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ORIGINAL ARTICLE

Radical scavenging and singlet oxygen quenching activity of extracts from Indian seaweeds

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Abstract Free radicals and singlet oxygen are responsible for oxidative stress related diseases and many natural compounds are known to have antioxidant properties. In this study, extracts from brown and red seaweeds of Indian origin were evaluated for their ability to scavenge different radicals and quench singlet oxygen. The crude extract in methanol and its fractions in different solvents were evaluated for their activity. The methanol extract and its fractions from brown seaweed exhibited higher 2,2′-azinobis(3-ethylbenzothizoline-6-sulfonic acid) radical scavenging activity with more than 90% scavenging in butanol and ethyl acetate fractions and correlated with polyphenol content. There was a significant difference ($p \leq 0.001$) in hydroxyl radical scavenging activity between different fractions of the same seaweed. Among the crude extracts, extract from *Gracilaria corticata* showed the highest (14.0%) activity. Crude extract from brown seaweeds showed higher peroxyl radical scavenging activity compared to red seaweeds. In fractions from brown seaweed extracts, highest activity was observed in ethyl acetate fraction (>88%) followed by hexane fraction (>40 %). Ethyl acetate fraction from crude extract showed higher inhibitory activity against hemoglobin induced linoleic acid oxidation. Singlet oxygen quenching activity of the crude extract from brown seaweed was lower (<13%) compared to red seaweeds (16.4–20.5%).

Keywords Seaweed · Polyphenol · Antioxidant · Free radical · Singlet oxygen

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Introduction

Seaweeds constitute one of the major components of diet in several Asian countries, particularly Japan, China and Korea. The consumption of seaweed as a part of diet has been shown to be one of the reasons for low incidence of breast and prostrate cancer in Japan and China compared to North America and Europe (Pisani et al. 2002). Seaweeds are rich source of variety of nutrients and bioactive components (Ito and Hori 1989). *In vivo* studies have demonstrated the anti-cancerous, anti-obesity, anti-inflammatory and anti-proliferative effects of seaweeds and their components (Miyashita 2006, Yuan and Walsh 2006).

Reactive oxygen species (ROS) that include free radicals such as superoxide, hydroxyl and peroxyl are responsible for manifestation of oxidative stress related diseases like cancer and arteriosclerosis (Darely-Usmer and Halliwell 1996). ROS are the important initiators of lipid oxidation in biological membranes, which lead to many diseases (Frei 1994). Thus, it is believed that the antioxidative components in seaweeds are responsible for their effects on disease protection. As the ROS are implicated in several diseases, antioxidants play an important role in preventing the interaction of reactive oxygen species with biological system (Ames et al. 1993).

In addition to being implicated in diseases and aging, ROS also play an important role in chemical deterioration of food. The lipid oxidation initiated by ROS can lead to unacceptability of food products in addition to formation of harmful lipid oxidation products (Stadler and Guillot 1997). Studies using model systems also reported that free radicals induce protein oxidation and prevention of protein oxidation by antioxidants has protective effects on lipid fractions (Baron et al. 2005). Antioxidants or ingredients having antioxidative properties are used extensively for improvement of food stability. With the focus is being shifting towards finding alternatives for synthetic food ingredients, natural substances having antioxidative properties need to be further explored.

Studies have been carried out on the antioxidative potential of different seaweeds mainly from the waters of China,

Korea and Japan (Yan et al. 1998, Lim et al. 2002, Ahn et al. 2004, Heo et al. 2005, Kuda et al. 2005). The presence of antioxidative substances in seaweeds is suggested to be an endogenous defense mechanism as a protection against oxidative stress due to extreme environmental conditions (Aguilera et al. 2002). The antioxidative components in seaweeds include chlorophyll and carotenoid pigments, vitamins like α-tocopherol and phenolic substances (Shahidi and Zhong 2007). The antioxidant activity of extracts from red algae, 'dulse' (*Palmaria palmate*) is associated with aqueous/alcohol soluble compounds characterized by phenolic functional groups with reducing activity (Yuan et al. 2005). Duan et al. (2006) reported that antioxidant activity of red seaweed extracts correlated with their polyphenol content. Yoshie et al. (2000) identified the presence of catechins in marine algae. Fucoxanthin, isolated from the brown seaweed Wakame exhibited various radical scavenging activities (Sachindra et al. 2007).

In India use of seaweeds as dietary constituents is limited. They are mainly used for the extraction of agar, alginate and carrageenan. However, attempts are being made to use seaweed powder in food products as functional ingredient. Addition of seaweed powders in fish and vegetable snack products improved the functional and sensory properties (Senthil et al. 2005, Mamatha et al. 2007). Besides improving the functional properties, addition of seaweeds in food products would also improve the nutritional quality due to the presence of many bioactive components like antioxidative substances. Chemical composition of seaweeds and resulting bioactivities are reported to be variable with species, habitat, maturity and environmental conditions (Ito and Hori 1989). This study was aimed at evaluating the antioxidative potential of 2 brown and 4 red seaweeds from India with respect to radical scavenging, singlet oxygen quenching and inhibition of linoeic acid oxidation. The brown seaweeds used were *Padina tetrastomatica, Turbinaria conoides*, while the red seaweeds were *Acanthophora spicifera, Kappaphycus alvarezi, Gracilaria edulis* and *Gracilaria corticata*.

Materials and methods

Chemicals: The chemicals 2, 2′-azinobis(3-ethylbenzothizoline-6-sulphoic acid) (ABTS), 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH), 1-3-diphenylisobenzofuran (DPIBF), peroxidase, luminol and pyrogallol red were from Sigma-Aldrich Chemie (Steinheim, Germany). All other solvents and chemicals used were of analytical grade.

Preparation of seaweed extracts: Dried seaweeds obtained from India were washed in running water for 15 min, drained and dried again at 50°C for 15 h. Dried seaweeds were powdered and soaked in methanol (1:20, w/v) overnight and filtered to collect the methanol fraction. The residue was extracted 2 more times and the filtrates were combined and concentrated to obtain the crude extract. The crude extract dissolved in methanol was fractionated successively with solvents of increasing polarity to get hexane, ethyl acetate, butanol and remaining aqueous fraction. All the fractions were concentrated by evaporating under vacuum in a rotary evaporator.

ABTS radical scavenging activity: ABTS radical solution was prepared by mixing 5 ml of ready-to-use ABTS solution with 100 ml of acetate buffer (0.05M, pH 4.5) and 5 units of peroxidase and incubating at 37°C for 15 h. The decolorization of ABTS radical solution was started by mixing 1.9 ml of ABTS solution with 0.1 ml of sample and incubating at 37°C for 1 h. The absorbance was measured at 734 nm initially and at the end of incubation period. Scavenging activity (SA) % was calculated as follows:

$$SA, \% = [1-(A_{sample} - A_{sample\ blank})/A_{control}] \times 100$$

Hydroxyl radical scavenging activity: Hydroxyl radical was generated by the Fenton system and the scavenging activity was measured by the luminometric method described by Komrskova et al. (2006) using ATTO Luminescencer 2300R (ATTO Corporation, Japan). The reaction mixture contained H_2O_2 (2 mM), $FeSO_4$ (0.1 mM), luminol (0.8 mM) and sample in acetonitrile in a total volume of 100 µl. Acetonitrile was used instead of samples in control. The 96-microwell plates with all the reagents except H_2O_2 were incubated at 37°C for 5 min in the instrument and the reaction was started by the automatic addition of H_2O_2. The resulting luminescence was measured every minute for 30 min. The total luminescence counts for the entire period were used to calculate the scavenging as follows:

$$\text{Scavenging, \%} = 100 - \frac{(100 \times \text{Luminescence count of sample})}{\text{Luminescence count of control}}$$

Peroxyl radical scavenging activity: Peroxyl radical scavenging activity was measured by the inhibition of pyrogallol red (PGR) oxidation by the peroxyl radicals generated from AAPH (Lopez-Alarcon and Lissi 2005); 1.0 ml of 60 µM PGR was mixed with 0.1 ml of sample and 0.015 ml of 600 mM AAPH and incubated at 37°C for 2 h in a water bath. The reaction mixture was chilled immediately after incubation and the absorbance was measured at 540 nm. Reaction mixture without AAPH served as blank. The scavenging rate (SR%) was calculated as follows:

$$SR, \% = 100 - [(100/(A_{ControlBlank} - A_{ControlTest}) \times (A_{SampleBlank} - A_{SamplelTest})]$$

Inhibition of hemoglobin induced linoleic acid oxidation: Inhibition of hemoglobin induced linoleic acid oxidation was determined by the method of Kuda et al. (2005). The reaction mixture contained 0.1 ml sample, 0.1 M linoleic acid in ethanol (0.025 ml) and 0.2 M phosphate buffer (0.075 ml, pH 7.2). Oxidation was initiated by addition of 0.05 ml of 0.08% hemoglobin. After incubation for 60 min at 37°C, oxidation was stopped by the addition of 5 ml of

0.6% HCl/ethanol. The peroxide value of the reaction mixture (0.2) was measured by mixing with 0.02 ml of 20 mM $FeCl_2$ and 0.01 ml of 30% ammonium thiocyanate and reading the absorbance at 490 nm using a microplate reader.

Singlet oxygen quenching activity: Singlet oxygen was generated by a peroxidase, H_2O_2 and halide system (Piatt et al. 1977) and the oxidation of DPIBF by the singlet oxygen was measured at 420 nm. The reaction mixture in acetate buffer (pH 4.5, 50 mM) contained 1 unit of peroxidase, 0.25 mM KBr, 0.25 mM H_2O_2, 0.1 mM DPBIF solubilized in 0.05% Triton X-100 and the extract. The reaction was initiated by addition of H_2O_2 into the thermostatically maintained cuvette containing the reaction mixture at 37°C, and the decrease in absorbance at 420 nm was noted at 30 sec interval for 5 min. The quenching % was calculated from the reduction in absorbance in the presence and absence of extract.

Determination of total polyphenol content: Total polyphenols in the extracts was measured by Folin-Ciocalteu method; 0.1 ml of the sample solution in methanol was mixed with 0.75 ml of 10% Folin-Ciocalteu reagent and incubated at 37°C. After 5 min 0.75 ml of 6% sodium carbonate solution was added and allowed to stand for 30 min at 37°C and the colour developed was measured at 750 nm. Pyrocatechol was used as standard and the total polyphenol content was expressed as catechol equivalents.

Statistical analysis: The experiments were carried in 3 replicates. The difference between samples in their ability to scavenge radicals and quench singlet oxygen was assessed statistically by ANOVA and Duncan's multiple range tests using STATISTICA software (Statsoft Inc 1999).

Results and discussion

The crude extract and its fractions from brown seaweeds, *T. conoides* and *P. tetrastomatica* showed higher ABTS radical scavenging activity compared to other 4 red seaweeds (Table 1). Both butanol and ethyl acetate fractions from crude extract of brown seaweeds showed scavenging activity above 90%. Among red seaweeds, extract from *A. spicifera* showed highest ABTS radical scavenging activity (Table 1).

The crude extract, from *G. corticata* showed the highest hydroxyl radical scavenging activity (14.0%) followed by *K. alvarezi* (10.3%) and *T. conoides* (10.2%) (Table 1). However, generally all the fractions except aqueous showed higher activity than crude extract. Hexane fraction showed higher than 66% hydroxyl radical scavenging activity. Eventhough higher scavenging (>90%) was observed in butanol fraction of brown seaweed extracts, the activity of butanol fractions from red seaweeds was lower (4.4–23.6%)

Table 1 Radical scavenging activity of seaweed extracts

Seaweed	Crude extract	Hexane	Ethyl acetate	Butanol	Aqueous
ABTS radical scavenging, %					
P tetrastomatica[1]	88.4 ± 4.79^{ap}	47.2 ± 9.84^{bp}	91.0 ± 5.35^{ap}	91.6 ± 5.89^{ap}	70.3 ± 8.30^{cp}
T conoides[1]	89.9 ± 3.18^{ap}	50.4 ± 7.96^{bp}	90.0 ± 6.97^{ap}	94.6 ± 3.18^{ap}	72.4 ± 6.18^{cp}
A spicifera[2]	32.5 ± 6.50^{aq}	36.6 ± 3.24^{abr}	44.4 ± 9.35^{bq}	36.9 ± 10.82^{abq}	32.7 ± 10.65^{aq}
K alvarezi[2]	8.2 ± 3.07^{ar}	38.9 ± 5.55^{bpr}	27.4 ± 1.55^{cr}	8.9 ± 0.48^{ar}	6.2 ± 1.09^{ar}
G edulis[2]	10.2 ± 2.16^{abr}	3.4 ± 1.07^{bs}	14.4 ± 2.58^{acs}	20.0 ± 1.17^{as}	7.9 ± 3.16^{bcr}
G corticata[2]	16.1 ± 5.29^{acr}	13.8 ± 1.86^{acs}	38.6 ± 5.07^{bq}	21.5 ± 1.58^{acs}	8.5 ± 2.38^{cr}
Hydroxyl radical scavenging, %					
P tetrastomatica[1]	9.9 ± 1.85^{ap}	79.3 ± 1.01^{bp}	85.1 ± 3.13^{cp}	96.4 ± 1.11^{dp}	11.3 ± 0.87^{ap}
T conoides[1]	10.2 ± 2.38^{ap}	87.9 ± 3.12^{bq}	84.3 ± 4.12^{bp}	92.3 ± 3.11^{cp}	14.4 ± 1.90^{ap}
A spicifera[2]	9.4 ± 0.96^{ap}	71.0 ± 0.51^{br}	75.3 ± 1.29^{bq}	21.0 ± 1.66^{cq}	2.7 ± 0.63^{dq}
K alvarezi[2]	10.3 ± 2.12^{ap}	82.1 ± 4.87^{bp}	43.7 ± 3.13^{cr}	23.6 ± 3.00^{dq}	2.4 ± 0.57^{eqr}
G edulis[2]	4.9 ± 0.98^{aq}	66.5 ± 3.53^{bs}	49.2 ± 0.13^{cr}	4.4 ± 0.36^{ar}	0.3 ± 0.03^{ar}
G corticata[2]	14.0 ± 3.20^{ap}	68.5 ± 5.03^{brs}	75.3 ± 5.03^{cq}	8.2 ± 1.82^{dr}	2.1 ± 0.25^{eqr}
Peroxyl radical scavenging, %					
P tetrastomatica[1]	15.3 ± 2.78^{ap}	40.9 ± 8.34^{bp}	89.8 ± 5.57^{cp}	17.9 ± 4.72^{ap}	7.9 ± 0.87^{ap}
T conoides[1]	17.7 ± 2.21^{ap}	47.9 ± 7.12^{bq}	88.8 ± 4.17^{cp}	20.2 ± 3.17^{ap}	9.2 ± 1.18^{ap}
A spicifera[2]	12.6 ± 1.67^{ap}	52.9 ± 7.56^{bq}	35.4 ± 4.24^{cq}	7.8 ± 0.87^{aq}	7.8 ± 2.24^{ap}
K alvarezi[2]	3.2 ± 0.61^{aq}	40.1 ± 7.12^{bp}	5.9 ± 0.74^{acr}	6.9 ± 1.92^{aq}	11.3 ± 0.53^{cp}
G edulis[2]	3.9 ± 1.16^{aq}	31.0 ± 9.52^{br}	26.9 ± 6.45^{bs}	5.2 ± 0.87^{aq}	2.8 ± 0.40^{ap}
G corticata[2]	2.1 ± 0.10^{aq}	43.2 ± 1.57^{bp}	30.4 ± 1.63^{cqs}	3.3 ± 0.23^{aq}	9.1 ± 0.24^{ap}

Values with different superscripts in rows (a,b,c,d,e) and columns (p,q,r,s) differ significantly ($p \leq 0.05$) (n=3).
[1]Brown and [2]red seaweeds, ABTS: 2,2` azinobis (3-ethylbenzothizoline-6-sulfonic acid)

than hexane (66.5–82.1%) and ethyl acetate (43.7–75.3%) fractions (Table 1).

Crude extract from brown seaweeds showed higher peroxyl radical scavenging activity (17.7% and 15.3% in *T. conoides* and *P. tetrastomatica,* respectively) compared to the crude extract from red seaweeds (Table 1). In fractions from brown seaweed extracts, the highest activity was observed in ethyl acetate fraction (>88%) followed by hexane fraction (>40 %). However, among the 4 red seaweeds, highest activity was observed in hexane fraction (31.0–52.9%) (Table 1).

Oxidation of linoleic acid was induced by hemoglobin and resultant hydroperoxide were determined in the presence and absence of 100 µg extract. Ethyl acetate fraction generally showed higher inhibitory activity than other fractions and aqueous fraction showed the lowest activity (Table 2).

Singlet oxygen quenching activity of hexane fraction from all the seaweeds was higher (25.4–49.6%) compared to other fractions (Table 3). Quenching activity of the crude extract from brown seaweed was relatively lower (10–12%) compared to red seaweeds (16.4–20.5%) and the highest activity (49.6%) was observed in hexane fraction of *K. alvarezi*.

Highest polyphenol content (27.2 mg/g catechol equivalent) was observed in butanol fraction from the brown seaweed *T. conoides* (Table 4). Among the red seaweeds, *A. spicifera* crude extract and its fractions contained the highest amount of polyphenol (9.1–15.1 mg/g). In general, polyphenol content was higher in ethyl acetate and butanol fractions.

Many reports are available in terms of different *in vitro* and *in vivo* evaluation of antioxidative activity of seaweeds. ABTS assay is a simple indirect method for determining the activity of natural antioxidants. In the absence of phenolics, ABTS radical is rather stable, but it reacts energetically with a H-atom donor such as phenolics, being converted into a

Table 2 Inhibition of hemoglobin induced linoleic acid oxidation (% Inhibition) by seaweed extracts

	Crude extract	Hexane	Ethyl acetate	Butanol	Aqueous
P tetrastomatica[1]	39.4 ± 4.57^{ap}	35.9 ± 5.72^{ap}	44.8 ± 6.07^{ap}	41.8 ± 2.57^{ap}	21.9 ± 3.02^{bp}
T conoides[1]	42.3 ± 3.19^{ap}	36.1 ± 6.23^{ap}	50.2 ± 4.18^{bpq}	39.1 ± 3.75^{ap}	20.3 ± 2.29^{cp}
A spicifera[2]	30.5 ± 5.17^{aq}	45.2 ± 1.38^{bq}	55.3 ± 3.28^{cq}	20.8 ± 1.81^{dq}	13.3 ± 1.00^{eq}
K alvarezi[2]	19.3 ± 3.54^{ar}	35.1 ± 0.89^{bp}	33.2 ± 4.85^{br}	24.9 ± 5.75^{aq}	1.6 ± 0.70^{cr}
G edulis[2]	31.1 ± 2.11^{aq}	23.2 ± 0.73^{br}	30.5 ± 6.88^{ar}	22.6 ± 1.08^{bq}	13.0 ± 1.30^{cq}
G corticata[1]	28.4 ± 3.10^{aq}	17.9 ± 1.41^{br}	31.4 ± 3.84^{ar}	30.9 ± 4.07^{aq}	14.5 ± 2.02^{bq}

Values with different superscripts in rows (a,b,c,d) and columns (p,q,r) differ significantly ($p \leq 0.05$) (n=3).
[1]Brown and [2]red seaweeds.

Table 3 Singlet oxygen quenching activity (% quenching) of seaweed extracts

	Crude extract	Hexane	Ethyl acetate	Butanol	Aqueous
P tetrastomatica[1]	10.7 ± 0.54^{ap}	35.5 ± 1.06^{bp}	20.5 ± 1.72^{cp}	10.6 ± 1.18^{apq}	14.3 ± 2.29^{apq}
T conoides[1]	12.2 ± 0.78^{ap}	39.4 ± 0.91^{bq}	23.3 ± 0.87^{cp}	12.2 ± 1.82^{aq}	12.3 ± 1.23^{aq}
A spicifera[2]	16.4 ± 1.67^{aq}	25.4 ± 2.31^{br}	22.1 ± 1.69^{bp}	8.4 ± 1.51^{cp}	7.0 ± 1.34^{cr}
K alvarezi[2]	20.5 ± 2.90^{ar}	49.6 ± 3.85^{bs}	27.1 ± 0.88^{cq}	15.9 ± 1.64^{dr}	19.1 ± 1.60^{ads}
G edulis[2]	20.3 ± 1.83^{ar}	34.2 ± 2.99^{bp}	17.7 ± 2.22^{ap}	17.3 ± 1.69^{ars}	17.7 ± 2.24^{aps}
G corticata[2]	18.4 ± 1.86^{aqr}	33.8 ± 2.51^{bp}	31.1 ± 2.55^{br}	19.9 ± 2.37^{as}	19.6 ± 3.10^{as}

Values with different superscripts in rows (a,b,c,d) and columns (p,q,r,s) differ significantly ($p \leq 0.05$) (n=3).
[1]Brown and [2]red seaweeds

Table 4 Total polyphenol content (mg/g catechol equivalent) of seaweed extracts

	Crude extract	Hexane	Ethyl acetate	Butanol	Aqueous
P tetrastomatica[1]	11.6 ± 0.40^{ap}	7.1 ± 0.25^{bp}	12.5 ± 0.45^{ap}	21.8 ± 0.29^{cp}	7.9 ± 0.40^{bp}
T conoides[1]	14.2 ± 0.51^{aq}	8.1 ± 0.32^{bq}	14.2 ± 0.38^{aq}	27.2 ± 0.38^{cq}	8.1 ± 0.29^{bp}
A spicifera[2]	9.9 ± 0.38^{ar}	9.1 ± 0.75^{ar}	14.2 ± 0.22^{bq}	15.1 ± 0.50^{br}	10.6 ± 0.10^{aq}
K alvarezi[2]	2.9 ± 0.25^{as}	8.9 ± 2.05^{bq}	8.2 ± 0.35^{br}	1.2 ± 0.14^{cs}	0.32 ± 0.08^{cr}
G edulis[2]	5.1 ± 0.10^{at}	3.5 ± 0.40^{bs}	6.6 ± 0.44^{cs}	2.5 ± 0.50^{dt}	1.8 ± 0.73^{ds}
G corticata[2]	5.8 ± 0.18^{at}	4.4 ± 0.12^{bs}	8.7 ± 0.31^{cr}	5.4 ± 0.58^{au}	4.1 ± 0.05^{bt}

Values with different superscripts in rows (a,b,c,d) and columns (p,q,r,s,t,u) differ significantly ($p \leq 0.05$) (n=3).
[1]Brown and [2]red seaweeds

Table 5 Correlation coefficient (r) between total polyphenol content and different antioxidant assays for each fraction

Sample	ABTS radical scavenging	Peroxyl radical scavenging	Inhibition of linoleic acid oxidation	Hydroxyl radical scavenging	Singlet oxygen Quenching
Crude extract	0.82	0.86	0.80	0.02	-0.80
Hexane	0.75	0.40	0.84	0.56	0.20
Ethyl acetate	0.69	0.56	0.89	0.73	-0.15
Butanol	0.92	0.78	0.56	0.81	-0.76
Aqueous	0.69	-0.01	0.62	0.47	-0.83

ABTS: As in Table 1

non-colored form of ABTS (Roginsky and Lissi 2005). The results of the present study indicate that the extracts from brown seaweed exhibited higher ABTS radical activity compared to red seaweed. Relationship between ABTS radical scavenging and total polyphenol content showed a correlation of 0.69–0.92 (Table 5). Butanol fraction having higher polyphenol content showed the highest correlation (r=0.92) between these two parameters, indicating the crucial role of phenolics in ABTS radical scavenging. However, the limitations of ABTS assay, such as the capability of a sample to react with ABTS radical rather than to inhibit the oxidative process and slow reaction of many phenolics (Roginsky and Lissi 2005) necessitate compatible evaluation of antioxidant activity using other assays as well.

Hydroxyl and peroxyl radical are extremely reactive oxidizing radicals that can react with most of the biomolecules and these radicals are responsible for formation of other radicals such as alkoxyl radicals (Cheesman and Slater 1993). As the peroxyl radicals are the carriers of the chain-reaction, they can oxidize further PUFA molecules and initiate new chains, producing lipid hydroperoxides that can be broken down to yet more radical species and to a wide range of compounds, particularly aldehydes (Esterbauer et al. 1990, Porter 1990). Radical scavenging activity of varieties of seaweeds has been reported (Lim et al. 2002, Ahn et al. 2004, Kuda et al. 2005, Duan et al. 2006, Yuan and Walsh 2006). It is postulated that as marine algae are exposed to intense light and high oxygen concentration that lead to formation of free radicals and strong oxidizing agents, they protect themselves from these damaging effects by producing antioxidative substances (Dykens et al. 1992).

In the present study when different seaweeds were compared for their radical scavenging activity, in general no significant differences were observed. However, the activity differed when the crude extract was fractionated using solvents of different polarity. There are not many reports on comparative evaluation of antioxidant activity of seaweeds belonging to different groups. Nahas et al. (2007) reported the high antioxidant activity of extracts from brown seaweeds, comparable to that of commercial antioxidants. Anggadiredjal et al. (1997) reported that extracts from fresh seaweeds shows higher antioxidant activity compared to extracts from dry seaweeds and red seaweeds possess higher activity than brown seaweeds. Kuda et al. (2005) observed that the aqueous extract from brown seaweeds possesses stronger antioxidant activity compared to ethanol extract. However, in the present study it is observed that antioxidative substances present in the seaweeds are non-polar in nature as organic solvent fractions, particularly hexane and ethyl acetate fractions exhibit higher antioxidant activity.

The principal antioxidant components in seaweeds are thought to be polyphenols. Studies have demonstrated the correlation between polyphenol content and radical scavenging activity (Kuda et al. 2005). Phenolic terpenoids isolated from Mediterranean marine algae possess strong singlet oxygen quenching activity (Foti et al. 1994). In this study only ABTS radical activity had strong correlation with total polyphenol content (Table 5). Singlet oxygen quenching activity of the extracts did not exhibit any correlation to polyphenol content. The correlation between polyphenol content and different radical scavenging activities differed among fractions. Seaweeds also contain other antioxidative substances like tocopherol, chlorophylls and carotenoids, which get partitioned into organic solvents. The HPLC analysis of different fractions from the brown seaweeds showed that ethyl acetate fraction contains highest amount of fucoxanthin, a major carotenoid in brown seaweeds (data not shown) and fucoxanthin exhibits high radical scavenging activity (Sachindra et al. 2007). Thus the radical scavenging activity of hexane and ethyl acetate fractions were found to be higher in many cases. Thus it can be suggested that it is not just polyphenol content, but presence of other antioxidative components in seaweeds that impart the radical scavenging activity to the extracts from these seaweeds.

Conclusion

This study indicated that the dry seaweeds examined possess radical scavenging and singlet oxygen quenching activity. At present, in India, seaweeds are not considered as a dietary item. As seaweeds are known to contain antioxidative substances and other biomolecules of health importance, increased attempts should be made towards their better utilization in food. The use of seaweeds as an active ingredient in many food preparations would benefit the consumers with respect to availability of these important biomolecules.

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