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Post Harvest Technology and Food Biochemistry Laboratory, Department of Food Science and Technology, University of Karachi, Karachi, Pakistan

Antioxidant potential of seaweeds occurring at Karachi coast of Pakistan

Amna Tariq, Jehan Ara, Viqar Sultana, Syed Ehteshamul-Haque, Mohammad Athar*

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Summary

The oxidative damage caused by reactive oxygen species (ROS) caused damage to bio-molecules leading to various diseases such as cancer, coronary heart diseases, renal failure, diabetes, ageing etc. There is an increasing interest in natural antioxidants because of the safety and toxicity problems of synthetic antioxidant. In this study, antioxidant activity of aqueous and ethanol extracts of 15 different seaweeds and total phenolic contents were evaluated. DPPH (2, 2-Diphenyl-1-picrylhydrazyl) assay was used to determine free radical scavenging activity. The aqueous extracts showed more promising antioxidant activity as compare to ethanol extracts. Antioxidant activity of most of the seaweed reached at maximum after 180 to 220 minutes and then declined suddenly or gradually. Antioxidant activity of some seaweed was more or less equal to α- tocopherol used as standard antioxidant. After 180 minutes, the highest antioxidant activity was found in *Caulerpa taxifolia* (64.63%), *Stokeyia indica* (63.67%), *Ulva fasciata* (63.28%), *Dictyota dichotoma* var. *velutricata* (62.74%) as compared to 62.24% of α-tocopherol. All the test seaweeds were found to contain polyphenols at various concentrations. However, presence of polyphenol in some seaweeds did not show any correlation with antioxidant activity.

Introduction

Reactive oxygen species (ROS), such as superoxide radical (O_2^{*-}) hydroxyl radical (OH*), peroxyl redical (ROO*) and nitric oxide radical (NO*) attack biological molecules, such as lipids, proteins, enzymes and nucleic acids (DUAN et al., 2006; HALLIWELL et al., 1997). These free radicals are made by cells as a part of their normal functioning in the human body. These radicals have missing electrons and react to other molecules for taking electrons out of them, resulting in the development of several degenerative disease conditions, including cancer, cardiovascular diseases, rheumatoid arthritis, cataracts, immune system decline, liver diseases, diabetes mellitus, renal failure, brain dysfunction and aging (HALLIWELL et al., 1997; KEHRER, 1993; KUDA et al., 2005; VINAYAK et al., 2010). Usually balance between the formation of reactive species and antioxidant defenses is kept in the body, but oxidative stress may result when these systems fail to cope with the production of ROS/ RNS (Reactive nitrogen specie) (KIM et al., 2008). Antioxidants are effective in protecting the body against damage by reactive oxygen species. Antioxidants inhibit or prevent the oxidation of a substrate, and evolve to protect biological systems against damage induced by ROS (HWANG et al., 2010).

Over the years numerous food industries have effectively been adding antioxidants to food formations as a conventional way of decreasing the occurrence of lipid oxidation in their products (ZAHRA et al., 2007). Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertiary butyl hydroxyquinone (TBHQ) are some of the various synthetic antioxidants that are being used by several food industries for this purpose. However, some of these antioxidants have been suspected to be carcinogenic, hence their use as food ingredients has been restricted by regulatory bodies (HUANG and WANG, 2004). These implications alleviated the need for alternatives of the suspected carcinogenic synthetic antioxidants to be found, for use in food products. There is an increasing interest in natural antioxidants because of the safety and toxicity problems of synthetic antioxidant (AMAROWICZ et al., 2000). Natural antioxidants do not comprise any detrimental chemical combinations, so they are considered to be rather more safer for use in food products and are not subjected to any legal restrictions if they are "Generally Recognized as Safe (GRAS)" (HEO et al., 2005). Therefore, the search for natural antioxidants as alternatives to synthetic ones is of great interest. Among the sources of natural antioxidants, marine macro-algae (seaweed) are now being considered to be a rich source of antioxidants (CHANDINI et al., 2008; HWANG et al., 2010; KIM et al., 2008). In our previous studies, we have reported antifungal, nematicidal, cytotoxic, antibacterial and hyolipidaemic activities of seaweed occurring at Karachi coast (ARA et al., 1998; 1999; 2002 a, b; 2005). Antioxidant activity of some seaweed from Karachi coast has been reported by SABINA et al. (2006). The present report describes the antioxidant activity of ethanol and water extracts of some seaweed occurring at Karachi coast at different time intervals, using DPPH (2, 2-Diphenyl-1-picrylhydrazyl) assay. The total phenolic content of seaweeds was also estimated to find correlation between antioxidant activity and phenolic content in terms of gallic acid.

Material and method

Chemicals

Chemicals used in this study are DPPH (2, 2-Diphenyl-1-picrylhydrazyl) (sigma), alpha tocopherol (Fluka), DMSO (Fisher Scientific), ethanol (Merck). All chemicals and solvents were of analytical grade.

Seaweeds

Seaweeds, *Caulerpa taxifolia, Dictyota dichotoma,* var. *velutricata, Dictyota indica, Halimeda tuna, Iyengaria stellate, Melanothamnus afaqhusainii, Jolyna laminarioides, Padina pavonia, Sargassum swartzii, S. variegatum, Stoechospermum marginatum, Stokeyia indica, Solieria robusta, Ulva fasciata,* and *U. lactuca* were collected from Buleji Beach of Karachi coast at low tide and identified. They were washed with tap water and dried under shade. Dry seaweeds were ground into fine powder, packed in polyethylene bags and kept at room temperature for further use.

Preparation of Extracts

Aqueous extract

Dry powder of seaweed was homogenized with de-ionized water and filtered through cotton wool and Whatman filter paper. The filtrate was lyophilized using freeze dryer (Eyela FD-1) and stored at -10°C until used.

* Corresponding author

Ethanol extract

One hundred grams of dry powder of seaweed was extracted three times with ethanol and concentrated to dryness on rotary evaporator (Buchi R-200), weighed and stored at room temperature until used.

Free Radical Scavenging Activity (antioxidant assay)

The radical scavenging activity of seaweed extracts was determined using DPPH (2, 2-Diphenyl-1-picrylhydrazyl) assay (DUAN et al., 2006; ZUBIA et al., 2007). Where an aliquot of 200 µl of seaweed extract (lyophilized water extract or ethanol extract) (5mg/ml of aqueous ethanol with the ratio of 1:4) was mixed with 800 µl of 100 mM Tris-HCl buffer (pH 7.4). The mixture was added to 30 µM DPPH (dissolved in DMSO) and vortex. The absorbance was measured at 517 nm using UV-visible spectrophotometer, against aqueous ethanol, used as blank. One ml of aqueous ethanol with 1 ml of DPPH was used as control. The sample mixture was kept in dark for 20 minutes, and the absorbance was measured until the reading reached at plateau. α- tocopherol at concentration of 5 mg/ml was used as standard. The antioxidant activity was calculated using the following formula:

$$\text{Antioxidant activity} = \frac{\text{Absorbance of control-Absorbance of sample} \times 100}{\text{Absorbance of control}}$$

Determination of polyphenol in seaweeds

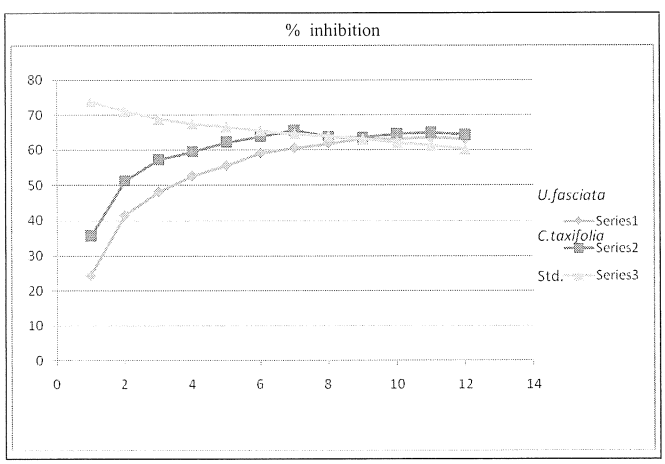
Polyphenol were determined in dry powder of seaweeds and lyophilized water extract. The extraction of polyphenol in dry seaweed powder was made by the method of JIMENEZ-ESCRIG et al., (2001). One gram ground seaweed powder was mixed with 40 ml methanol:water (50:50) plus HCl (to adjust pH at 2.0). The mixture was shaken thoroughly at room temperature for one hour. The mixture was then centrifuged at 1500 × g for 10 minutes and supernatant was separated. To the residue 40 ml acetone:water (70:30) was added and centrifuged. The two extracts were combined for polyphenol estimation. The polyphenol estimation was made by Folin-Ciocalteu phenol reagent as described by CHANDINI et al. (2008).

For estimation 100 µl aliquots of lyophilized water extract and extracted polyphenols were mixed with 2 ml of 2% Na_2CO_3 and allowed to stand for 2 minutes at room temperature. After incubation 100 µl of 50% Folin-Ciocalteu phenol reagent was added and mixture was mixed thoroughly and allowed to stand for 30 minutes at room temperature in dark. Absorbance of samples was recorded at 720 nm using spectrophotometer and phenolic content was expressed as gallic acid equivalents.

Results

Of the 15 seaweed species tested for antioxidant property, ethanol extract of *Solieria robusta, Stoechospermum marginatum, Caulerpa taxifolia* and *Jolyna laminarioides* showed significant antioxidant activity at varying time interval, 50% or more. The antioxidant activity of these seaweeds initially was weaker than standard α-tocopherol and then increased gradually with time. The activity was significantly ($p<0.05$) higher in water extracts of most of the seaweeds as compared to ethanol extract (Tab. 1 and 2). The antioxidant activity of both water and ethanol extract of *S. marginatum* and *C. taxifolia,* water extract of *Stokeyia indica, Sargassum variegatum, Ulva fasciata* and ethanol extracts of *S.robusta* gradually increased with time.

The lyophilized water extracts of seaweed *Stokeyia indica, Caulerpa taxifolia, Ulva fasciata, Dictyota dichotoma* var. *velutricata* and *Jolyna laminarioides* showed significant antioxidant activity more or less equal to α-tocopherol, a commercial antioxidant. Free radical scavenging activity of lyophilized water extracts was generally greater than the ethanol extracts of same seaweed. Furthermore, activity was reached upto 50% or more within 40-60 minutes and maintained up to 180 minutes in *Stokeyia indica*, *Caulerpa taxifolia, Ulva fasciata* and *Dictyota dichotoma* var. *velutricata.* Seaweeds, *Stoechospermum marginatum, Sargassum variegatum, Padina pavonia, Melanothamnus afaqhusinaii* and *Jolyna laminarioides* also showed significant free redical scavenging activity, more than 50% at 280 minutes or above (Tab. 2). Both lyophilized water extract and ethanol extract of *Caulerpa taxifolia* showed significant antioxidant activity (Tab. 1 and 2; Fig. 1). Polyphenol was found to be present in seaweeds at various concentrations, 0.02 to 11.3 mg%. However concentrations of polyphenoles was found higher in dry seaweeds as compared to water extracts (Tab. 3).



after: 1 = 0 minute of incubation of 20 min., 2 = 20 min., 3 = 40 min., 4 = 60 min., 5 = 80 min., 6 = 100 min., 7 = 120 min., 8 = 140 min., 9 = 160 min., 10 = 180 min., 11 = 200 min., 12 = 220 min.

Fig. 1: Antioxidant activity of water extracts of *Ulva fasciata*, *Caulerpa taxifolia* and α-tocopherol.

Discussion

Nowadays there is a growing interest on the discovery of natural antioxidants, mainly for two reasons: due to their protective role in the development of disease like cancer, atherosclerosis, arthritis, diabetes, Alzheimer's and aging, secondly phytochemicals are generally safer than synthetic chemicals. In this study, the ethanol and lyophilized water extracts of seaweeds exhibited the significant antioxidant activity by DPPH free radical scavenging. The activity was increased with time of incubation. Marine algae have remained in use by man in a variety of ways since medieval times (KUDA et al., 2005) and have conventionally been used as marine vegetables in the Far East and Pacific. Many algae have found to be producing a range of complex compounds including some of considerable medicinal value (ANGGADIREDJA et al., 1997). Moreover these algae being typical photosynthetic plants, under particular combinations of oxygen and light tend to produce free radicals and other strong oxidizing agents as well (JIMENEZ-ESCRIG et al., 2001). The present report of antioxidant activity of seaweeds of Karachi coast is in agreement with previous reports made from different geographical regions (CHANDINI et al., 2008; LIM et al., 2002; SANTOSO et al., 2004).

Tab. 1: Antioxidant activity (% inhibition[1]) of ethanol extract of seaweeds at different time intervals

Seaweeds	Time (minutes)																	
	0	20	40	60	80	100	120	140	160	180	200	220	240	260	280	300	320	340
Standard	73.8^{a}	71.1^{a}	68.9^{a}	67.53^{a}	66.8^{a}	65.5^{a}	64.6^{a}	64.09^{a}	63.1^{a}	62.2^{a}	61.32^{a}	60.33^{a}	59.43^{a}	58.54^{a}	57.74^{a}	56.92^{a}	56.28^{a}	55.78^{b}
Stoechospermu marginatum	10.05^{def}	26.68^{d}	31.6^{e}	36.8^{cd}	39.9^{cd}	42.9^{bc}	45.4^{bcd}	47.5^{bc}	48.8^{bcd}	50.6^{bcd}	51.87^{bc}	52.92^{bc}	53.2^{bc}	54.6^{ab}	55.52^{ab}	56.18^{ab}	57.07^{bc}	N.T
Stokeyia indica	10.1^{def}	13.46^{ef}	17.25^{g}	20.4^{f}	22.1^{f}	25.1e	26.8^{f}	28.4^{e}	30.3^{h}	31.6^{h}	32.54^{g}	34.92^{g}	35.86^{g}	37.29^{g}	38.78^{f}	39.38^{e}	N.T	N.T
Sargassum variegatum	1.87^{f}	4.48^{h}	6.7^{i}	7.8^{h}	9.02^{h}	10.58^{g}	10.8h	11.9^{g}	12.9^{k}	13.8^{g}	15.28^{i}	16.34^{i}	17.66^{i}	18.28^{i}	19.83^{h}	20.67^{g}	N.T	N.T
S. swartzii	29.4^{c}	41.14^{b}	43.7^{b}	45.0^{b}	46.1^{b}	46.6^{b}	46.9^{bc}	47.2^{be}	47.7^{cd}	47.9^{cde}	47.68^{cd}	48.42^{cd}	48.94^{cd}	49.24^{cd}	48.87^{cd}	49.41^{c}	N.T	N.T
Padina pavonia	3.25^{ef}	9.61^{fg}	12.8^{h}	14.3^{g}	16.2^{g}	17.9^{f}	19.7^{g}	20.6^{f}	22.0^{i}	23.6^{i}	25.21^{h}	26.37^{h}	27.93^{h}	28.88^{h}	30.17^{g}	30.77^{f}	31.85^{f}	N.T
Caulerpa taxifolia	14.5^{d}	33.43^{c}	37.4^{d}	38.8^{c}	43.3^{bc}	45.4^{b}	47.5^{b}	49.65^{b}	51.3^{bc}	52.5^{bc}	53.85^{b}	55.39^{ab}	56.59^{ab}	57.55^{a}	58.49^{a}	59.45^{a}	60.51^{a}	61.58^{a}
Iyengaria stellata	2.9^{ef}	6.02^{gh}	6.5^{i}	6.7^{h}	9.1^{h}	10.18^{g}	12.3^{h}	13.6^{g}	14.5^{j}	15.3j	16.37^{i}	17.56^{i}	18.51^{i}	19.92^{i}	20.5^{h}	20.68^{g}	21.50^{g}	22.06^{d}
Solieria robusta	15.2^{d}	33.53^{c}	38.1^{cd}	40.9^{bc}	45.1^{b}	47.08b	49.5^{b}	52.2^{b}	53.3^{b}	54.7^{b}	55.77^{b}	56.44^{ab}	57.26^{ab}	58.21^{a}	58.23^{a}	58.79^{a}	59.49^{ab}	59.37^{a}
Halemida tuna	11.2^{def}	15.8^{e}	21.01^{g}	22.1^{f}	23.3^{f}	27.7^{e}	30.24^{f}	32.36^{de}	33.9^{gh}	35.41^{gh}	37.90^{ef}	39.24^{fg}	41.08^{ef}	42.47^{ef}	43.25^{ef}	44.02^{d}	44.64^{e}	46.32^{c}
Ulva lactuca	7.26^{def}	15.01^{e}	20.8^{g}	23.3^{f}	26.7^{f}	28.9^{e}	30.8^{ed}	33.1d^{e}	34.7^{gh}	36.8^{gh}	39.0^{ef}	40.80^{ef}	42.32^{ef}	43.40^{ef}	44.49^{de}	45.58^{d}	46.35^{e}	48.16^{c}
Ulva fasciata	6.83^{def}	15.6^{e}	19.04^{g}	21.8^{f}	24.6^{f}	26.2^{e}	27.8^{f}	29.6^{e}	31.4^{h}	33.32^{gh}	34.83^{fg}	36.25^{fg}	37.88^{fg}	39.27^{fg}	41.37^{ef}	42.15^{de}	N.T	N.T
Dictyota dichtoma var. *velutricata*	13.1^{de}	25.9^{d}	32.6^{e}	36.1^{cd}	38.5^{cd}	38.8^{cd}	41.6^{cd}	42.6^{c}	42.6^{ef}	42.78^{ef}	43.12^{de}	45.47^{de}	45.88^{de}	46.35^{de}	45.26^{de}	45.26^{d}	N.T	N.T
Dictyota indica	26.6^{c}	23.2^{d}	26.7^{f}	29.9^{e}	33.1^{e}	34.7^{d}	35.7^{e}	36.9^{d}	38.0^{fg}	38.6^{fg}	38.66^{ef}	38.62^{fg}	38.89^{fg}	39.46^{fg}	39.49^{f}	N.T	N.T	N.T
Melanothamnus afaqhusainii	45.7^{b}	43.4^{b}	41.8^{bc}	29.9^{e}	N.T	N.T	N.T	N.T	N.T	N.T	N.T	N.T	N.T	N.T	N.T	N.T	N.T	N.T
Jolyna laminarioides	15.6^{d}	25.7^{d}	30.42^{ef}	33.4^{de}	36.9^{de}	39.05^{cd}	41.1^{d}	43.2^{c}	44.9^{d}e	46.9^{de}	48.25^{c}	49.35^{cd}	50.73cd	51.67^{bc}	52.73^{bc}	53.15^{b}	53.9^{d}	N.T
LSD0.05[2]	9.64	4.32	4.05	4.96	4.77	4.95	5.12	4.84	4.80	5.07	4.83	4.81	4.64	4.60	4.49	3.31	2.71	2.22

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

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

N.T= Not tested

Tab. 2: Antioxidant activity (% inhibition[1]) of water extract of seaweeds at different time intervals

Seaweeds	Time (minutes)																
	0	20	40	60	80	100	120	140	160	180	200	220	240	260	280	300	320
Standard	73.89^{a}	71.13^{a}	68.93^{a}	67.53^{a}	66.66^{a}	65.57^{a}	64.66^{a}	64.09^{a}	63.17^{a}	62.24^{a}	61.32^{b}	60.33^{b}	59.43^{b}	58.54^{cd}	57.74^{b}	56.92^{b}	56.28^{b}
Stoechospermum marginatum	21.7^{f}	37.45^{fg}	41.09^{d}	43.48^{de}	47.80^{e}	50.66^{cd}	51.17^{cd}	53.15^{b}	54.09^{b}	55.35^{bc}	56.46^{cd}	56.60^{cd}	59.96^{b}	61.01^{bc}	61.26^{a}	N.T	N.T
Stokeyia indica	26.49^{de}	45.5d^{e}	51.02^{c}	54.96^{c}	56.90^{c}	58.59^{b}	60.4^{b}	62.22^{a}	63.14^{a}	63.67^{a}	64.08^{a}	64.23^{a}	64.25^{a}	64.27^{a}	N.T	N.T	N.T
Sargassum variegatum	21.42^{f}	37.38^{fg}	41.58^{d}	44.68^{d}	47.47^{e}	49.91^{de}	51.84^{c}	53.44^{b}	55.31^{b}	56.46^{b}	57.51^{c}	58.58^{bc}	59.64^{b}	58.17^{cd}	60.88^{a}	61.53^{a}	N.T
S. swartzii	7.85^{ij}	13.14^{j}	15.59^{g}	17.11^{h}	18.93^{l}	20.86^{i}	22.05^{h}	22.83^{f}	24.10^{g}	25.01^{g}	26.66^{h}	27.63^{h}	28.75^{f}	28.8^{g}	30.62^{e}	30.95^{d}	N.T
Padina pavonia	12.55^{gh}	32.71^{g}	37.74^{d}	41.36^{de}	44.48^{f}	47.38^{e}	48.67^{de}	49.62^{c}	51.16^{cd}	53.18^{cd}	54.45^{de}	55.45^{de}	56.81^{c}	58.53^{cd}	59.77^{a}	N.T	N.T
Caulerpa taxifolia	35.65^{b}	51.21^{c}	57.22$_{b}$	59.54^{b}	62.29^{b}	63.82^{a}	65.63^{a}	63.87^{a}	63.59^{a}	64.63^{a}	64.93^{a}	64.36^{a}	N.T	N.T	N.T	N.T	N.T
Iyengaria stellata	8.36^{hij}	13.45^{j}	14.30^{g}	16.36^{h}	17.94^{i}	18.68^{i}	20.35^{h}	21.14^{f}	22.07^{g}	23.03^{g}	23.84^{i}	23.99^{i}	26.72^{f}	28.8^{g}	30.49^{e}	N.T	N.T
Solieria robusta	5.73^{j}	11.61^{j}	13.71^{g}	15.60^{h}	17.64^{i}	18.53^{i}	20.79^{h}	22.16^{f}	22.94^{g}	24.05^{g}	25.57^{hi}	25.43^{hi}	28.41^{f}	29.61^{g}	N.T	N.T	N.T
Halemida tuna	11.16^{ghi}	19.26^{i}	22.52^{f}	24.54^{g}	26.84^{h}	28.42^{h}	29.78^{g}	31.56^{e}	32.30^{f}	33.76^{f}	35.34^{g}	36.78^{g}	37.12^{e}	40.63^{f}	41.83^{d}	N.T	N.T
Ulva lactuca	4.87^{j}	6.2^{k}	7.09^{h}	8.06^{i}	10.32^{j}	11.07^{j}	12.23^{i}	13.38^{g}	15.12^{h}	15.84^{h}	17.36^{j}	18.08^{j}	18.98^{g}	19.83^{h}	20.78^{f}	21.64^{e}	N.T
U. fasciata	24.40^{ef}	41.80^{ef}	48.28^{c}	52.78^{c}	55.68^{cd}	59^{b}	60.66^{b}	61.82^{a}	63.14^{a}	63.28^{a}	63.69^{ab}	63.21^{a}	62.73^{a}	62.88^{ab}	N.T	N.T	N.T
Dictyota Dichotoma var. *velutricata*	33.48^{bc}	57.99^{b}	59.90^{b}	62.56^{b}	63.16^{b}	64.35^{a}	63.23^{ab}	62.97^{a}	62.42^{a}	62.74^{a}	N.T	N.T	N.T	N.T	N.T	N.T	N.T
D. indica	29.39^{cd}	48.84^{cd}	51.77^{c}	52.85^{c}	53.01	53.10^{c}	52.85^{c}	53.39^{b}	53.37^{bc}	53.72^{bcd}	52.76^{e}	N.T	N.T	N.T	N.T	N.T	N.T
Melanothamnus afaqhusainii	22.91^{ef}	34.50^{g}	37.73^{d}	40.34^{e}	43.37^{f}	44.44$_{f}$	46.63^{e}	47.94^{c}	49.85^{d}	51.11^{d}	52.36^{e}	54.04^{e}	54.96^{c}	55.82^{d}	56.81^{b}	57.29^{b}	57.6^{a}
Jolyna laminarioides	14.28^{g}	26.28^{h}	29.95^{e}	33.03^{e}	35.95^{g}	38.23^{g}	40.19^{f}	42.26^{d}	44.84^{e}	45.93^{e}	47.08^{f}	48.69^{f}	49.79^{d}	50.67^{e}	51.54^{b}	52.34^{c}	53.46^{c}
LSD0.05[2]	4.23	4.53	3.70	3.30	2.83	2.79	2.72	2.81	2.75	2.75	2.37	2.34	2.26	2.76	2.03	1.74	1.17

[1] Mean values in column bearing same superscript letters are not significantly different (P<0.05) according to Duncan's multiple range test.
[2] Mean values in column showing differences greater than LSD values are significantly different at p< 0.05.
N.T= Not tested

Tab. 3: Antioxidant activity of seaweeds (% inhibition) after 180 minutes and polyphenol determined in dry powder of seaweeds and lyophilized water extracts (1 g).

No.	Seaweed	Antioxidant activity % inhibition after 180 minute		Phenolic contents mg% gallic acid	
		Water extract	Ethanol extract	Lyophilized water extract	Phenols extracted from dry powder
1	Standard(α-tocopherol)	62.24	62.2	--	--
	Brown				
2	*Dictyota dichotoma* var, *velutricata*	62.74	42.78	2.57	10.03
3	*Dictyota indica*	53.72	38.6	1.95	8.47
4	*Jolyna laminarioides*	45.93	46.9	1.37	2.49
5	*Iyengaria stellata*	23.03	15.3	0.077	2.54
6	*Padina pavonia*	53.18	23.6	1.95	6.5
7	*Sargassum variegatum*	56.46	13.8	1.89	11.3
8	*Sargassum swartzii*	25.01	47.9	1.6	5.27
9	*Stoechospermum marginatum*	55.35	50.6	1.28	5.17
10	*Stokeyia indica*	63.67	31.6	2.26	8.4
	Green				
11	*Caulerpa taxifolia*	64.63	52.5	2.5	8.59
12	*Halimeda tuna*	33.76	35.41	0.63	2.55
13	*Ulva fasciata*	63.28	33.32	0.32	2.23
14	*Ulva lactuca*	63.28	36.8	0.02	2.07
	Red				
15	*Melanothamnus afaqhusainii*	51.11	N.T	1.38	6.12
16	*Solieria robusta*	24.05	54.7	0.15	1.95

NT= not tested

In this study, although, the activity of seaweed extracts were initially weaker than α-tocopherol but gradually increased with increased in incubation time and reached equivalent to α-tocopherol. The free redical scavenging activity in water extract of *Caulerpa taxifolia* (64.93 at 200 min) *Stokeyia indica* (64.27% at 260 min), *Sargassum variegatum* (61.53% at 300 min), *Ulva fasciata* (63.21% at 220 min) and *Melanothamnus afaqhusainii* (57.6% at 320 min) were maximum. Whereas the ethanol extract of *Stoechospermum marginatum* (57.07% at 320 min), *Caulerpa taxifolia* (61.58% at 340 min) and *Solieria robusta* (59.37% at 340 min) also showed significant activity. YUAN et al. (2005) also reported the antioxidant activity of seaweed with increased in time. In this study both water and ethanol extracts of *Caulerpa taxifolia* showed significant activity. These findings suggest that some seaweeds could be a good source of natural antioxidant. The ability of seaweeds to reduce free radical over a long period of time may also have benefits for extending the shelf life of processed foods during storage and distribution (YUAN et al., 2005).

Of the dietary phytochemicals, it has been suggested that polyphenols prevent oxidative damage to important biological membrane (DECKER, 1995) and plant food (ROBARDS et al., 1999). In this study almost all test seaweeds were found to contain polyphenol at various concentrations. There are reports that many algal species contain polyphloroglucinol, phenolics, phlorotannins (SHIBATA et al., 2008; NAKAMURA et al., 1996; PAVIA and ABERG, 1996) and in many cases the antioxidant activity of algae could be due to these compounds (RAGAN and GLOMBITZA, 1986). However in this study, concentration of polyphenols and antioxidant activity did not show any correlation in most of the seaweed. PAYET et al. (2005) reported free radical scavenging activity in DPPH assay, and suggested that besides, polyphenoles other compounds may contribute to the free radical scavenging activity and no correlation or weak correlation was found between antioxidant activity and phenolic content. Similarly, HEO et al. (2005) reported that, although some seaweeds contain high amount of phenolics but they did not show antioxidant activity. This study suggests that other materials in seaweeds such as low molecular weight polysaccharides, pigments or proteins may influence the activity. Within the traditional Japanese diet, seaweeds are commonly used as sushi wrappings, seasonings, condiments and vegetables and thus constitute between 10-25% of food intake by most Japanese (SKIBOLA, 2004). The low incidence of chronic diseases among the Japanese as compared to people having low to zero seaweed intake is attributed to significant dietary differences between the population (YUAN et al., 2006). Future of seaweeds as a natural antioxidant food supplement seems enormous.

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Addresses of the authors:

Ph.D. student Amna Tariq, Post Harvest Technology and Food Biochemistry Laboratory, Department of Food Science and Technology, University of Karachi, Karachi-75270, Pakistan.

Prof. Dr. Jehan Ara, Post Harvest Technology and Food Biochemistry Laboratory, Department of Food Science and Technology, University of Karachi, Karachi-75270, Pakistan.

Prof. Dr. Viqar Sultana, Biotechnology and Drug Development Laboratory, Department of Biochemistry, University of Karachi, Karachi-75270, Pakistan.

Prof. Dr. Syed Ehteshamul-Haque, Agricultural Biotechnology and Phyto-pathology Laboratory, Department of Botany, University of Karachi, Karachi-75270, Pakistan.

Dr. Mohammad Athar, California Department of Food and Agriculture, Meadowview Operation Center, 3288 Meadowview Road, Sacramento, CA 95832, USA. E-mail: atariq@cdfa.ca.gov)