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Biochemical evaluation of antioxidant activity in extracts and polysaccharide fractions of seaweeds

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ABSTRACT: In the present study ethanol and water extracts of 15 seaweeds, *Dictyota dichotoma* var. *velutricata, Dictyota indica, Iyengaria stellata*, *Padina pavonia, Sargassum swartzii, Sargassum variegatum, Stoechospermum marginatum, Stokeyia indica, Jolyna laminarioides, Caulerpa taxifolia*, *Halimeda tuna, Ulva fasciata, Ulva lactuca*, *Solieria robusta,* and *Melanothamnus afaqhusainii,* were evaluated for their antioxidant potential by ABTS, superoxide and total antioxidant capacity (TAC) assays. The activity was concentration dependent and the variation in antioxidant potential was also observed by different assays in both extracts. Ethanol extract of *D. dichotoma* var. *velutricata, D. indica* and *S. marginatum* demonstrated highest activity by TAC assay. The antioxidant potential in organic solvent fractions of seaweeds namely *P. pavonia*, *S. swartzii*, *S. marginatum* and *M. afaqhusainii* was also determined and chloroform fraction of all the four seaweeds showed highest activity by superoxide assay. Antioxidant activity of extracted fractions of polysaccharides from *S. indica*, *C. taxifolia* and *D. dichotoma* var. *velutricata* was also evaluated by superoxide method. Polysaccharide fractions of *S. indica* obtained from HCl (at 70 ^{0}C and room temperature) and water extract demonstrated highest activity respectively. All the polysaccharide fractions of *C. taxifolia* showed excellent activity except $CaClF_{70\,^{\circ}C}$. Polysaccharide fractions of *D. dichotoma* var. *velutricata* also exhibited very good activity.

Keywords: *Antioxidant activity, Karachi coast, Polysaccharide fractions, Seaweeds*

INTRODUCTION

Membrane lipid peroxidation is induced by free radicals or reactive oxygen species, which are formed in the body as a result of many biochemical reactions as well as in electron transport chain (Ewing *et al*., 1989). These free radicals damage cells and begin a series of chain reaction leading to oxidation of various compounds like proteins of cell membrane, which impair the function of various enzymes that are responsible for maintaining membrane integrity and neutralizing metabolic oxidative products. Antioxidants play a vital role against various diseases like cancer, cardiovascular diseases, inflammation, ageing process, rheumatoid arthritis, diabetes, as well as disease associated with cartilage and Alzheimer's disease (Chauhan and Chauhan, 2006). Another field that is strongly affected by lipid peroxidation is the food sector, where the free radicals not only affect the quality of lipid in raw or processed food but also lead to loss of nutritional value. In the past decade search for natural antioxidant compounds has gained considerable attention and a number of publications on antioxidants from natural sources appeared (Huang *et al*., 2004). Antioxidants from natural sources are preferred by consumers (Kranl *et al*., 2005), due to concern on the toxic and carcinogenesis effects of synthetic antioxidants (Ito *et al*., 1986; Safer and Al-Nughamish, 1999). Natural antioxidants have a low capacity to reduce oxidative reactions, but their price is relatively high (Haliwell *et al*., 1988). Therefore, there is a need to develop potent, cheaper and safer natural antioxidants.

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Antioxidative properties of seaweeds have been studied in several geographic regions. Marine algae are rich source of dietary fiber, minerals, proteins and vitamins (Yan *et al.*, 1998). Different reports are available on the antioxidant ability of natural products using different assays due to various mechanisms of antioxidant action (Frankel and Meyer, 2000; Prior and Cao, 1999), as no single assay will accurately reflect all the radical sources or all antioxidants in a mixed or complex system (Prior *et al*., 2005).

Polysaccharides from some seaweeds have been reported to possess biological activity of potential medicinal value. These polysaccharides have become very important products in the food industry (Usov, 1998; Usov *et al*., 2002). Sulfonated polysaccharides are found in varying amount in three major divisions of marine algal groups, *Rhodophyta*, *Phaeophyta*, and *Chlorophyta*. These compounds found in *Rhodophyta* are mainly galactans consisting entirely of galactose or modified galactose unit (Fonseca *et al*., 2008; Shanmugam and Mody, 2000). The general sulfated polysaccharides of *Phaeophyta* are called fucan. The major polysaccharides in *Chlorophyta* are polydisperse heteropolysaccharides, although homopolysaccharide may also be found (Farias *et al.*, 2008; Hayakawa *et al*., 2000; Matsubara *et al*., 2001). Sulfated polysaccharides from algae possess important pharmacological activities such as antioxidant, anticoagulant, anti-inflammatory, antiproliferative, antitumoral, anticomplementary, antiviral, antipeptic and antiadhesive (Azevedo *et al*., 2009; Cumashi *et al*., 2007; Damonte *et al*., 2004). There are only few studies available on the antioxidant activity of polysaccharides from seaweeds.

In our previous report (Tariq *et al*., 2011) we reported the antioxidant activity of 15 seaweeds in ethanol and water extract from Karachi coast belonging to *Chlorophyta*, *Phaeophyta* and *Rhodophyta* by DPPH method. This study was aimed to evaluate the antioxidant capacity of same 15 seaweeds at different concentration on the basis of ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) cation radical scavenging assay, superoxide anion scavenging assay and total antioxidant capacity assay in ethanol and water extract. Additionally four seaweeds viz. *Padina pavonia*, *Sargassum swartzii*, *Stoechospermum marginatum* and *Melanothamnus afaqhusainii* were selected for fractionation by organic solvents to evaluate the nature of natural antioxidants present in these seaweeds. The selection was made on the basis of antioxidant potential of ethanol extracts as demonstrated by any or all the four methods viz. DPPH, ABTS, superoxide and total antioxidant capacity assay. The study also describes the antioxidant activity of fractions of polysaccharides from three selected seaweed viz. *Stokeyia indica*, *Caulerpa taxifolia* and *Dictyota dichotoma* var. *velutricata*. This is the first report on antioxidant activity of fractions of polysaccharides from Karachi coast.

MATERIALS AND METHODS

Chemicals used in the study were DPPH (2,2-Diphenyl-1-picrylhydrazyl), TrisHCl, Dimethyl sulfoxide (DMSO), ABTS [2,2'azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt] (Fluka),potassium persulphate, sodium phosphate, pyrogallol (Fisher scientific), HCl, sulfuric acid, di-sodium phosphatehypta-hydrate, ammonium molybdate, ethanol (Merck), -tocopherol (Fluka), BHA (butylated hydroxyanisole) (Avonchem), BHT (butylated hydroxytoulene) (Avonchem), gallic acid, ascorbic acid (Merck). All chemicals and solvents were of analytical grade.

In the present study, we evaluated the antioxidant potential of ethanol and water extract of seaweeds belonging to *Phaeophyta* namely, *Dictyota dichotoma* var. *velutricata, Dictyota indica, Iyengaria stellata*, *Padina pavonia, Sargassum swartzii, Sargassum variegatum, Stoechospermum marginatum, Stokeyia indica* and *Jolyna laminarioides.* Seaweeds studied belonging to Chlorophyta were *Caulerpa taxifolia*, *Halimeda tuna, Ulva fasciata,* and *Ulva lactuca,* whereas *Solieria robusta* and *Melanothamnus afaqhusainii* belonged to Rhodophyta. All the seaweeds were collected from Buleji Beach of Karachi coast at low tide and were identified in the lab. Seaweeds were dried under shade and grounded into fine powder of 250µm, packed in polyethylene bags and kept at room temperature.

Preparation of Water extract

Fifty grams of seaweeds were homogenized with distilled water. The extract was filtered through cotton wool and filter paper Whatman No.1 respectively. The filtrate was lyophilized using freeze dryer (Eyela FD-1) and stored at -10° C till used.

Preparation of Ethanol extract

Dry powder of seaweeds (100 g) was soaked in ethanol (100%) for one week. The extract was filtered

through Whatman No.1, and the process was repeated thrice. All the three filtrates were collected and concentrated to dryness on rotary evaporator (Buchi R-200). The extracts were stored at room temperature until used.

Fractionation of ethanol extract

Ethanol extract of four seaweeds *Stoechospermum marginatum*, *Sargassum swartzii*, *Padina pavonia* and *Melanothamnus afaqhusainii* were selected for fractionation by organic solvents. The selection was made on the basis of antioxidant potential demonstrated by one or more methods used. The ethanol extract of each seaweed was first passed through *n*-hexane, and *n*-hexane soluble and insoluble portions were separated. *n*-Hexane insoluble portion was then passed through chloroform, and chloroform soluble and insoluble portions were separated. Finally chloroform insoluble portion was passed through methanol and methanol soluble and insoluble portions were separated. All the fractions were concentrated under vacuum using rotary evaporator. The antioxidant activity of these fractions was evaluated by the DPPH, and superoxide anion assay.

Determination of Antioxidant Activity

The antioxidant activity in extracts and fractions of selected seaweeds was determined by following assays.

ABTS cation radical scavenging assay

The $ABTS^+$ radical cation scavenging activity of extracts and standards: -tocopherol, BHA, BHT gallic acid and ascorbic acid was determined according to modified assay of Re *et al.,* (1999). Five ml of 7.0 mM ABTS was reacted with 88.0 µl of 140 mM potassium persulfate overnight in the dark to yield the $ABTS^+$ radical cation. Prior to use in the assay, the $ABTS^+$ radical cation was diluted with 50% ethanol for an initial absorbance of 0.700 (±0.02). 100 µl of ABTS reagent was mixed with 1 µl of sample at the concentration of 1 mg/ml and 5 mg/ml. A microplate reader (Bio-Rad) was used to read the absorbance at 415 nm against blank, respective solvents were used as blank. A mixture of ABTS reagent and respective solvent was used as control. Absorbance was measured at 0, 1, 2, 3, 4 and 5 min intervals. The inhibition percentage was calculated by the following formula:

Scavenging activity (%) = {1-absorbance of sample/ absorbance of control} × 100

Superoxide anion radical scavenging assay

The superoxide assay was determined by the modified method of Authkorala *et al.,* (2003). The assay mixture contained 2.6 ml of phosphate buffer (pH 8.24, 50 mM) and 0.3 ml of algal extract at the concentration of 0.5 and 1 mg/ml and fractions @ 0.5 mg/ml were incubated for 10 minutes at room temperature. Freshly prepared 0.1 ml of pyrogallol (3 mM pyrogallol in 10 mM HCl) was added to this mixture and the reaction proceeded for 4 min at room temperature. Auto-oxidation of pyrogallol was measured at 325 nm. The absorbance of extract was recorded after every minute to obtain maximum inhibition point of the reaction. Increment of absorbance was calculated by the difference of absorbance (absorbance at maximum inhibition point - absorbance at zero time). The scavenging activity was calculated as:

{1-Ai-Aj/Ac} × 100

Where:

Ai= absorbance measured with extract and pyrogallol

Aj= absorbance measured with extract and without pyrogallol

Ac= absorbance of control with particular solvent (without extract)

Total antioxidant capacity (TAC) assay

Total antioxidant capacity assay was conducted according to the method of Prieto *et al.*, (1999). An aliquot of 0.1 ml of sample at the concentration of 1 mg/ml and 3 mg/ml was mixed with 1 ml reagent solution (containing 0.6 M sulfuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate). The reaction mixture was incubated at 95 °C for 90 min. The samples were cooled to room temperature and absorbance was measured at 695 nm against blank. A mixture of 1 ml of reagent solution and 0.1 ml of respective solvent was used for blank.

DPPH free radical scavenging assay

The free radical scavenging activity of seaweeds extracts in different fractions was determined by using DPPH (2, 2-Diphenyl-1-picrylhydrazyl) assay (Duan *et al.*, 2006; Zubia *et al.*, 2007), where an aliquot of 200 µl of seaweed fraction was mixed with 800 µl of 100 mM Tris-HCl buffer (pH 7.4). 30 µM DPPH (dissolved in DMSO) was added to the mixture and vortex. The absorbance was recorded at 517 nm against blank by using UV-visible spectrophotometer. One ml ethanol in 1 ml DPPH was used as control. The mixture was kept in

dark for 20 minutes, and the absorbance was measured until the absorbance reached at plateau. The antioxidant activity was calculated by using the following formula: Antioxidant activity= (Absorbance of control-Absorbance of sample/ Absorbance of control) × 100

Extraction of Polysaccharides

Polysaccharides were extracted from three seaweeds: *Stokeyia indica*, *Dictyota dichotoma* var. *velutricata* and *Caulerpa taxifolia*. 100 g of each seaweed powder was extracted with 80% aqueous ethanol (500 ml) under mechanical stirring at room temperature and at 70 ^{0}C for 24 hours. Filtrates of ethanol extract at room temperature (EF_R) and at 70 ^{0}C (EF_{70}) were collected separately. Residues was divided into three equal portions and extracted with 240 ml of water, dilute hydrochloric acid (at pH 2), and 2% calcium chloride separately with mechanical agitation for 7 hour at room temperature. The extracts were filtered in order to obtain following fractions:

WF_R (water fraction at room temperature)
$HClF_R$ (Hydrochloric acid fraction at room temperature)
$CaClF_R$ (Calcium chloride fraction at room temperature)

The residue of each solvent was re extracted with respective solvents i.e. water, HCl and $CaCl_2$ at 70 ^{0}C for 7 hour with mechanical stirring and following fractions were obtained:

$WF_{70\,°C}$ (water fraction at70 ^{0}C)
$HClF_{70\,°C}$ (Hydrochloric acid fraction at 70 °C)
$CaClF_{70\,°C}$ (Calcium chloride fraction at70 °C)

All the six fractions were concentrated on rotary evaporator, dialyzed and freeze dried (Ponce *et al.*, 2003).

Determination of Antioxidant Activity of polysaccharide fractions

The antioxidant activity in polysaccharides fractions of selected seaweeds was determined by superoxide anion assay method (Authukorala *et al.*, 2003).

Analysis of data

All the experiments were conducted with 4 replicates. Data were analyzed and subjected to Analysis of Variance (ANOVA) and means were separated using Duncan's multiple range test according to Armitage and Berry (1999).

RESULTS

ABTS cation radical scavenging assay

The antioxidant activity in ethanol extract of the fifteen seaweeds at the concentration of 1 mg/ml and 5 mg/ml by ABTS method are presented in Table 1. The activity was concentration dependent. The activity in all the seaweeds was lower than the standards BHA, BHT and -tocopherol at both concentrations tested. The highest activity at 5 mg/ml was demonstrated by *M. afaqhusainii* (46.03%), followed by *D. indica* (32.37%).

The activity in water extract of same seaweeds was also investigated by ABTS method and was found concentration dependent (Table 2). The antioxidant potential of some seaweeds at 5 mg/ml was comparable with standards ascorbic acid (94.41%) and gallic acid (93.30%). *S. variegatum* (86.93%), *D. dichotoma* var. *velutricata* (76.87%), *D. indica* (73.16%), and *C. taxifolia* (71.32%) demonstrated more than 70% activity. *S. marginatum* (67.83%), *S. swartzii* (61.27%), *I. stellata* (54.87%), and *P. pavonia* (53.42%) also showed significant activity at 5 mg/ml. However it was observed that activity in water extract by ABTS method was much higher than ethanol extracts of all seaweeds.

Superoxide anion radical scavenging assay

The results of ethanol extract by superoxide assay are given in Table 3. Two concentration levels i.e. 0.5 mg/ml and 1 mg/ml were used for extracts. The activity was concentration dependent. Out of fifteen seaweeds tested, five seaweed belonging to Phaeophyta namely *S. swartzii* (99.03%), *S. marginatum* (97.79%), *P. pavonia* (97.12%), *D. indica* (96.73%), *D. dichotoma* var. *velutricata* (90.99%) showed highest activity at the concentration of 1 mg/ml. The rest of the seaweeds also showed potent antioxidant activity, but lower than these five.

The antioxidant potential in water extract by superoxide assay was determined at two concentration level 0.5 mg/ml and 1 mg/ml (Table 4). The activity was concentration dependent. If we compare the activity of water extracts at the concentration of 0.5mg/ml, it was much lower than the standards ascorbic acid (0.05mg/ml) and gallic acid (0.05mg/ml). Two seaweeds *S. indica* (93.82%) and *S. swartzii* (90.79%) showed activity equivalent to standard ascorbic acid. At the concentration of 1 mg/ml the activity was much higher and seven out of 15 seaweeds tested demonstrated more than 80% activity. The activity was in the order of *S. indica* > *S. swartzii* > *D. dichotoma* var. *velutricata* > *S. variegatum* > *J. laminarioides* > *D. indica* > *H. tuna* > *C. taxifolia* > *S. marginatum* > *U. fasciata* > *M.*

Table 1: Antioxidant activity of ethanol extract of seaweeds by ABTS cation radical scavenging assay.

Sample	Antioxidant Activity (%) Concentration (mg/ml)	
	1	5
Standards:		
BHA	$93.85^{a} \pm 0.14$	$94.21^{a} \pm 0.09$
BHT	$92.97^{a} \pm 0.36$	$94.19^{a} \pm 0.08$
-tochopherol	$51.92^{b} \pm 1.31$	$76.28^{b} \pm 1.82$
Brown:		
Dictyota dichtoma var. *velutricata*	$2.08^{lm} \pm 0.50$	$5.8^{i} \pm 0.7$
Dictyota indica	$3.03^{jkl} \pm 1.10$	$32.37^{d} \pm 1.87$
Iyengaria stellata	$1.03^{m} \pm 0.25$	$6.61^{i} \pm 1.85$
Padina pavonia	$6.93^{fg} \pm 0.5$	$13.48^{g} \pm 1.27$
Sargassum swartzii	$13.38^{c} \pm 1.28$	$20.86^{f} \pm 0.07$
Sargassum variegatum	$5.76^{gh} \pm 0.35$	$7.36^{hi} \pm 0.46$
Stoechospermum marginatum	$9.49^{d} \pm 1.52$	$24.42^{e} \pm 1.63$
Stokeyia indica	$3.84^{ijk} \pm 0.16$	$8.25^{hi} \pm 2.35$
Jolynala minarioides	$2.50^{klm} \pm 0.71$	$5.68^{i} \pm 2.18$
Green:		
Caulerpa taxifolia	$8.70^{de} \pm 0.53$	$20.10^{f} \pm 2.50$
Halemida tuna	$4.76^{hi} \pm 1.34$	$9.88^{h} \pm 1.03$
Ulva fasciata	$0.95^{m} \pm 0.80$	$3.05^{j} \pm 0.16$
Ulva lactuca	$3.29^{ijkl} \pm 1.37$	$5.94^{i} \pm 0.56$
Red:		
Solieria robusta	$4.54^{hij} \pm 0.91$	$6.34^{i} \pm 0.59$
Melanothamnus afaqhusainii	$7.80^{ef} \pm 0.35$	$46.03^{c} \pm 2.57$
LSD 0.05 Seaweed= 1.41[1] Concentration= 0.47[2]		

Mean values in column bearing same superscript letter are not significantly different according to Duncan's multiple range test (one way ANOVA).
[1]Mean values in column showing difference greater than LSD values are significantly different at $p<0.05$(two way ANOVA).
[2]Mean values in row showing difference greater than LSD values are significantly different at $p<0.05$(two way ANOVA).
Values are means ±Standard deviation with n=4.

afaqhusainii > *P. pavonia* >*I. stellata* > *S. robusta* > *U. lactuca*. Seven brown, three green and one red seaweed exhibited more than 70 % activity.

Total antioxidant capacity assay

The results of total antioxidant capacity assay for ethanol and water extracts are shown in Tables 5 and 6 respectively. The activity in ethanol extract was determined at two concentration level i.e. 1 mg/ ml and 3 mg/ml (Table 5). The activity was concentration dependent both in standards and extracts. *D. indica*, *D. dichotoma* var. *velutricata* and *S. marginatum* showed maximum activity at both concentrations of ethanol extract. Variation in antioxidant potential of all the seaweeds was observed at both concentration levels.

The results of water extract by total antioxidant capacity method at two concentration levels are shown in Table 6. The activity was concentration dependent. *D. dichotoma* var. *velutricata* (129.45 mg equivalent of ascorbic acid / g extract), *D. indic*a (113.48 mg equivalent of ascorbic acid / g extract) and *S. variegatum* (83.99 mg equivalent of ascorbic acid / g extract) showed high activity and were ranked among top three with respect to activity at 1 mg/ml by total antioxidant capacity method. At 3 mg/ml *D. dichotoma* var. *velutricata* showed highest activity (325 mg equivalent of ascorbic acid / g extract). A variation in activity was found in different species.

Antioxidant activity of fractions

Four seaweeds *P. pavonia*, *S. swartzii*, *S.*

Table 2: Antioxidant activity of water extract of seaweeds by ABTS cation radical scavenging assay.

Sample	Antioxidant Activity (%) Concentration (mg/ml) 1	5
Standards:		
Ascorbic acid	$94.27^{a} \pm 0.21$	$94.41^{a} \pm 0.29$
Gallic acid	$92.3^{a} \pm 0.42$	$93.30^{a} \pm 0.08$
Brown:		
Dictyota dichtoma var. *velutricata*	$50.29^{b} \pm 1.67$	$76.87^{c} \pm 1.77$
Dictyota indica	$23.93^{d} \pm 1.45$	$73.16^{d} \pm 0.15$
Iyengaria stellata	$8.61^{f} \pm 2.24$	$54.87^{g} \pm 2.73$
Padina pavonia	$15.83^{e} \pm 2.00$	$53.42^{g} \pm 2.5$
Sargassum swartzii	$16.69^{e} \pm 1.99$	$61.27^{f} \pm 0.07$
Sargassum variegatum	$23.55^{d} \pm 1.83$	$86.93^{b} \pm 2.76$
Stoechospermum marginatum	$15.02^{e} \pm 1.48$	$67.83^{e} \pm 2.11$
Stokeyia indica	$14.08^{e} \pm 2.43$	$46.20^{h} \pm 1.92$
Jolynala minarioides	$8.43^{fg} \pm 1.02$	$24.54^{j} \pm 1.24$
Green:		
Caulerpa taxifolia	$27.00^{c} \pm 0.81$	$71.32^{d} \pm 1.65$
Halemida tuna	$1.03^{i} \pm 0.92$	$3.32^{l} \pm 2.53$
Ulva fasciata	$7.67^{fg} \pm 2.70$	$34.22^{i} \pm 2.51$
Ulva lactuca	$6.03^{fg} \pm 2.10$	$17.99^{k} \pm 1.41$
Red:		
Solieria robusta	$3.21^{hi} \pm 0.0$	$26.49^{j} \pm 2.30$
Melanothamnus afaqhussainii	$5.64^{gh} \pm 0.0$	$17.02^{k} \pm 1.96$
LSD 0.05 Seaweed= 2.02[1] Concentration= 0.69[2]		

Mean values in column bearing same superscript letter are not significantly different according to Duncan's multiple range test (one way ANOVA).
[1]Mean values in column showing difference greater than LSD values are significantly different at $p<0.05$(two way ANOVA).
[2]Mean values in row showing difference greater than LSD values are significantly different at $p<0.05$(two way ANOVA).
Values are means ±Standard deviation with n=4.

marginatum and *M. afaqhusainii* were selected for further investigation of active fraction. The antioxidant potential of fractions was determined by superoxide assay (Table 7) and DPPH free radical scavenging method (Table 8). The hexane (83.93%) and chloroform (83.81%) soluble fractions of ethanol extract of *P. pavonia* showed maximum activity by superoxide radical scavenging assay. Ethanol extract (73.30%) showed better activity than methanol soluble fraction (39.72%). Remarkable high activity was observed in all fractions of *S. swartzii.* The activity in chloroform soluble fraction, ethanol extract, hexane soluble fraction and methanol soluble fraction was 95.38%, 94.44 %, 88.08 % and 83.31% respectively. Hexane (77.83%) and chloroform soluble fractions (77.75%) had higher activity than ethanol extract of *S. marginatum* (69.46%). Chloroform (89.11%) and methanol soluble fraction (82.63%) of *M. afaqhusainii* demonstrated potent activity as compared to ethanol extract (76.37%), (Table7).

The activity in different fractions of four selected seaweeds by DPPH method is presented in Table 8. All the fractions and ethanol extract demonstrated <50% activity in *P. pavonia*. The activity was in the order of chloroform fraction > methanol fraction >

Table 3: Antioxidant activity of ethanol extract of seaweeds by superoxide anion radical scavenging assay.

Sample	Antioxidant Activity (%) Concentration (mg/ml) 0.5	1
Standards:		
BHA	6.82 ± 0.62(conc:0.05mg/ml)	N.T
BHT	26.56 ± 1.56(conc:0.05mg/ml)	N.T
-tochopherol	12.29 ± 0.61(conc:0.05mg/ml)	N.T
Brown:		
Dictyota dichtoma var. *velutricata*	72.78^{d}± 2.04	90.99^{b} ± 1.06
Dictyota indica	79.87^{b}±1.75	96.73^{a} ± 1.89
Iyengaria stellata	23.95^{m} ± 0.63	29.31^{k} ± 1.45
Padina pavonia	73.30^{d} ± 2.47	97.12^{a} ± 2.54
Sargassum swartzii	94.44^{a} ± 1.26	99.03^{a} ± 0.53
Sargassum variegatum	36.02^{k} ± 0.09	55.45^{j} ± 0.79
Stoechospermum marginatum	69.46^{e} ± 1.31	97.79^{a}± 1.31
Stokeyia indica	65.71^{fg} ± 1.43	75.95^{g} ± 0.55
Jolynala minarioides	27.04^{l} ± 0.22	76.99^{fg} ± 1.79
Green:		
Caulerpa taxifolia	66.28^{f}± 2.26	81.9^{e} ± 0.55
Halemida tuna	62.89^{g}± 1.90	72.25^{h} ± 1.63
Ulva fasciata	40.16^{j} ± 2.46	88.25^{c} ± 2.60
Ulva lactuca	57.86^{h} ± 0.74	78.95^{f} ± 1.05
Red:		
Solieria robusta	51.78^{i} ± 1.78	68.52^{i} ± 2.31
Melanothamnus afaqhusainii	76.37^{c} ± 2.62	85.38^{d} ± 1.78
LSD 0.05 Seaweed= 1.78[1] Concentration= 0.59[2]		

Mean values in column bearing same superscript letter are not significantly different according to Duncan's multiple range test (one way ANOVA).
[1]Mean values in column showing difference greater than LSD values are significantly different at p<0.05(two way ANOVA).
[2]Mean values in row showing difference greater than LSD values are significantly different at p<0.05(two way ANOVA).
Values are means ±Standard deviation with n=4.

hexane fraction > ethanol extract. The activity in ethanol extract as well as in fractions of *S. swartzii* was less than 50%. The highest activity was recorded in ethanol extract (49.42%) followed by methanol (44.47%), hexane (37.07%) and chloroform (22.28%) soluble fraction. In *S. marginatum* ethanol extract (57.08%) had higher activity than fractions. All the fractions and ethanol extract showed less than 50% activity.

The activity in extracts and fractions of all the four seaweeds tested by DPPH assay was lower than superoxide anion radical scavenging assay.

Antioxidant activity of polysaccharide fractions

Three seaweeds *S. indica*, *C. taxifolia*, and *D. dichotoma* var. *velutricata* were selected for sequential extraction of polysaccharides on the basis of their high antioxidant activity in water extract by DPPH method. Further the antioxidant activity in different fractions of polysaccharides was estimated by superoxide method.

Stokeyia indica

The results of water extract and fractions of polysaccharide of *S. indica* are presented in Table 9.

Table 4: Antioxidant activity of water extract of seaweeds by superoxide anion radical scavenging assay.

Sample	Antioxidant Activity (%) Concentration (mg/ml)	
	0.5	1
Standards:		
Ascorbic acid	93.65 ± 2.81(conc:0.05mg/ml)	N.T
Gallic acid	96.17 ± 1.27(conc:0.05mg/ml)	N.T
Brown:		
Dictyota dichtoma var. *velutricata*	71.87^{c} ± 1.99	91.8^{b}± 1.29
Dictyota indica	71.47^{c} ± 0.32	86.18^{cd} ± 0.40
Iyengaria stellata	50.33^{f} ± 0.58	60.58^{f} ± 2.88
Padina pavonia	56.91^{e} ± 0.87	69.64^{e} ± 1.87
Sargassum swartzii	90.79^{b} ± 1.51	92.54^{b} ± 1.78
Sargassum variegatum	72.77^{c} ± 1.25	91.39^{b} ± 1.56
Stoechospermum marginatum	47.86^{f} ± 1.61	72.83^{e} ± 2.65
Stokeyia indica	93.82^{a} ± 2.6	97.05^{a} ± 1.96
Jolynala minarioides	39.91^{g} ± 1.61	87.50^{c} ± 0.83
Green:		
Caulerpa taxifolia	60.11^{d} ± 1.11	82.82^{d} ± 2.68
Halemida tuna	34.88^{h} ± 0.96	85.67^{cd} ± 0.58
Ulva fasciata	34.65^{h} ± 2.4	72.32^{e} ± 1.52
Ulva lactuca	13.1^{k} ± 1.99	24.20^{h} ± 2.90
Red:		
Solieria robusta	24.2^{j} ± 1.6	37.67^{g} ± 2.52
Melanothamnus afaqhussainii	29.03^{i} ± 1.40	72.13^{e} ± 1.63
LSD 0.05 Seaweed= 2.07[1]	Concentration= 0.71[2]	

Mean values in column bearing same superscript letter are not significantly different according to Duncan's multiple range test (one way ANOVA).
[1]Mean values in column showing difference greater than LSD values are significantly different at $p<0.05$(two way ANOVA).
[2]Mean values in row showing difference greater than LSD values are significantly different at $p<0.05$(two way ANOVA).
Values are means ±Standard deviation with n=4.

The results shows that HCl fraction at 70 °C (HCl $F_{70°C,}$ 97.44±0.48%) and fraction in HCl at room temperature (HClF$_{R,}$ 96.99±0.6 %) possessed more or less the same activity as found in water extract (97.05±1.96%). The activity in HCl fractions as estimated by superoxide method at 70 °C (HCl $F_{70°C,}$97.44± 0.48%) and at room temperature (HClF$_{R,}$ 96.99±0.6%), were followed by activity in polysaccharide water fraction at 70 °C (WF$_{70,}$ 84.08±1.51%) and at room temperature (WF$_{R,}$ 76.36±1.76). The activity in fractions of ethanol at 70 °C (EF$_{70°C}$78.96 ±2.22%) was significantly higher than fraction at room temperature (EF$_{R}$ 5.40±0.35). Maximum activity was observed in fractions extracted with HCl at 70 °C (HCl $F_{70°C,}$ 97.44± 0.48%) and at room temperature (HClF$_{R,}$ 96.99±0.6%), which was followed by fractions in water at 70 °C (WF$_{70°C,}$ 84.08±1.51%) and at room temperature (WF$_{R,}$ 76.36±1.76).

Caulerpa taxifolia

The antioxidant activity of fractions of polysaccharide of *C. taxifolia* is shown in Table 10. The highest superoxide anion scavenging activity was demonstrated by ethanol fraction at room temperature (EF$_{R,}$ 98.69±0.0%) followed by water fraction at 70 °C

Table 5: Antioxidant activity of ethanol extract of seaweeds by total antioxidant capacity assay.

Sample	Antioxidant Activity (mg -tocopherol equivalents/g extract) Concentration (mg/ml)	
	1	3
Brown:		
Dictyota dichtoma var. *velutricata*	481.29[d]±1.15	648.25[d]±0.89
Dictyota indica	484.66[c]±0.44	652.44[c]±1.04
Iyengaria stellata	13.45[p]±0.85	22.50[p]±2.61
Padina pavonia	112.69[m]±1.44	242.57[i]±1.8
Sargassum swartzii	295.44[f]±0.88	299.88[g]±0.55
Sargassum variegatum	149.31[k]±0.8	227.49[k]±0.59
Stoechospermum marginatum	437.83[e]±1.89	580.51[e]±0.81
Stokeyia indica	61.22[o]±0.23	233.77[j]±1.11
Jolynala minarioides	10.09[q]±2.89	32.87[o]±0.95
Green:		
Caulerpa taxifolia	143.41[l]±0.95	186.86[m]±2.95
Halemida tuna	290.96[g]±0.77	303.06[f]±0.78
Ulva fasciata	76.98[n]±0.85	154.36[n]±1.29
Ulva lactuca	175.88[i]±0.95	187.90[m]±0.52
Red:		
Solieria robusta	215.17[h]±0.63	256.64[h]±1.21
Melanothamnus afaqhusainii	165.88[j]±0.76	202.11[l]±1.50
LSD 0.05 Seaweed= 1.64[1] Concentration= 0.56[2]		

Mean values in column bearing same superscript letter are not significantly different according to Duncan's multiple range test (one way ANOVA).
[1]Mean values in column showing difference greater than LSD values are significantly different at $p<0.05$(two way ANOVA).
[2]Mean values in row showing difference greater than LSD values are significantly different at $p<0.05$(two way ANOVA).
Values are means ±Standard deviation with n=4.

($WF_{70°C}$ 95.59±1.06%) and HCl fraction at room temperature ($HCLF_R$, 92.59±2.46%). All the fractions of *C. taxifolia* showed potent an ion scavenging activity greater than water extract (82.82±2.68%) except calcium chloride fraction at 70 °C ($CaClF_{70°C}$, 67.46±0.0%).

Dictyota dichotoma var. *velutricata*

Results obtained for the scavenging activity by *D. dichotoma* var. *velutricata* (Table 11) showed that all the fractions of polysaccharide possessed more than 80% activity except $HClF_R$ (72.73±2.33%). $WF_{70°C}$ showed higher activity (97.15±0.05%) than water extract (91.8±1.29%).

DISCUSSION

The antioxidant activity in ethanol and water extract by ABTS and superoxide assay was concentration dependent. Rana *et al.*, (2010) reported correlation between concentration of the extract and % inhibition of free radicals in different models including DPPH and ABTS radical scavenging activity. Reducing power increased with increasing concentration was also observed by Ganesan *et al.*, (2008). Same trend was observed by Kumaran and Karunakaran (2007) in methanol extract of higher plants. This property is associated with the presence of reductones that are reported to be terminators of free radical chain reaction (Duh, 1998).

The ability of seaweed extracts to behave as antioxidant in ethanol and water extracts by ABTS method was variable in different seaweeds, however the activity was many fold less in ethanol extract than water extract. The difference is probably due to the characteristics of the antioxidant components extracted

Table 6: Antioxidant activity of water extract of seaweeds by total antioxidant capacity assay.

Sample	Antioxidant activity (mg ascorbic acid equivalents/g extract)	
	Concentration (mg/ml)	
	1	3
Brown:		
Dictyota dichtoma var. *velutricata*	129.45^{b}±0.63	325.24^{b}±2.87
Dictyota indica	113.48^{c}±0.83	211.53^{d}±1.02
Iyengaria stellata	26.26^{i}±1.03	66.47^{l}±1.11
Padina pavonia	48.83^{f}±0.29	127.94^{g}±0.43
Sargassum swartzii	39.02^{g}±0.63	91.79^{j}±1.05
Sargassum variegatum	83.99^{d}±0.63	138.88^{e}±0.47
Stoechospermum marginatum	64.41^{e}±0.76	106.69^{i}±0.25
Stokeyia indica	65.28^{e}±0.13	134.83^{f}±0.87
Jolyna laminarioides	15.66^{j}±0.25	52.49^{n}±2.36
Green:		
Caulerpa taxifolia	49.68^{f}±2.15	123.04^{h}±0.49
Halemida tuna	26.73^{i}±0.60	56.11^{m}±0.4
Ulva fasciata	33.08^{h}±0.75	71.72^{k}±2.65
Ulva lactuca	4.29^{l}±2.64	15.76^{p}±0.38
Red:		
Solieria robusta	13.35^{k}±1.23	42.99^{o}±1.58
Melanothamnus afaqhusainii	82.76^{d}±1.64	233.34^{c}±0.75
LSD 0.05 Seaweed= 1.51[1] Concentration= 0.53[2]		

Mean values in column bearing same superscript letter are not significantly different according to Duncan's multiple range test(one way ANOVA).
[1]Mean values in column showing difference greater than LSD values are significantly different at $p<0.05$(two way ANOVA).
[2]Mean values in row showing difference greater than LSD values are significantly different at $p<0.05$(two way ANOVA).
Values are means ±Standard deviation with n=4.

Table 7: Antioxidant activity of fractions by superoxide anion radical scavenging assay.

Seaweeds	Antioxidant activity (%)			
	Ethanol extract	Hexane fraction	Chloroform fraction	Methanol fraction
Brown:				
Padina pavonia	73.30^{b} ± 2.47	83.93^{a}± 0.37	83.81^{a} ± 0.58	39.72^{c} ± 0.57
Sargassum swartzii	94.44^{a} ± 1.26	88.08^{b} ± 1.05	95.38^{a}± 0.27	83.31^{c} ± 0.36
Stoechospermum marginatum	69.46^{b} ± 1.31	77.83^{a}± 2.18	77.75^{a} ± 0.55	-
Red:				
Melanothamnus afaqhusainii	76.37^{c} ± 2.62	70.98^{d} ± 0.76	89.11^{a}± 0.42	82.63^{b} ± 0.61
LSD	2.47	1.60	2.45	2.66

Mean values in column bearing same superscript letters are not significantly different ($p<0.05$) according to Duncan's multiple range test. [1]Mean values in row showing difference greater than LSD values are significantly different at $p<0.05$. N.T. =Not tested. Values are means ±Standard deviation with n=4.

from different seaweeds (Lai *et al*., 2001). Duffy and Power (2001) reported little antioxidant activity in ethanol extract of some Chinese plants namely goldthread rhizome (rhizomza *Captidix trifolia*), skullcap root (radix *Scutellaria lateriflora*), milkvetch root (radix *Astragali*), big head root (*Bighead*

Table 8: Antioxidant activity of fractions by DPPH assay.

Seaweeds	Antioxidant activity (%)			
	Ethanol extract	Hexane fraction	Chloroform fraction	Methanol fraction
Brown:				
Padina pavonia	$31.85^{c} \pm 1.16$	$32.59^{c} \pm 0.21$	$44.09^{a} \pm 0.18$	$34.86^{b} \pm 0.18$
Sargassum swartzii	$49.42^{a} \pm 1.37$	$37.07^{c} \pm 0.37$	$22.28^{d} \pm 0.62$	$44.47^{b} \pm 0.39$
Stoechospermum marginatum	$57.08^{a} \pm 2.85$	$39.83^{c} \pm 0.27$	$46.34^{b} \pm 0.17$	-
Red:				
Melanothamnus afaqhusainii	$45.78^{a} \pm 1.27$	$43.19^{b} \pm 0.26$	$36.31^{c} \pm 0.47$	$42.82^{b} \pm 0.22$
LSD	1.14	1.50	2.70	1.32

Mean values in column bearing same superscript letters are not significantly different ($p<0.05$) according to Duncan's multiple range test. [1]Mean values in row showing difference greater than LSD values are significantly different at $p<0.05$.
N.T. =Not tested.
Values are means ±Standard deviation with n=4.

Table 9: Antioxidant activity of polysaccharides fractions of *Stokeyia indica* by superoxide anion radical scavenging assay.

Polysaccharides fractions	Antioxidant activity (%)
$HClF_{70°C}$	$97.44^{a} \pm 0.48$
H_2O Ext.	$97.05^{a} \pm 1.96$
$HClF_{R}$	$96.99^{a} \pm 0.6$
$WF_{70°C}$	$84.08^{b} \pm 1.51$
$EF_{70°C}$	$78.96^{c} \pm 2.22$
WF_{R}	$76.36^{d} \pm 1.76$
$CaCl_2F_{R}$	$72.83^{e} \pm 2.60$
$CaCl_2F_{70°C}$	$51.05^{f} \pm 0.37$
EF_{R}	$5.40^{g} \pm 0.35$
$LSD^{1}_{0.05}$	2.53

Mean values in column bearing same superscript letters are not significantly different ($p<0.05$) according to Duncan's multiple range test.
[1]Mean values in column showing difference greater than LSD values are significantly different at $p<0.05$.
Values are means ±Standard deviation with n=4.
WF_R= Water fraction at room temperature; $WF_{70°C}$= Water fraction at 70°C; EF_R= Ethanol fraction at room temperature; $EF_{70°C}$= Ethanol fraction at 70°C; $HClF_R$= HCl fraction at room temperature; $HClF_{70°C}$= HCl fraction at 70°C; $CaCl_2F_R$= $CaCl_2$ fraction at room temperature; $CaCl_2F_{70°C}$= $CaCl_2$ fraction at 70 °C.

Table 10: Antioxidant activity of polysaccharides fractions of *Caulerpa taxifolia* by superoxide anion radical scavenging assay.

Polysaccharides fractions	Antioxidant activity (%)
EF_{R}	$98.69^{a} \pm 0.0$
$WF_{70°C}$	$95.59^{b} \pm 1.06$
$HClF_{R}$	$92.59^{c} \pm 2.46$
$EF_{70°C}$	$89.80^{cd} \pm 1.01$
WF_{R}	$89.30^{d} \pm 1.73$
$CaCl_2F_{R}$	$88.35^{de} \pm 1.61$
$HClF_{70°C}$	$85.65^{ef} \pm 2.77$
H_2O Ext.	$82.82^{fg} \pm 2.68$
$CaCl_2F_{70°C}$	$67.46^{h} \pm 0.0$
$LSD^{1}_{0.05}$	2.90

Mean values in column bearing same superscript letters are not significantly different ($p<0.05$) according to Duncan's multiple range test.
[1]Mean values in column showing difference greater than LSD values are significantly different at $p<0.05$.
Values are means ±Standard deviation with n=4.
WF_R= Water fraction at room temperature; $WF_{70°C}$ = Water fraction at 70°C; EF_R= Ethanol fraction at room temperature; $EF_{70°C}$ = Ethanol fraction at 70°C; $HClF_R$= HCl fraction at room temperature; $HClF_{70°C}$= HCl fraction at 70°C; $CaCl_2F_R$= $CaCl_2$ fraction at room temperature; $CaCl_2F_{70°C}$ = $CaCl_2$ fraction at 70 °C.

atractylodes rhizome), Chinese white peony root (radix *Paeonniae alba*), tangerine peel (pericardium *Citri reticulatae*), pine needle (*Pinus tabulaeformis*), medicated leaven (*Massa fermentata medicinalis*), hawthorn fruit (wild) (*Crataegus cunteata*) and hawthorn fruit (cultivate) (*Crataegus cunteata*). Boonchum *et al*., (2011) in their study revealed that aqueous extract was a better source of antioxidants. Water extract of seaweeds contained higher antioxidant activity (Kuda and Ikemori, 2009). Thus it can be assumed that different seaweeds possess different antioxidant potential in different extraction medium. This may be due to different polarities of each antioxidant compound group present in the seaweeds (Marinova and Yanishlieva, 1997).

Antioxidant property as demonstrated by superoxide assay method in ethanol and water extracts was in the range of 29.31-99.03% and 24.20-97.05%, respectively at the concentration of 1 mg/ml. The ranking of antioxidant activity of the extracts may vary with analysis method.

Table 11: Antioxidant Activity of Polysaccharide Fractions of *Dictyota dichotoma* var. *velutricata* by superoxide anion radical scavenging assay.

Polysaccharides fractions	Antioxidant activity (%)
$WF_{70°C}$	97.15[a] ± 0.05
WF_R	93.78[b] ± 2.34
$CaCl_2F_{70°C}$	91.87[b] ± 0.75
H_2O Ext.	91.8[b] ± 1.29
$CaCl_2F_R$	87.33[c] ± 0.67
$EF_{70°C}$	86.51[c] ± 2.77
$HClF_{70°C}$	86.37[c] ± 1.35
EF_R	84.96[c] ± 0.14
$HClF_R$	72.73[d] ± 2.33
$LSD^1_{0.05}$	2.65

Mean values in column bearing same superscript letters are not significantly different ($p<0.05$) according to Duncan's multiple range test.

[1]Mean values in column showing difference greater than LSD values are significantly different at $p<0.05$.

Values are means ±Standard deviation with n=4.

WF_R=Water fraction at room temperature; $WF_{70°C}$= Water fraction at 70°C; EF_R= Ethanol fraction at room temperature; $EF_{70°C}$=Ethanol fraction at 70°C; $HClF_R$= HCl fraction at room temperature; $HClF_{70°C}$= HCl fraction at 70°C; $CaCl_2F_R$= $CaCl_2$ fraction at room temperature; $CaCl_2F_{70°C}$= $CaCl_2$ fraction at 70°C.

Antioxidant activity of ethanol extract ranked differently when analyzed by ABTS and superoxide method also reported (Martinez-Valverde *et al.,* 2002).

Most of the seaweeds demonstrated very high activity, 90% or more both in water and ethanol extracts by superoxide assay. While very low activity in comparison with standards was observed in ethanol extract by ABTS method. Many reports are available emphasizing on carrying out more than one type of antioxidant activity measurement to take into account the various mechanisms of antioxidant action (Frankel and Meyer, 2000; Prior and Cao, 1999), as single assay may not accurately reflect all the radical sources or all antioxidants in a mixed or complex system (Prior *et al.,* 2005). These methods differ in terms of their assay principles and experimental conditions; consequently, in different methods antioxidants in particular have varying contribution to total antioxidant potential (Cao and Prior, 1998). Total antioxidant capacity method showed varied activity in different species of seaweed in ethanol and water extract and was concentration dependent. However, ethanol extract demonstrated better activity than water extract (El-Hajaji *et al*., 2010), moreover the activity in extracts was much lower than standards.

Four seaweeds viz. *P. pavonia*, *S. swartzii*, *S. marginatum* and *M. afaqhusainii* were selected on the basis of their activity in one or any of the four assays used to evaluate their activity in ethanol extract. All fractions of selected seaweeds demonstrated excellent activity except for methanol soluble fraction (39.72%) of *P. pavonia* by superoxide assay. Hexane and chloroform soluble fraction of *P. pavonia* and *S. marginatum* had much higher activity than other fractions. Chloroform soluble fraction and ethanol extract of *S. swartzii* exhibited significantly higher activity. Chloroform and methanol soluble fraction of *M. afaqhusainii* were most active. If we compare the activity in different fractions, chloroform fraction of all 4 tested seaweed was found most active.

The results of DPPH assay of fractions showed that chloroform fraction of *P. pavonia* was most active, whereas the ethanol extract of the rest of the seaweeds was most active than fractions. Furthermore the activity in ethanol extract and fractions of all four seaweeds was lower by DPPH method than superoxide assay. Higher activity in fractions by superoxide assay may be due to the interference of other compounds present in crude extract; it has been reported that solvent used for extraction have dramatic effect on the chemical species (Yuan *et al.*, 2005). Further no single assay will accurately reflect all the radical sources or all antioxidants in a mixed or complex system (Prior *et al.*, 2005). These methods differ in terms of their assay principles and experimental conditions; consequently, in different methods antioxidants in particular have varying contribution to total antioxidant potential (Cao and Prior, 1998). It could be concluded that seaweeds can be utilized as a source of natural antioxidant compounds as their crude extracts and fractions exhibited antioxidant activity. The results of fractions of ethanol extract by superoxide assay indicate that different solvent fractions exhibit higher antioxidant activity as compared to crude extract. This could be due to fact that crude extract tend to have more interfering substances as compared to fractions.

The antioxidant activity of polysaccharide fractions from *S. indica*, *C. taxifolia* and *D. dichotoma* are shown in Tables 9-11 respectively. The highest activity in fractions of polysaccharides from *S. indica* was demonstrated by HCl $F_{70°C}$ (97.44 %) and H_2O extract. (97.05%), followed by $HClF_R$ (96.99%), and WF_{70} (84.08%) (Table 9). The HCl treated fraction contains fucoidan (Ponce *et al.*, 2003). The general sulfated

polysaccharides of Phaeophyta are called fucan. Fucan represents a family of water soluble, sulfated polysaccharides, rich in sulfated L-fucose extracted from extracellular matrix of these weeds (Costa *et al.*, 2011; Li *et al.*, 2008). Fucoidan, the sulfated -L-fucan (term often interchangeably used with fucan) has demonstrated a wide range of pharmaceutical activities. Brown seaweeds (Phaeophyta) are known to produce different polysaccharides, namely alginates, laminaran and fucoidan (Painter, 1983; Percival and McDowell, 1967). Fucoidans usually containd large proportions of L-Fucose and sulfate, together with minor amounts of other sugars like xylose, galactose, mannose, and glucuronic acid (Duarte *et al.,* 2001). Several biological activities (Patankar *et al.,* 1993; Renn, 1993) have been attributed to the fucoidan (Blondin *et al*., 1994; Chevolot *et al.*, 2001; Mauray *et al.*, 1995). In last few years sulfated polysaccharide from marine algae have been reported to have antioxidant activities. Ruperez *et al.*, (2002) found that sulfated polysaccharide from *Fucus vesiculosus* showed antioxidant activity by ferric reducing power assay; sulfated polysaccharides from *Laminaria japonica* and *Ecklonia kurome* were also demonstrated to have free radical scavenging activities. *Stokeyia indica* is included among the major fucan yielding brown seaweeds genera (Castro-Gonzalez *et al.*, 1996).

The antioxidant activity of polysaccharide fractions isolated from *C. taxifolia* are shown in Table 10. The activity in different fractions was in the order of EF_R> $WF_{70°C}$ > $HClF_R$> $EF_{70°C}$ $_>WF_R$>$CaCl_2F_R$> $HClF_{70°C}$ > H_2O ext.> $CaCl_2F_{70°C.}$ The highest antioxidant activity in EF_R (98.69%) and $WF_{70°C}$ (95.59%) fractions was observed. It was demonstrated in some studies that green algae are composed of ~11% protein, ~36% carbohydrate, ~ 53% ash and are rich in minerals like calcium, iron, phosphorus and chloride (Castro-Gonzalez *et al.*, 1996). Carbohydrates include cell-wall water soluble sulphated ulvan (Bobin-Dubigeon *et al.*, 1997; Lahaye and Ray, 1996; Lahaye *et al.*, 1997; Ray and Lahaye, 1995a, b). Being a water soluble polysaccharide, ulvan can be effectively extracted with water (Alves *et al.*, 2010; Lahaye and Ray, 1995b). Some studies report that hot water extraction results in good extraction yields (McKinnell and Percival, 1962; Robic *et al.*, 2009; Yamamoto, 1980). The high antioxidant activity in $WF_{70°C}$ is in agreement with this finding, since sulfated polysaccharide from algae possess important pharmacological activities such as antioxidant, anticoagulant, antiproliferative, antitumoral, antiinflamatory and antiviral (Azevedo *et al.*, 2009; Cumashi *et al.*, 2007; Damonte *et al.*, 2004).

The antioxidant activity of fractions of polysaccharide from *Dictyota dichotoma* var. *velutricata* are given in Table 11. The results showed that all the fractions of polysaccharide possessed more than 80% activity except $HClF_R$ (72.73±2.33%). $WF_{70°C}$ showed higher activity (97.15±0.05%) than water extract (91.8±1.29%). Some fractions showed high antioxidant potential including $WF_{70°C}$, WF_R, $CaCl_2F_{70°C}$, H_2O ext.(>90%) and some showed reasonable antioxidant potential (72.73-87.33%). These results clearly indicate the beneficial effect of sulfated polysaccharides from seaweeds in antioxidant status of consumers (Castro-Gonzalez *et al*., 1996).

The antioxidant potential of polysaccharides fractions extracted from *S. indica*, *C. taxifolia* and *D. dichotoma* var. *velutricata* showed that some fractions had excellent antioxidant potential (> 90%), whereas other had reasonable activity. The variation in antioxidant potential may be attributed to the structure of sulfated polysaccharides (Alasalvar *et al.*, 2010). The monomeric constitution, degree of sulfation and their position, type of glycosidic linkage were held chief determining factors for variation in activity. High sulfate content and low molecular size were studied to exert stronger radical scavenging activities. Sulfation is critical for efficacy of fucoidan (Frenette and Weiss, 2000).

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