contents of *S. wightii* and *U. lactuca* were evaluated using the Folin-Ciocalteu method and were expressed as GAE. The brown seaweed *S. wightii* showed significantly higher phenolic content than the green seaweed *U. lactuca*. Matahjun *et al*[22] observed the similar result that the brown seaweeds contained higher phenolic content than the green and red seaweeds. In agreement with previous study[20] there was a significant correlation between antioxidant activity and phenolic content of these seaweeds. Many algal species contain phytoconstituents such as phenols, flavonoids and tannins which could be responsible for the antioxidant activity in preventing number of disease through free radical scavenging activity[23] and in this study the antioxidant activity of algae could be due to these compounds.

There are many methods to determine antioxidant capacity and they differ in terms of their assay principles and experimental conditions. Consequently, in different methods antioxidants in particular have varying contributions to total antioxidant potential[24]. The two methods of FRAP assay which can react with iron (II) and SH-group containing antioxidants[25] and DPPH method for organic radicals[26], are expected to accurately reflect all of the antioxidants in a sample. In this study, antioxidant activities were tested using three different assays *i.e.* total antioxidant activity, DPPH radical scavenging activity and FRAP assay. These three methods represented different mechanisms of antioxidant action. Published reports on total antioxidant activity of seaweeds are limited. However, Kumaran and Karurnakaran[27] have reported total antioxidant activity in the range of 245 to 376 mg ascorbic acid equivalents/g in *Phyllanthus* species. Ye *et al*[28] noticed higher antioxidant activity (30.50 μmol $FeSO_4$/mg) in ethanol extract of brown seaweed *Sargassum pallidum*. It has been reported that solvents used for extraction have dramatic effect on the chemical changes[29]. In methanolic extracts of *Phyllanthus* species also same trend has also been reported by Kumaran and Kurnakaran[27].

In the present study, the methanol extracts of seaweeds were found to possess strong antioxidant activity. The antioxidant mechanisms of seaweed extracts may be attributed to their free radical-scavenging ability. Radical scavenging is an important antioxidant activity. A stable free radical, DPPH, was used for another assay of antioxidant activity. Decolorization of DPPH suggests the presence of electron and/or hydrogen donors in algal extracts. The radical scavenging activities (RSA) for the aqueous methanol extracts of the two tested seaweeds (*S. wightii* and *U. lactuca*) were comparable with known antioxidant such as ascorbic acid. The RSA% of brown seaweed (*S. wightii*) was 108.06% that is higher than that the RSA% of green seaweed (*U. lactuca*) that was found to be only 14.20% of DPPH. This could be attributed to the higher amount of total phenol compound found in brown algae than green algae. Our results are similar with Monsuang *et al*[30] who also reported that brown seaweed *Sargassum* sp, showed significantly higher phenolic content and antioxidant activities than the red and green seaweeds.

In the FRAP assay, antioxidants in the sample reduce Fe^{3+}- tripyridyltriazine complex (Fe^{3+}-TPTZ), present in stoichiometric excess, to the blue-colored ferrous form (Fe^{2+}-TPTZ). The antioxidant potential is proportional to the combined (total) ferric reducing/antioxidant power (FRAP value) of the antioxidants in the sample[31]. The reducing power property indicates that the antioxidant compounds are electron donors and can reduce the oxidized intermediates of the lipid per oxidation process, so that they can act as primary and secondary antioxidants[13] We found that the brown seaweed *S. wightii* was more reactive than the green seaweed *U. lactuca* through FRAP assay.

On the basis of the results obtained, seaweeds can be used for a variety of beneficial chemo-preventive effects.

Conflict of interest statement

We declare that we have no conflict of interest

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References

[1] FAO. *State of world aquaculture: 2006*. Rome: FAO; 2006, p. 134.
[2] Dhargalkar VK, Pereira N. Seaweed: promising plant of the millennium. *Sci Cult* 2005; **71**: 60-66.
[3] Barlow CJ. Marine by-products as functional food ingredients. *Food Technol Int* 2007; 1-5. [Online] Available from: www.food tech- international.com [Accessed on 21 September, 2011]
[4] Bhaskar N, Miyashita K. Lipid composition of *Padina tetratomatica* (Dictyotales, Pheophyta), a brown seaweed of the west coast of India. *Indian J Fish* 2005; **52**: 263-268.
[5] Chandini SK, Ganesan P, Suresh PV, Bhaskar N. Seaweeds as a source of nutritionally beneficial compounds-a review. *J Food Sci Technol* 2008; **45**(1): 1-13.
[6] Huang HL, Wang BG. Antioxidant capacity and liphophilic contents of seaweeds collected from the Qingdao coastline. *J Agric.Food Chem.*2004; **52**: 4993-4997.
[7] Heo SJ, Park EJ, Lee KW, Jeon YJ. Antioxidant activites of enzymatic extracts from brown seaweeds. *Biosour Technol* 2005; **96**: 1613-1623.
[8] Newman DJ, Cargg GM, Snader KM. Natural products as source of new drugs over the period 1981-2002. *J Nat Prod* 2003; **66**: 1022-1037.
[9] Zhou J, Hu N, Wu YL, Pan YJ, Sun CR. Preliminary studies on the chemical characterization and antioxidant properties of acidic polysaccharides from *Sargassum fusiforme*. *J Zhejiang Univ Sci B* 2008; **9**: 721-727.
[10] Ananthi S, Ragavendran HR, Sunil AG, Gayathri V, Ramakrishnan G, Vasanthi HR. *In vitro* antioxidant and *in vivo* anti-inflammatory potential of crude polysaccharide from *Turbinaria ornata* (marine brown alga). *Food Chem Toxicol* 2010; **48**: 187-192.
[11] Velioglu YS, Mazza G, Gao L, Oomah BD. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *J Agric Food Chem* 1998; **46**: 4113-4117.
[12] Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal Biochem* 1999; **269**: 337-341.
[13] Yan GC, Chen HY. Antioxidant activity of various tea extracts in relation to their antimutagenecity. *J Agri Food Chem* 1995; **43**: 27-37.
[14] Oyaizu M. Studies on product of browning reaction prepared from glucose amine. *Jpn J Nutr* 1986; **44**: 307-315.
[15] Fuesetani N. *Drugs from the sea*. Basel: Karger; 2000, p. 1-5.
[16] Latha S, Daniel M. Phenolic antioxidants of some common pulses. *J Food Sci Technol* 2001; **38**: 272-273.
[17] Ruberto G, Baratta MT, Biondi DM, Amico V. Antioxidant activity of extracts of the marine algal genus *Cystoseira* in a micellar model system. *J Appl Phycol* 2001; **13**: 403-407.
[18] Sugihara N, Arakawa T, Ohnishi M, Furuno K. Anti- and pro-oxidative effects of flavonoids on metal induced lipid hydroperoxide-dependent lipid peroxidation in cultured hepatocytes located with alphalinolenic acid. *Free Radic Biol Med* 1999; **27**: 1313-1323.
[19] Kahkonen MP, Hopia A, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, et al. Antioxidant activity of plant extracts containing phenolic compounds. *J Agric Food Chem* 1999; **47**: 3954-3962.
[20] Duan XJ, Zhang WW, Li XM, Wang BG. Evaluation of antioxidant property of extract and fractions obtained from a red alga, *Polysiphonia urceolata*. *Food Chem* 2006; **95**: 37-43.
[21] Kuda T, Tsunekawa M, Goto H, Araki Y. Antioxidant properties of four edible algae harvested in the Noto Peninsula, Japan. *J Food Composit Anal* 2005; **18**: 625-633.
[22] Matanjum P, Mohamed S, Mustapha NM, Muhamad K, Ming CH. Antioxidant activites and phenolics content of eight species of seaweeds from north Borneo. *J Appl Phycol* 2008; **20**: 367-373.
[23] Athiperumalsamy T, Rajeswari VD, Poorna SH, Kumar V, Jesudas LL. Antioxidant activity of seagrasses and seaweeds. *Bot Mar* 2010; **53**: 251-257.
[24] Cao G, Prior RL. Comparison of different analytical methods for assessing total antioxidant capacity of human serum. *Clin Chem* 1998; **44**: 1309-1315.
[25] Benzie IF, Strain JJ. The ferric reducing ability of plasma as a measure of antioxidant power: the FRAP assay. *Anal Biochem* 1996; **239**: 70-76.
[26] Chandrasekar D, Madhusudhana K, Ramakrishna S, Diwan PV. Determination of DPPH free radical scavenging activity by reversed-phase HPLC: a sensitive screening method for polyherbal formulations. *J Pharm Biomed Anal* 2006; **40**: 460-464.
[27] Kumaran A, Karunakaran RJ. *In vitro* antioxidant activities of methanol extracts of five *Phyllanthus* species from India. LWT-Food Sci *Technol.* 2007; **40**: 344-352.
[28] Ye H, Zhou CH, Sun Y, Zhang X, Liu J, Hu QH, et al. Antioxidant activities *in vitro* of ethanol extract from brown seaweed *Sargassum pallidum*. *Eur Food Res Technol* 2009; **230**: 101-109.
[29] Yuan YV, Bone DE, Carrington MF. Antioxidant activity of dulse (*Palmaria palmata*) extract evaluated *in vitro*. *Food Chem* 2005; **91**: 485-494.
[30] Yangthong M, Hutadilok-Towatana N, Phromkunthong W. Antioxidant activities of four edible seaweeds from the southern coast of Thailand. *Plant Foods Hum Nutr* 2009; **64**: 218-223.
[31] Benzie IFF, Szeto YT. Total antioxidant capacity of teas by the ferric reducing/antioxidant power assay. *J Agric Food Chem* 1999; **47**: 633-636.