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COMPARISON OF THE ANTIOXIDATIVE COMPONENTS OF SOME MARINE MACROALGAE FROM TURKEY

GAMZE YILDIZ*, EGEMEN DERE AND ŞÜKRAN DERE

Uludag University, Science and Arts Faculty, Biology Department, 16059, Bursa, Turkey.
**Corresponding author's e-mail:gamze@uludag.edu.tr*

Abstract

There is an increasing demand for natural antioxidant molecules to replace the synthetic additives currently used in the food industry. Therefore, in this study, the total phenolic contents, total antioxidant capacities (lipid-soluble and water-soluble), total protein contents, vitamin E contents and vitamin C contents of *Ulva rigida* C. Agardh*, Codium tomentosum* Stackhouse*, Gracilaria gracilis* (Stackhouse) M. Steentoft, L.M. Irvine *et* W.F. Farnham and *Sphaerococcus coronopifolius* Stackhouse were determined. According to our data, among the algae studied, the highest levels of bioactive components were found in *U. rigida,* followed by *C. tomentosum, G. gracilis* and *S. coronopifolius*. In particular, *U. rigida* and *C. tomentosum* showed the highest values for all of the parameters studied. Therefore, *U. rigida* and *C. tomentosum* could be considered as species possessing significant natural antioxidant molecules which might be useful for the food industry.

Introduction

Reactive oxygen species (ROS) such as hydrogen peroxide, superoxide radicals, hydroxyl radicals and singled oxygen are physiological metabolites formed by aerobic organisms as a result of the metabolism of oxygen. ROS are unstable and highly reactive. Excessive production of such molecular compounds can cause damage to proteins, lipids, DNA and cell membranes through chain reactions. In the human body, such damage can contribute to a number of diseases, such as atherosclerosis, rheumatoid arthritis, diabetes, muscular dystrophy, pulmonary dysfunction, myocardial infarction, Alzheimer's disease and some types of cancer (Ruberto *et al.,* 2001).

ROS are scavenged by antioxidant molecules such as ascorbate, glutathione and tocopherol and by enzymes such as superoxide dismutase, catalase, ascorbate peroxidase and glutathione reductase (Collen & Davison, 2001). The presence of antioxidant compounds in the diet can help provide protection from ROS-linked diseases. Butylated hydroxyanisol and butylated hydroxytoluene are synthetic antioxidants commonly used for the maintenance of foodstuffs (Safer & Al-Nughamish, 1999). However, in most countries, there are some limitations on the use of synthetic antioxidant compounds in food products due to their side effects (Kabouche *et al.,* 2007). Thus, it is essential to develop and utilize effective natural antioxidant molecules. Over the past several decades, plants and their extracts have been demonstrated to have strong antioxidant activity (Al-Juhaimi & Ghafoor 2011; Kanwal *et al.,* 2011; Sultana *et al*., 2013).

Seaweeds are considered to be an important source of bioactive compounds, as they are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities. Compounds with cytostatic, antiviral, antihelminthic, antifungal, and antibacterial activities have been detected in green, brown and red algae (Ambreen *et al.,* 2012; Newmann *et al.,* 2003). Proteins with anti-oxidative properties, phenolic compounds such as flavonoids, coumarins and tocopherols, and nitrogen-containing compounds including alkaloids, chlorophyll derivates, amino acids and amines, as well as other compounds such as carotenoids, ascorbic acid, glutathione, and uric acid, are powerful antioxidant molecules in macroalgae (Lordan *et al.,* 2011). However, the chemical and nutritional composition of a particular seaweed depends on many factors, including species, geographic origin, environmental and physiological variation (Bocanegra *et al.,* 2009).

The increasing demand for natural antioxidant compounds has led to a search for new sources. Seaweed extracts from Turkey are one of the potential sources, but existing reports on their antioxidant properties are very limited. Therefore, to identify new sources of natural antioxidant molecules, the total phenolic contents, total antioxidant capacities (lipid-soluble and water-soluble), total protein contents, vitamin E contents and vitamin C contents for *Ulva rigida, Codium tomentosum, Gracilaria gracilis* and *Sphaerococcus coronopifolius* were determined in this study.

Materials and Methods

Four seaweed species, including two red algae (*Gracilaria gracilis* (Stackhouse) M. Steentoft, L.M. Irvine *et* W.F. Farnham*, Sphaerococcus coronopifolius* Stackhouse) and two green algae (*Ulva rigida* C. Agardh*, Codium tomentosum* Stackhouse*),* were collected in June 2009 from the south shore of the sea of Marmara in Turkey (40º 22.8'N, 28º 48.0' E) by scuba diving at a depth of 5-10 m. Collected samples were kept in seawater until they arrived at the laboratory. After arrival, the samples were immediately rinsed with tap water and with distilled water to remove epiphyte, salt and dirt particles and then dried at room temperature for 3 days. To minimize the loss of active components, no heat treatment was applied. Dried samples were stored in the dark until analyzed. Dried algal samples (10 g) were extracted with 80% methanol using a Soxhlet apparatus for 24 h. The extraction was repeated three times. The three extracts were combined, and the methanol was evaporated in a rotary vacuum evaporator at 40˚C until the samples were dry. Yields of extracted matter were calculated as the percentage (w/w) of dried algal powder.

The phenolic contents of the crude methanol extracts were measured using Folin-Ciocalteau's method, as described by Taga *et al.,* (1984). A 100 µl aliquot of each sample was mixed with 2 ml of 2% Na_2CO_3 and allowed to stand for 2 min at room temperature. After incubation, 100 µl of 50% Folin-Ciocalteu's phenol reagent was added, and the mixture was combined thoroughly and allowed to stand in the dark for 30 min at room temperature. Absorbance of the mixture was then measured at 720 nm, and total phenolic content was calculated with a Gallic acid (GA) standard and expressed as mg of GA equivalent (GAE) per g dry weight (DW).

The samples were homogenized with hexane and shaken for 1 h at 4˚C in the dark. After centrifugation at 6000 g for 10 min, the supernatant was transferred to new tubes. Samples of hexanic extracts (200 µl) were placed in Eppendorf tubes, dried out and re-dissolved in 200 µl ethanol. These ethanolic solutions were mixed with 1 ml of phosphomolybdenum reagent (32 mM sodium phosphate, 4 mM ammonium molybdate, 0.6 M sulfuric acid), and then, the samples were incubated at 95˚C for 90 min. Finally, the absorbance at 695 nm was measured. Lipid-soluble antioxidant capacity was expressed as mg of α-tocopherol equivalent per g DW (Prieto *et al.,* 1999).

Samples of water extracts (200µl) were mixed with 1ml phosphomolybdenum reagent, and then, the samples were incubated at 95˚C for 90 min. Afterwards, the absorbance at 695 nm was measured. Water-soluble antioxidant capacity was expressed as mg of L-ascorbic acid equivalent per g DW (Prieto *et al.,* 1999).

Vitamin E content was determined using a method described by Prieto *et al.,* (1999). A 0.1 ml hexanic extract of algae was mixed with 1 ml phosphomolybdenum reagent solution and incubated at 37˚C for 90 min with vigorous shaking. The absorbance was then measured at 695 nm. Vitamin E content was expressed as mg of α-tocopherol equivalent per g DW. Ascorbic acid concentrations were determined using the titrimetric Association of Official Analytical Chemists (AOAC) method no. 967.21 using 2, 6-dichlorophenol indophenol as a titrant (Anon., 1990). Vitamin C content was expressed as mg ascorbic acid (AA) per g DW.

Total protein content was determined spectrophotometrically at 595 nm, and concentrations were calculated by comparing the sample measurements with a calibration curve of bovine serum albumin (Bradford, 1976). Total protein content was expressed as mg bovine serum albumin (BSA) per g DW.

The data were presented as the mean ± standard deviation. Data were tested for normality (Kolmogorov-Smirnov test) and subjected to Levene's test to verify the homogeneity of variances among groups. One way analysis of variance (ANOVA) was used to compare all of the parameters among species, and a post-hoc test (Tukey's HSD) was performed when the data revealed significant differences at a level of $p<0.05$. These analyses were carried out with the commercial software program SPSS 11.5. All of the experiments were conducted four times.

Results and Discussion

Extract yield: The antioxidant capacity of a food is usually measured using extracts obtained with aqueous organic solvents (methanol, ethanol, chloroform *etc.*). The extraction yield and the antioxidant activity of the extracts are strongly dependent on the polarity of the solvent (Mohammedi & Atik, 2011). Due to the variation in polarity among antioxidants, there is no one solvent that would be entirely satisfactory for the extraction of all of the antioxidants present in a food (Perez-Jimenez *et al.,* 2008). However, previous studies have reported that methanol is specifically effective in extracting polyphenol (Pinelo *et al.,* 2004), and methanol extracts exhibit the highest antioxidant activity in many seaweed species (Yan *et al.,* 1999). In our lab, seaweeds were extracted with methanol, ethanol, acetone and diethyl ether to select the most suitable solvent for achieving high extraction yields. The highest yield values for all of the studied species were obtained with methanol (data not shown). The high yields obtained by using methanol as a solvent indicate that the compounds found in seaweed extracts were polar. The yields of the total methanolic extracts of four seaweeds are given in Fig. 1. Considerable variations in extraction yield were found among different seaweed species. The highest recorded extraction yield was from *Codium tomentosum* (23.83%), followed by *Sphaerococcus coronopifolius* (17.25%), *Ulva rigida* (11.42%) and *Gracilaria gracilis* (5.79%), successively.

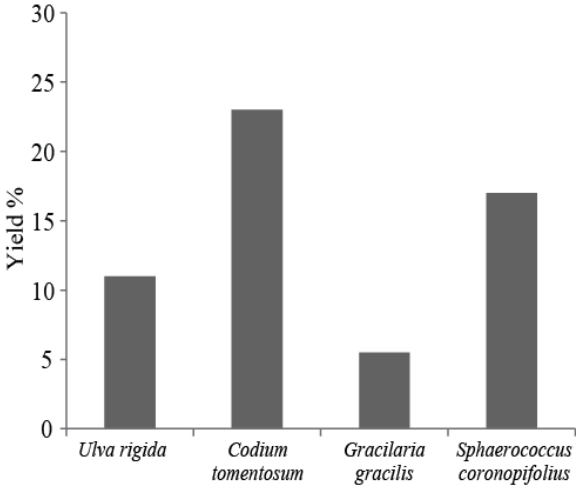

Fig. 1. Yield of total methanolic extract (as % w/w of seaweed on dry weight basis).

Total phenolic content: Using the Folin-Ciocalteau method, the phenolic contents of the seaweeds were investigated and expressed as mg GAE/g DW (Table 1). In this study, the phenolic contents varied among the four species, ranging between 0.70 and 2.26 mg GAE/g DW. The highest recorded phenolic content was from *Codium tomentosum*, followed by *Ulva rigida, Sphaerococcus coronopifolius* and *Gracilaria gracilis,* successively.

Table 1. The contents of bioactive molecule and antioxidant capacity of studied seaweeds.

	Ulva rigida	*Codium tomentosum*	*Gracilaria gracilis*	*Sphaerococcus coronopifolius*
Total phenol (mg GA / g)	1.68 ± 0.29	2.26 ± 0.08	0.70 ± 0.08	1.05 ± 0.03
LSAC (mg α-tocopherol / g)	6.47 ± 0.66	5.24 ± 0.61	1.48 ± 0.22	3.41 ± 0.44
WSAC (mg AA / g)	4.08 ± 1.69	4.68 ± 1.28	0.19 ± 0.03	2.65 ± 0.14
Vitamin E (mg α-tocopherol/g)	3.27 ± 0.22	3.08 ± 0.45	0.77 ± 0.08	0.59 ± 0.16
Vitamin C (mg AA / g)	2.05 ± 0.33	1.38 ± 0.19	0.24 ± 0.01	0.78 ± 0.07
Total protein (mg BSA / g)	4.06 ± 0.11	1.26 ± 0.13	0.11 ± 0.02	0.64 ± 0.05

Mean ± SD based on dry weight, n = 4

Table 1 illustrates that the total phenolic content of *G. gracilis* was significantly lower than that of the other seaweed species ($p<0.05$). The total phenolic contents of *U. rigida* and *C. tomentosum* were found to be nearly 2.5 times greater than that of *G. gracilis*. However, the total phenolic content of *G. gracilis* in our study (0.70 mg GAE/g) was relatively high compared to the value reported by Zhang *et al.,* (2007) (0.10 mg GAE/g). Compared to the other studied species, *S. coronopifolius* exhibited a moderate amount of phenolic content. All four of the studied species exhibited higher total phenolic contents than *Codium fragile* (0.27 mg GAE/g*), Dictyopteris divaricata* (0.96 mg GAE/g) and *Scytosiphon lomentaria* (0.52 mg GAE/g), described by Zhang *et al.,* (2007).

Previous studies have noted that the concentration of polyphenols in seaweeds exhibits seasonal and geographic variations but also varies among different thalli, such as old versus new thalli (Johnson & Mann, 1986). In addition, seasonal studies on the phenolic content of seaweeds have shown that the phenolic content is highest during the summer (Connan *et al.,* 2004). Therefore, we believe that the high phenolic contents of our samples compared to those of previous studies could be due to the collection of the samples in June and the geographical properties of the Sea of Marmara.

Phenolic compounds such as flavonoids, phenolic acids and tannins are considered to be major contributors to the antioxidant capacity of plants. These antioxidants also take part in diverse biological activities, including anti-inflammatory, anti-atherosclerotic and anti-carcinogenic activities (Li *et al.,* 2007). In addition, Celikler *et al.,* (2008, 2009) have reported that *U. rigida* and *C. tomentosum,* which are collected from the Sea of Marmara, are strong antigenotoxic agents in human lymphocytes *In vitro*. In our study, green seaweeds *(U. rigida* & *C. tomentosum)* revealed higher levels of polyphenols than red seaweeds. Green seaweeds are known to contain high levels of water-soluble components such as sulfated polysaccharides and protein (Dere *et al.,* 2003). Therefore, we think that the strong antigenotoxic activity of *U. rigida* and *C. tomentosum* may be due to the high phenolic contents of these species.

Total antioxidant capacity (water and lipid-soluble): The total water-soluble and lipid-soluble antioxidant capacities of the seaweed species are presented in Table 1. The highest total water-soluble antioxidant capacity was found in *C. tomentosum*. However, *C. tomentosum*'s capacity was not significantly different from that of the other green algae, *U. rigida*. Similarly, no significant difference was found between the green algae and the red algae species *S. coronopifolius*. In contrast, *G. gracilis* revealed a significantly lower total water-soluble antioxidant capacity than all of the species that were studied ($p<0.05$).

Codium species are a significant source of sulfated galactans (Farias *et al.,* 2008), which is one reason for the high water-soluble antioxidant capacity of *C. tomentosum*. A high water-soluble antioxidant capacity was also observed for *U. rigida*. The water-soluble polysaccharides from *Ulva* species, called Ulvans, are complex sulfated heteropolysaccharides and represent the major polymeric fraction of the algal cell wall (Lahaye & Robic, 2007). Until recently, these sulfated polysaccharides were largely ignored as sources of antioxidant activity. However, studies over the last several years have revealed that sulfated polysaccharides from a number of seaweeds have significant antioxidant capability (Jiao *et al.,* 2011). Thus, we believe that the high water-soluble antioxidant capacity of *U. rigida* may be related to its ulvans.

The total lipid-soluble antioxidant capacity of *U. rigida* was significantly higher than those of the red seaweeds ($p<0.05$). However, no difference was found between the capacities of *U. rigida* and *C. tomentosum*. As with water-soluble antioxidant capacity, *S. coronopifolius* possessed a moderate level of lipid-soluble antioxidant capacity among the studied species, whereas *G. gracilis* was significantly lower than the other studied seaweeds ($p<0.05$).

The results of this experiment demonstrate that the studied seaweed species contain different levels of phenolic content and possess diverse antioxidant properties. In this study, *U. rigida* and *C. tomentosum* exhibited remarkably high antioxidant capacities and phenolic contents. The positive correlation between the polyphenolic content of algae and its antioxidant activity is well documented (Velioglu *et al.,* 1998). Therefore, we believe that the high antioxidant capacities of *U. rigida* and *C. tomentosum* may be a result of their high phenolic contents.

Vitamin-E and vitamin-C contents: Vitamin E is the major lipid-soluble antioxidant responsible for protecting the polyunsaturated fatty acids in membranes against lipid

peroxidation, free radicals and singlet oxygen species (Machlin & Bendich, 1987). In nature, α-tocopherol is the most common form of vitamin E. It is also the most biologically active form. In this study, we found that the vitamin E contents of *U. rigida* and *C. tomentosum* were remarkable higher than those of the two red seaweeds, a statistically significant difference (3.27 and 3.08 mg tocopherol/g DW, respectively) ($p < 0.05$). High levels of vitamin E in *U. rigida* and *C. tomentosum* are nutritionally important because of vitamin E's potential role in the prevention of heart disease and cancer (Kaur *et al.,* 2007), which is related to its antioxidant properties. Aside from the antioxidant properties of vitamin E, other biological activities have recently been reported, such as the regulation of cellular signaling and gene activity, the modulation of immune function and the induction of apoptosis (Azzi *et al.,* 2002).

Ascorbic acid, also referred to as L-Ascorbic acid or vitamin C, is a water-soluble vitamin whose main use in therapy is to resist infections in cells (Matei & Magearu, 2004). In addition, ascorbic acid has been identified as having important functions in photosynthesis, such as in the protection of the photosynthetic apparatus from the oxygen radicals and H_2O_2 that are formed from the Mehler reaction during photosynthesis. According to our data, *Ulva rigida* has a vitamin C content approximately three times higher in value than those of the other study species, with the exception of *C. tomentosum* (Table 1). The vitamin C content of *U. rigida* was significantly different from the contents of the other species ($p<0.05$). *U. rigida* is a fast-growing alga, and exhibits high photosynthetic performance compared to the other studied species. Because of its high photosynthetic rate, oxygen radicals and H_2O_2 are also produced at a higher rate. Thus, for fast growing species such as *U. rigida,* increases in antioxidants and free radical scavengers are needed to deal with these toxic products of photosynthesis (Asada, 1999). In this case, the result is a relatively high vitamin C content for *U. rigida*.

Total protein: The highest protein content was identified in *U. rigida*. *C. tomentosum, G. gracilis* and *S. coronopifolius* possessed lower total protein contents, with only 1.26 mg BSA/g, 0.11 mg BSA/g and 0.64 mg BSA/g, respectively (Table 1). Yildiz *et al.,* (2009) have claimed that inorganic pollution in seawater has a great impact on the protein contents of some algal species. Therefore, we posit that the high protein content of *U. rigida* may be due to inorganic pollution in the study area. In addition, *U. rigida* is a nitrophilic and opportunistic species (Gordillo *et al.,* 2001). Opportunistic species often have high nitrogen incorporation rates (Perez-Mayorga *et al.,* 2011) compared to other, more specialized, algae (Pedersen & Borum, 1997). *Ulva* species are characterized by a simple thallus with a high surface-area–to-volume ratio (Rosenberg & Ramus, 1984), allowing for a rapid and efficient response to changes in ambient conditions such as nutrient inputs, resulting in high rates of growth and nutrient uptake (Pedersen & Borum, 1997), which most likely includes protein uptake.

Conclusions

In this study, the biologically active compounds were compared between four seaweeds, including two green and two red algae. According to our data, among the studied seaweeds, the highest levels of bioactive components were found in *U. rigida*, followed by *C. tomentosum*, *G. gracilis* and *S. coronopifolius*, respectively. Green seaweeds possessed significantly higher values than red seaweeds for all of the studied parameters. In practice, this study suggests that methanolic extracts of *U. rigida* and *C. tomentosum* could be utilized as natural sources of antioxidants. Aside from the direct health benefits, antioxidants from natural sources combat the lipid oxidation of foods, especially during processing and storage. Moreover, *U. rigida* and *C. tomentosum* are easily accessible sources of natural antioxidant molecules for use in food supplements or in pharmaceutical applications. However, no compositional analysis of these extracts was performed. The results of this study could be utilized by other researchers in future investigations of the characterization of the biologically active molecules that are responsible for the antioxidant activity in *U. rigida* and *C. tomentosum*.

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References

Al-Juhaimi, F. and K. Ghafoor. 2011. Total phenols and antioxidant activities of leaf and steam extracts from coriander, mint and parsley grown in Saudi Arabia. *Pak. J. Bot.,* 43(4): 2235-2237.

Ambreen, K. Hira, A. Tarıq, Ruqqia, V. Sultana and J. Ara. 2012. Evaluation of biochemical component and antimicrobial activity of some seaweeds occuring at Karachi coast. *Pak. J. Bot.,* 44(5): 1799-1803.

Anonymous. 1990. Official Methods of Analysis of the Association of Official Analytical Chemists. Williams S, (Ed) 15th ed. Arlington, Virginia.

Asada, K. 1999. The water-water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. *A. Rev. Plant Physiol. Plant Mol. Biol.*, 50: 601-639.

Azzi, A., R. Ricciarelli and J.M. Zingg. 2002. Non-antioxidant molecular functions of alpha-tocopherol (vitamin E). *FEBS Letters,* 22: 8-10.

Bocanegra, A., S. Bastida, J. Benedi, S. Rodenas and F.J. Sanchez-Muniz. 2009. Characteristics and nutritional and cardiovascular-health properties of seaweeds. *J. Med. Food.,* 12: 236-258.

Bradford, M. 1976. A rapid and sensitive method for the quantification of micrograms quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.,* 72: 248-54.

Celikler, S., G. Yildiz, O. Vatan and R. Bilaloglu. 2008. *In vitro* antigenotoxicity of *Ulva rigida* C. Agardh (Chlorophyceae) extract against induction of chromosome aberration, sister chromatid exchange and micronuclei by mutagenic agent MMC. *Biomed. Envir. Sci.,* 21: 492-498.

Celikler, S., O. Vatan, G. Yildiz and R. Bilaloglu. 2009. Evaluation of anti-oxidative, genotoxic and antigenotoxic potency of Codium tomentosum Stackhouse ethanolic extract in human lymphocytes *In vitro*. *Food and Chem. Toxicol.*, 47: 796-801.

Collen, J and I.R. Davison. 2001. Seasonality and thermal acclimation of reactive oxygen metabolism in *Fucus vesiculosus* (Phaeophyceae). *J. Phycol.*, 37: 474-481.

Connan, S., F. Goulard, V. Stiger, E. Deslandes and E. Ar Gall. 2004. Interspecific and temporal variation in phlorotannin levels in an assemblage of brown algae. *Bot. Mar.*, 47: 410-416.

Dere, Ş., N. Dalkiran, D. Karacaoglu, G. Yildiz and E. Dere. 2003. The Determination of total protein, total soluble carbohydrate and pigment contents of some macroalgae collected from Gemlik-Karacaali (Bursa) and Erdek-Ormanlı (Balıkesir) in the Sea of Marmara, Turkey. *Oceanologia.,* 45(3): 453-471.

Farias, E.H.C., V.H. Pomin, A.P. Valente, H.B. Nader, H.A.O. Rocha and P.A.S. Mourao. 2008. A prepanderently 4-sulfated, 3 linked galactan from the gren algae *Codium isthmacladum*. *Glycobiology.,* 18: 250-259.

Gordillo, F.J.L., F.X. Niell and F.L. Figueroa. 2001. Nonphotosynthetic enhancement of growth by high CO_2 level in the nitrophilic seaweed *Ulva rigida* C. Agardh (Chlorophyta). *Planta.,* 213: 64-70.

Jiao, G., G. Yu, J. Zhang and S. Ewart. 2011. Chemical structures and bioactives of sulfated polysaccharides from marine algae. *Mar. Drugs.,* 9: 196-223.

Johnson, C.R. and K.H. Mann. 1986. The importance of plant defence abilities to the structure of subtidal seaweed communities: the kelp *Laminaria longicruris* de la Pylaie survives grazing by the snail *Lacuna vincta* (Montagu) at high population densities. *J. Exp. Mar. Biol. Ecol.,* 97: 231-267.

Kabouche, A., Z. Kabouche, M. Ozturk, U. Kolak and G. Topçu. 2007. Antioxidant abietane diterpenoids from *Salvia barrelieri*. *Food Chem.,* 102: 1281-1287.

Kanwal, S., N. Ullah, Ihsan-Ul-Haq, I., Afzal and B. Mirza. 2011. Antioxidant, antitumor activities and phytochemical investigation of Hedera nepalensis K.Koch, an important medicinal plant from Pakistan. *Pak. J. Bot.,* 43: 85-89.

Kaur, C., K. Kumar, D. Anil and H.C. Kapoor. 2007. Variations in antioxidant activity in Broccoli (*Brassica oleracea* L.) cultivars. *J. Food Biochem.,* 31: 621-638.

Lahaye, M. and A. Robic. 2007. Structute and functional properties of ulvan, a polysaccharide from gren seaweeds. *Biomacromolecules,* 8: 1765-1774.

Li, H.B., K.W. Cheng, C.C. Wong, K.W. Fan, F. Chen and Y. Jiang. 2007. Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. *Food Chem.,* 102: 771-776.

Lordan, S., R.P. Ross and C. Stanton. 2011. Marine Bioactives as Functional Food Ingredients: Potential to Reduce the Incidence of Chronic Diseases. *Mar. Drugs,* 9: 1056-1100.

Machlin, L.J. and A. Bendich. 1987. Free radical tissue damage: protective role of antioxidant nutrients. *FASEB.,* 1: 441-445.

Matei, N. and V. Magearu. 2004. Determination of vitamin C from some natural products preserved under different storage conditions. Analele Universităţii din Bucureşti, *Chimie, Anul XIII (serie nouă),* vol. I-II: 65-68.

Mohammedi, Z. and F. Atık. 2011. Impact of solvent extraction type on total polyphenols content and biological activity from *Tamarix aphylla* (L.) Karst. *Int. J. Pharm. Bio. Sci.,* 2(1): 609-615.

Newmann, D.J., G.M. Cragg and K.M. Snader. 2003. Natrural products as source of new drugs over the period 1981-2002. *J. Nat. Prod.,* 66: 1022-1037.

Pedersen, M.F. and J. Borum. 1997. Nutrient control of estuarine macroalgae: growth strategy and the balance between nitrogen requirements and uptake. *Mar. Ecol. Prog. Ser.,* 161: 155-163.

Perez-Jimenez, J., S. Arranz, M. Tabernero, M.E. Diaz-Rubio, J. Serrano, I. Gonı and F. Saura-Calixto. 2008. Updated morphology to determine antioxidant capacity in plant foods, oils and beverages: Extraction, measurement and expression of results. *Food Res. Int.,* 41: 274-285.

Perez-Mayorga, D.M., L.B. Ladah, J.A. Zertuche-Gonzalez, J.J. Leichter, A.E. Filonov and M.F. Lavin. 2011. Nitrogen uptake and growth by opportunistic macroalga *Ulva lactuca* (Linnaeus) during the internal tide. *J. Exp. Mar. Biol and Ecol.,* 406: 108-115.

Pinelo, M., L. Manzocco, M.J. Nunez and M.C. Nicoli. 2004. Solvent effect on quercetin antioxidant capacity. *Food Chem.,* 88: 201-207.

Prieto, P., M. Pineda and M. Aguilar. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin-E. *Anal. Biochem.,* 269: 337-341.

Rosenberg, G. and J. Ramus. 1984. Uptake of inorganic nitrogen and seaweed surface area: volume ratios. *Aquat. Bot.,* 19: 65-72.

Ruberto, G., M.T. Barata, D.M. Biondi and V. Amico. 2001. Antioxidant activity of extracts of the marine algal genus *Cystoseira* in a micellar model system. *J. Appl. Phycol.,* 13: 403-407.

Safer, A.M. and Al-Nughamish. 1999. Hepatotoxicity induced by the antioxidant food additive butylated hydroxytoluene (BHT) in rats: an electron microscopical study. *Histol. Histopathol.,* 14: 391-406.

Sultana, B., Z. Hussaim, M. Hameed and M. Mushtaq. 2013. Antioxidant activity among different parts of Aubergine (*Solanum melongena* L.). *Pak. J. Bot*., 45(4): 1443-1448.

Taga, M.S., E.E. Miller and D.E. Pratt. 1984. Chia seeds as a source of natural lipid antioxidants. *JAOCS,* 61: 928-931.

Velioglu, Y.S., G. Mazza, L. Gao and B.D. Oomah. 1998. Antioxidant activity and total phenolic in selected fruits, vegetables and grain products. *J. Agr. Food Chem.,* 46: 4113-4117.

Yan, X., Y. Chuda, M. Suzuki and T. Nagata. 1999. Fucoxanthin as the major antoxidant in *Hijikia fusiformis*, a common edible seaweed. *Biosci. Biotechnol. Biochem.,* 63: 605-607

Yildiz, G., Ş. Dere and E. Dere. 2009. The Effect of Environmental Factors on Some Nutritional Contents of *Ulva rigida* C. Agardh. *Int. J. Phycol. Phycochem.,* 5: 1-6.

Zhang, W.W., X.J. Duan, H.L. Huang, Y. Zhang and B.G. Wang. 2007. Evaluation of 28 marine algae from the Quingdao coast for antioxidative capacity and determination of antioxidant efficiency and total phenolic content of fractions and subfractions derived from *Symphyocladia latiuscula* (Rhodomelaceae). *J. Appl. Phycol.,* 19: 97-108.

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