COMPARISON OF THE ANTIOXIDATIVE COMPONENTS OF SOME MARINE MACROALGAE FROM TURKEY

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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, antihelmintic, 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 (40° 22.8’N, 28° 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% Na₂CO₃ and allowed to stand for 2 min at room temperature. After incubation, 100 µl of 50% Folin-Ciocalteau’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) equivalent 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 (Pinelo 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).](image)

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.
than studied species exhibited higher total phenolic contents and moderate amount of phenolic content. All four of the polyphenols than red seaweeds. Green seaweeds are may be due to the high phenolic contents of these species. Antigenotoxic activity of (Dere 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 (Conan 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

<table>
<thead>
<tr>
<th></th>
<th>Ulva</th>
<th>Codium tomentosum</th>
<th>Gracilaria gracilis</th>
<th>Sphaerococcus coronopifolius</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total phenol (mg GA / g)</td>
<td>1.68 ± 0.29</td>
<td>2.26 ± 0.08</td>
<td>0.70 ± 0.08</td>
<td>1.05 ± 0.03</td>
</tr>
<tr>
<td>LSAC (mg α-tocopherol / g)</td>
<td>6.47 ± 0.66</td>
<td>5.24 ± 0.61</td>
<td>1.48 ± 0.22</td>
<td>3.41 ± 0.44</td>
</tr>
<tr>
<td>WSAC (mg AA / g)</td>
<td>4.08 ± 1.69</td>
<td>4.68 ± 1.28</td>
<td>0.19 ± 0.03</td>
<td>2.65 ± 0.14</td>
</tr>
<tr>
<td>Vitamin E (mg α-tocopherol/g)</td>
<td>3.27 ± 0.22</td>
<td>3.08 ± 0.45</td>
<td>0.77 ± 0.08</td>
<td>0.59 ± 0.16</td>
</tr>
<tr>
<td>Vitamin C (mg AA / g)</td>
<td>2.05 ± 0.33</td>
<td>1.38 ± 0.19</td>
<td>0.24 ± 0.01</td>
<td>0.78 ± 0.07</td>
</tr>
<tr>
<td>Total protein (mg BSA / g)</td>
<td>4.06 ± 0.11</td>
<td>1.26 ± 0.13</td>
<td>0.11 ± 0.02</td>
<td>0.64 ± 0.05</td>
</tr>
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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).
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 H2O2 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 H2O2 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**


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