International Journal of Food Properties, 15:1182–1189, 2012

ISSN: 1094-2912 print / 1532-2386 online
DOI: 10.1080/10942912.2010.517341

Taylor & Francis
Taylor & Francis Group

DETERMINATION OF THE ANTI-OXIDATIVE CAPACITY AND BIOACTIVE COMPOUNDS IN GREEN SEAWEED *ULVA RIGIDA* C. AGARDH

Gamze Yildiz, Serap Celikler, Ozgur Vatan, and Sukran Dere
Biology Department, Science and Arts Faculty, Uludag University, Bursa, Turkey

There is an increasing demand for natural antioxidant molecules in order to replace the synthetic additives in the food industry. Therefore,* Ulva rigida *C. Agardh was analyzed to determine its bioactive components, including the total phenolic content, antioxidant capacity (lipid and water-soluble), vitamins (A, E, and C), protein, carbohydrate, and pigments. As a result,* Ulva rigida *showed a high total phenolic, vitamin E, and total carotene content. Hence,* U. rigida *could be considered as a plant possessing natural antioxidant molecules and might be useful for the food industry.* U. rigida *can also be used for curing diseases arising from oxidative deterioration.

Keywords: *Antioxidant molecules, Pigments, Total phenolic content,* Ulva rigida*, Vitamins.*

INTRODUCTION

Reactive oxygen species (ROS), such as hydrogen peroxide, superoxide radicals, hydroxyl radicals, and singled oxygen, are physiological metabolites formed during aerobic life as a result of the metabolism of oxygen. ROS are toxic to cells. Excessive production of such molecular compounds can cause damage to proteins, lipids, DNA, and cell membranes. This damage can induce different kinds of diseases in the human body, such as atherosclerosis, rheumatoid arthritis, diabetes, muscular dystrophy, pulmonary dysfunction, mycocardial infarction, Alzheimer's disease, and some types of cancer.[1]

ROS is scavenged by antioxidant molecules, such as ascorbate, glutathione, and tocopherol, and by enzymes, such as superoxide dismutase, catalase, ascorbate peroxidase, and glutathione reductase.[2] The antioxidant molecules are essential for the body system. Many researchers have reported that plant-derived antioxidants have a beneficial effect on human health.[3,4] The presence of antioxidant compounds in the human diet can be helpful to protect us from these diseases. Over the past several decades, seaweeds and their extracts have been demonstrated to have strong antioxidant activity and they have been studied to produce antioxidant compounds.[5]

The increasing demand for natural antioxidant compounds results in the search for new sources of natural antioxidant molecules. Furthermore, *Ulva rigida* is used for human nutrition. Because of the potential benefits listed above, there is a great interest in studying and determining the amount of antioxidant components in this seaweed. In addition, reports

Received 20 May 2010; accepted 15 August 2010.
Address correspondence to Gamze Yildiz, Biology Department, Science and Arts Faculty, Uludag University, Bursa 16059, Turkey. E-mail: gamze@uludag.edu.tr

on the antioxidant properties of seaweed extracts from Turkey are very limited. Therefore, in this study total phenolic content, total antioxidant capacity (in soluble of lipid and water), total protein, total carbohydrate, vitamins (A, C, and E), and pigments (chlorophyll-a, chlorophyll-b, and carotene) contents were determined in order to identify new sources of natural antioxidant molecules.

MATERIALS AND METHODS

Collection of Samples

Ulva rigida was freshly collected from the Marmara Sea coast of Turkey during November 2007. Samples were kept in seawater until they arrived at the laboratory. The samples were immediately washed with top and distilled water to remove epiphytes, salt, and dirty particles after arrival. The clean algae were frozen and stored at -20°C. For the analyses, the frozen samples were used.

Chemicals

Folin-Ciocalteau reagent (F-9252), gallic acid (G-7384), and alfa-tocopherol (T-3251), L-ascorbic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). 2,6-Dichlorophenol indophenol, trichloroacetic acid were obtained from Merck (Darmstadt, Germany). All other solvents and chemicals were of analytical grade.

Total Phenolic Content

Frozen samples were extracted with methanol for the total phenol analysis. Phenolic contents were measured using Folin Ciocalteu's method as described by Taga et al.[6] An aliquot of 100 μl of the 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 reaction mixture was mixed thoroughly and allowed to stand for 30 min at room temperature in the dark. The absorbance was measured at 720 nm and total phenolic content was calculated with a Gallic acid standard and expressed as mg Gallic acid equivalent per 100 gram of fresh tissue.

Determination of Lipid-Soluble Antioxidant Capacity

Frozen 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 the same volume of ethanol. These ethanolic solutions were supplemented with 1 ml of phosphomolybdenum reagent (32 mM sodium phosphate, 4 mM ammonium molybdate, 0.6 M sulfuric acid) and incubated at 95°C for 90 min. Finally, the absorbance at 695 nm was measured. Lipid-soluble antioxidant capacity was expressed as equivalents of α-tocopherol in micromoles of α-tocopherol per gram of fresh tissue.[7]

Determination of Water-Soluble Antioxidant Capacity

Frozen samples of water extracts (200 μl) were supplemented with 1 ml of phosphomolybdenum reagent and incubated at 95°C for 90 min. Finally, the absorbance at

695 nm was measured. Water-soluble antioxidant capacity was expressed as the equivalent of L-ascorbic acid in micromoles of L-ascorbic acid per gram of fresh tissue.[7]

Determination of Vitamins E, C, and A

Vitamin E content was determined by using a method described by Prieto et al.[7] Hexanic extract of frozen algal tissues (0.1 ml) was mixed with 1 ml of phosphomolybdenum reagent solution and incubated at 37°C for 90 min with vigorous shaking. The absorbance was measured at 695 nm. Vitamin E content was expressed as α-tocopherol equivalents per 100 grams of fresh tissue. Ascorbic acid concentrations were determined by the titrimetic Association of Official Analytical Chemists (AOAC) method No. 967.21. Frozen samples were homogenized with metaphosphoric acid solution and then centrifuged at 6000 g for 10 min. Supernatants were titrated with 2,6-dichlorophenol indophenol as a titrant.[8] Vitamin C content was expressed as mg L-ascorbic acid per 100 grams of fresh tissue. Vitamin A content was determined and calculated by using a method described by Rutkowski and Grzegorczyk.[9] Frozen samples were extracted with hexane. An aliquot of 1 ml of hexanic extract and 1 ml of KOH solutions were mixed in a vortex for 1 min and incubated in a water bath at 60°C for 20 min. Cooled samples were mixed with 1 ml of xylene and centrifuged at 1500 g for 10 min. Absorbances of the organic phase were determined both before and after irration with UV light at 335 nm against xylene.

Determination of Total Soluble Carbohydrate and Protein

Total soluble carbohydrate was assayed by the anthrone-sulphuric acid method, which involved extraction with 15% trichloroacetic acid.[10] The absorbance was measured at 620 nm.

Total protein content was determined spectrophotometrically at 595 nm and concentrations were calculated by comparing with a calibration curve of bovine serum albumin.[11]

Determination of Pigments (Chlorophyll-*a*, Chlorophyll-*b*, and Total Carotene)

Chl *a* and Chl *b* contents for *Chlorophyta* were determined in accordance with the Jeffrey and Humphrey method by using 90% acetone as solvent.[12] Car was determined according to the Lichtentaler and Wellburn method by using 90% acetone.[13]

Statistical Analysis

Three samples were prepared for each experiment. The data were presented as mean $\pm$ standard deviation.

RESULTS AND DISCUSSION

Total Phenolic Content

Using the Folin Ciocalteu method phenolic content of *Ulva rigida* was investigated and expressed as mg gallic acid 100 g^{-1} FW (Table 1). Phenolic compounds are

Table 1 The contents of bioactive molecules and antioxidant capacity of *Ulva rigida* based on fresh weight (Mean ± SD), ($n = 3$).

	Ulva rigida C. Agardh
CALT (μmol α-tocopherol g^{-1})	130.91 ± 24.56
CAHT (μmol L-ascorbic acid g^{-1})	375.59 ± 61.63
Vitamin E (mg α-tocopherol 100 g^{-1})	147.00 ± 0.28
Vitamin A (μM)	0.91 ± 0.47
Vitamin C (mg L-ascorbic acid 100 g^{-1})	46.00 ± 0.17
Total phenol (mg gallic acid 100 g^{-1})	73.00 ± 0.13
Total protein (%)	52.33 ± 4.51
Total carbohydrate (%)	34.98 ± 20.39
Chlorophyll-a (mg 100 g^{-1})	51.13 ± 25.69
Chlorophyll-b (mg 100 g^{-1})	43.40 ± 19.32
Total carotene (mg 100 g^{-1})	9.67 ± 3.18

CAHT: Water soluble antioxidant capacity; CALT: lipid soluble antioxidant capacity.

Table 2 Total phenolic content of *Ulva rigida*[a] and of some other seaweeds[b].

Seaweeds	Total phenolic (mg gallic acid g^{-1})
Ulva rigida	0.73 ± 0.13
Codium fragile	0.27 ± 0.02
Dictyopteris divaricata	0.96 ± 0.01
Scytosiphon lomentaria	0.52 ± 0.01
Gracilaria gracilis	0.10 ± 0.00
Ceramium kondoi	0.44 ± 0.01

[a]Mean values, $n = 3$, fresh weight basis.
[b]*Source*: Zhang et al.[15] Values are based on dry weight.

commonly found in plants and have been reported to have several biological activities including the antioxidant activity. The major part of antioxidant molecules are polyphenolic compounds.[14] Therefore, a number of studies have focused on the biological activities of phenolic compounds.

In this study, the green algae *Ulva rigida* was found to contain a total phenolic compound of 73 mg gallic acid 100 g^{-1} FW. This value was compared with the corresponding data for several seaweeds, which was reported by Zhang et al.[15] They are also included in Table 2. Table 2 shows that the amount of total phenolic content of *U. rigida* was relatively higher than other seaweeds. Phenolic compounds have been highly prized for their important dietary roles as antioxidant molecules and chemo-preventive agents.[16] Celikler et al. have also reported that *Ulva rigida* have strong antigenotoxic activity in human lymphocytes *in vitro*[17] and antihyperglicemic effect in *in vivo*.[18]

Total Antioxidant Capacity (Water and Lipid-Soluble)

Total water-soluble and lipid-soluble antioxidant capacity of *U. rigida* is presented in Table 1. Mohamed et al.[19] observed the highest levels of water-soluble antioxidant capacity of 277.7 μmol L-ascorbic acid g^{-1} in wheat germ and the highest level of lipid-soluble antioxidant capacity of 118.5 μmol α-tocopherol g^{-1} in chili pepper seeds. Total water and lipid-soluble antioxidant capacity of *U. rigida* (375.59 μmol L-ascorbic acid g^{-1} FW and 130.91 μmol α-tocopherol g^{-1} FW, respectively) is relatively higher

when compared with these plants. The positive correlation between polyphenolic content of algae and its antioxidant activity is well documented.[20] In this study, *U. rigida* showed a remarkable antioxidant capacity. Therefore, we think that this might be a result of its high polyphenolic content.

Vitamin E, C, and A 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.[21] In addition, α-tocopherol is the most common form of Vitamin E present in nature and it is the most biologically active form. Ching and Mohamed[22] investigated α-tocopherol content in 62 edible tropical plants. In their study, the highest α-tocopherol content was found to be 79.65 mg α-tocopherol 100 g^{-1} in *Sauropus androgynus*. According to our data, *Ulva rigida* has a remarkably higher value (147 mg α-tocopherol 100 g^{-1} FW) than that of *Sauropus androgynus*. High content of Vitamin E in *U. rigida* is important because of its potential role in the prevention of heart disease and cancer.[23]

Except for antioxidant properties of Vitamin E, recently other biological activities have been reported, such as the regulation of cellular signaling and gene activity, modulation of immune function, and induction of apoptosis.[24] Ascorbic acid, also referred to as L-ascorbic acid or vitamin C, is a water-soluble vitamin and it is largely used in therapy for anti-infections in cells.[25] The content of vitamin C in *U. rigida* is 46 mg of ascorbic acid 100 g^{-1} FW and this value is higher than the values in *Gracilaria changgi*[26] and some of the consumable vegetables.[27] The content of vitamin A in *U. rigida* (0.91 μM) is presented in Table 1. Vitamin A or retinol is an essential nutrient for humans and animals since it cannot be synthesized within the body. A deficiency of vitamin A can be lead to a number of health problems.

Total Carbohydrate and Protein Contents

Marine plants characteristically contain sulfated polysaccharides that are not found in land plants. In recent years, sulfated polysaccharides from marine algae have been reported to have antioxidant activity.[28] Their activity depends on several structural parameters, such as the degree of sulfation, the molecular weight, and the sulfation positions. In this study, *U. rigida* showed high carbohydrate content (Table 1). However, Ortiz et al.[29] found that carbohydrate content of *U. lactuca* was 61.5%, which is higher than our result (34.98%). The mean protein content found in this study is in agreement with values reported for various macroalgae.[30] *U. rigida* showed a high protein content (52.33%) similar to traditional high protein plant sources.[26,29] This high protein content of *U. rigida* makes up a valuable source for human nutrition.

Pigments (Chlorophyll-a, Chlorophyll-b, and Total Carotene)

Chlorophyll-a is the main green pigment found in most algae. It is one of the significant biomolecules that is lately being studied for its antioxidant properties. Its absorption by human intestinal cells supports its potential importance for human health.[31] The second kind of chlorophyll is chlorophyll-b, which occurs only in green algae and the plants.

U. rigida also contains excessive levels of both chlorophyll-a (51.13 mg 100 g^{-1} FW) and chlorophyll-b (43.40 mg 100 g^{-1} FW, Table 1).

Carotene, especially β-carotene, is an important nutrient with provitamin A value. The protective role of carotenoids is based on the effective quenching and prevention of singlet oxygen formation. In our study, *U. rigida* showed higher total carotene content (9.67 mg 100 g^{-1} FW) when it is compared with green chilies (2410 μg 100 g^{-1}), tomato (3090 μg 100 g^{-1}), and maize (1782 μg 100 g^{-1}).[32] Many investigators have studied the relation between dietary carotenoid intake and health. Anti-ageing effects of carotenoids were also demonstrated by Cutler.[33]

CONCLUSIONS

Ulva rigida, a green algae found in Turkey, was analyzed in terms of its bioactive components. In this study, the nutritional value and biologically active compounds were compared with the values previously found in several plants. According to this study, it can be concluded that seaweeds can be utilized as a source of natural antioxidant compounds. They can enhance the antioxidant defense system of the human body. The present study appears to be useful for leading the development of therapeutic products to protect people against certain diseases. The results of this study may be well used by other researchers to analyze the characterization of the biologically active molecules that are responsible for the antioxidant activity in *U. rigida*.

ACKNOWLEDGMENTS

This investigation was supported by a grant from The Scientific and Technical Research Council of Turkey, Ankara (TUBITAK, project no. 107T279 (TBAG/HD-304).

REFERENCES

1. Ruberto, G.; Barata, M.T.; Biondi, D.M.; Amico, V. Antioxidant activity of extracts of the marine algal genus *Cystoseira* in a micellar model system. Journal of Applied Phycology **2001**, *13*, 403–407.
2. Collen, J.; Davison, I.R. Seasonality and thermal acclimation of reactive oxygen metabolism in *Fucus vesiculosus* (Phaeophyceae). Journal of Phycology **2001**, *37*, 474–481.
3. Gungor, N.; Sengul, M. Antioxidant activity, total phenolic content and selected physicochemical properties of white mulberry (*Morus alba* L.) fruits. International Journal of Food Properties **2008**, *11*, 44–52.
4. Okmen, B.; Sigva, H.O.; Mutlu, S.; Doganlar, S.; Yemenicioglu, A.; Frary, A. Total antioxidant activity and total phenolic contents in different Turkish eggplant (*Solanum melongena* L.) cultivars. International Journal of Food Properties **2009**, *12*, 616–624.
5. Yuan, Y.V.; Walsh, N.A. Antioxidant and antiproliferative activities of extracts from a variety of edible seaweeds. Food and Chemical Toxicology **2006**, *44*, 1144–1150.
6. Taga, M.S.; Miller, E.E.; Pratt, D.E. Chia seeds as a source of natural lipid antioxidants. Journal of American Oil Chemistry Society **1984**, *61*, 928–931.
7. Prieto, P.; Pineda, M.; Aguilar, M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Analytical Biochemistry **1999**, *269*, 337–341.
8. AOAC. *Official Methods of Analysis*, 16th Ed.; Association of Official Analytical Chemistry: Arlington, VA, 1995.

9. Rutkowski, M.; Grzegorzczyk, K.; Gendek, E.; Kedziora, J. Laboratory convenient modification of Bessey method for vitamin A determination in blood plasma. Journal of Physiology and Pharmacology **2006**, *57* (suppl. 2), 221.
10. Laurentin, A.; Edwards, C.A. A microtiter modification of the Anthrone-sulphuric acid colorimetric assay for glucose-based carbohydrates. Analytical Biochemistry **2003**, *315*, 143–145.
11. Bradford, M. A rapid and sensitive method for the quantification of micrograms quantities of protein utilizing the principle of protein-dye binding. Analaytical Biochemistry **1976**, *72*, 248–254.
12. Jeffrey, S.W.; Humprey, G.F. New spectrophometric equations for determining chlorophyll a, b, c_1, and c_2 in higher plants, algae and natural populations. Biochemie und Physiologie der Pflanzen **1975**, *167*, 191–194.
13. Lichtenthaler, H.K.; Wellburn, A.R. Determination of total carotenoids and chlorophylls a and b of leaf in different solvents. Biochemical Society Transactions **1983**, *11*, 591–592.
14. Cao, G.; Sofic, E.; Prior, R.L. Antioxidant and pro-oxidant behavior of flavanoids: Structure-activity relationship. Free Radical Biology and Medicine **1997**, *22*, 749–760.
15. Zhang, W.W.; Duan, X.J.; Huang, H.L.; Zhang, Y.; Wang, BG. 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). Journal of Applied Phycology **2007**, *19*, 97–108.
16. Bravo, L. Polyphenols: Chemistry, dietary source, metabolism, and nutritional significance. Nutrition Reviews **1998**, *56*, 317–333.
17. Celikler, S.; Yildiz, G.; Vatan, O.; Bilaloglu, R. *In vitro* antigenotoxicity of *Ulva rigida* C.Agardh (Chlorophyceae) extract against induction of chromosome aberration, sister chromatid exchange and micronuclei by mutagenic agent MMC. Biomedical and Environmental Sciences **2008**, *21*, 492–498.
18. Celikler, S.; Tas, S.; Vatan, O.; Ziyanok-Ayvalik, S.; Yildiz, G.; Bilaloglu, R. Anti-hyperglycemic and antigenotoxic potential of *Ulva rigida* ethanolic extract in the experimental diabetes mellitus. Food and Chemical Toxicology **2009**, *47*, 1837–1840.
19. Mohamed, R.; Pineda, M.; Aguilar, M. Antioxidant capacity of extracts from wild and crop plants of the Mediterranean region. Journal of Food Science **2007**, *72*, 59–63.
20. Velioğlu, Y.S.; Mazza, G.; Gao, L.; Oomah, B.D. Antioxidant activity and total phenolic in selected fruits, vegetables and grain products. Journal of Agricultural and Food Chemistry **1998**, *46*, 4113–4117.
21. Machlin, L.J.; Bendich, A. Free radical tissue damage: Protective role of antioxidant nutrients. Federation of American Societies for Experimental Biology **1987**, *1*, 441–445.
22. Ching, L.S.; Mohamed, S. Alpha-tocopherol content in 62 edible tropical plants. Journal of Agricultural Food Chemistry **2001**, *49*, 3101–3105.
23. Kaur, C.; Kumar, K.; Anil, D.; Kapoor, H.C. Variations in antioxidant activity in broccoli (*Brassica oleracea* L.) cultivars. Journal of Food Biochemistry **2007**, *31*, 621–638.
24. Azzi, A.; Ricciarelli, R.; Zingg, J.M. Non-antioxidant molecular functions of alpha-tocopherol (vitamin E). FEBS Letters **2002**, *22*, 8–10.
25. Matei, N.; Magearu, V. Determination of vitamin C from some natural products preserved under different storage conditions. Analele Universităţii din Bucureşti–Chimie, Anul XIII (serie nouă), **2004**, *I–II*, 65–68.
26. Norziah, M.H.; Ching, C.Y. Nutritional composition of edible seaweed *Gracilaria changgi*. Food Chemistry **2000**, *68*, 69–76.
27. Tee, E.S.; Mohd Ismail, N.; Mohd Nasir, A.; Khatijah, I. Nutrient composition of Malaysian foods. Kuala Lumpur: ASEAN Sub-Committee on Protein, Food Habits Research and Development, 1988.
28. Qi, H.; Zhang, Q.; Zhao, T.; Hu, R.; Zhang, K.; Li, Z. In vitro antioxidant activity of acetylated and benzoylated derivatives of polysaccharide extracted from *Ulva pertusa* (Chlorophyta). Bioorganic & Medicinal Chemistry Letters **2006**, *16*, 2441–2445.

29. Ortiz, J.; Romero, N.; Robert, P.; Aaya, J.; Lopez-Hernandez, J.; Bozzo, C.; Navarrete, E.; Osorio, A.; Rios, A. Dietary fiber, amino acid, fatty acid and tocopherol contents of the edible seaweeds *Ulva lactuca* and *Durvillaea antarctica*. Food Chemistry **2006**, *99*, 98–104.
30. Yildiz, G.; Dere, E.; Dere, Ş.; Ünübol, H. Determination of the biochemical composition of *Enteromorpha* from Marmara Sea. International Journal of Phycology and Phycochemistry **2008**, *4* (1), 1–4.
31. Ferruzzi, M.L.; Failla, M.L.; Schwartz, S.J. Assessment of degradation and intestinal cell uptake of carotenoids and chlorophyll derivatives from spinach puree using an in vitro digestion and caco-2 human cell model. Journal of Agricultural and Food Chemistry **2001**, *49*, 2082–2089.
32. Kandlakunta, B.; Rajendran, A.; Thingnganing, L. Carotene content of some common (cereals, pulses, vegetables, spices and condiments) and unconventional sources of plant origin. Food Chemistry **2008**, *106*, 85–89.
33. Cutler, R.G. Carotenoids and retinal: Their possible importance in determining longevity of prunate species. Proceedings of the National Academy of Sciences of the United States of the America **1984**, *81*, 7627–7631.