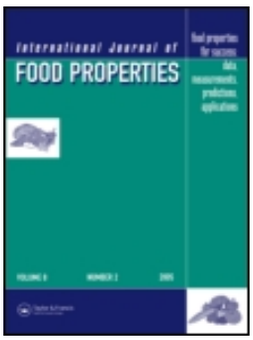

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DETERMINATION OF THE PHENOLIC COMPOUNDS AND ANTIOXIDATIVE CAPACITY IN RED ALGAE *GRACILARIA BURSA-PASTORIS*

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There is an increasing demand for natural antioxidant molecules in order to replace the synthetic additives in the food industry.* Gracilaria bursa-pastoris *(Gmelin) Silva was analyzed to determine its bioactive components including; the total phenolic content, total antioxidant capacity (lipid and water-soluble), vitamins (A, E and C), total protein and total carbohydrate content. In addition, the bioactive components of* Gracilaria bursa-pastoris *were compared with some plants and seaweeds having antioxidant capacity. This study showed that* Gracilaria bursa-pastoris *contained a high total phenolic content, vitamin E, vitamin C and the antioxidant capacity.* Gracilaria bursa-pastoris *can be utilized as a source of natural antioxidant molecules and could be useful for food industry.

Keywords: ***Gracilaria, Total phenolic content, Vitamins, Antioxidant molecules.***

INTRODUCTION

Reactive oxygen species (ROS) such as hydrogen peroxide, superoxide radicals, hydroxyl radicals and singlet oxygen are physiological metabolites formed during aerobic life as a result of the metabolism of oxygen. Excessive production of such molecular compounds can cause damage to bio-molecules such as proteins, lipids, DNA and cell membranes etc. This damage can induce different kinds of diseases in human body.[1] ROS is scavenged by antioxidant molecules; as ascorbate, glutathione, and tocopherol and by enzymes; such as superoxide dismutase, catalase, and ascorbate peroxidase and glutathione reductase.[2] For these reasons, many products with antioxidant properties are widely used in order to minimize oxidative damage to living cells and to prevent oxidative deterioration of food.

Many researches have reported that plant-derived antioxidants have beneficial effect on human health.[3,4] The human diet has an important role in protection against oxidative stress because many crucial antioxidants cannot be synthesized by human body. Therefore, the antioxidant molecules must be obtained through diet.

Seaweeds are potential renewable resource in marine environment. In addition, seaweeds provide excellent source of bioactive compounds such as vitamins, minerals, dietary fiber and various functional polysaccharides.[5] The observations suggest that these bioactive compounds have strong antioxidant properties. In addition, most seaweeds also contain

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sulphated polysaccharides with biological properties in cell wall, whereas most terrestrial plants do not.[6] The antioxidant molecules play a very important role in the food industry for the prevention of lipid peroxidation. BHA (butylated hydroxyanisol) and BHT (butylated hydroxytoluene) are synthetic antioxidants, commonly used for maintenance of foodstuff.[7] However, in most countries there are some limitations in using synthetic antioxidant compounds in the food products because of their side effects.[8] Thus, it is essential to develop and utilize effective natural antioxidant molecules. Natural antioxidant such as a-tocopherol, phenols and b-carotene found in higher plants are being use in the food industry to inhibit lipid peroxidation and they can protect the human body from free radicals and retard the progress of many chronic diseases.[9] The reports on antioxidant properties of seaweeds extracts from Turkey are also very limited. Therefore, in this study total phenolic content, total antioxidant capacity (in soluble of lipid and water), total protein, total carbohydrate and vitamin (A, C and E) contents were determined in *Gracilaria bursa-pastoris* (Gmelin) Silva, in order to identify the new resources of natural antioxidant molecules.

MATERIALS AND METHODS

Collection of Samples

G. bursa-pastoris was freshly collected from the Marmara Sea coast of Turkey during November, 2007. The samples were kept in seawater until they arrive to the laboratory. The samples were immediately washed with tap water and with distilled water to remove epiphytes, salt and dirty particles after arrival. The clean algae were frozen and stored at −20°C until used for experiments.

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

Total phenolic contents of crude methanol extract were measured using Folin Ciocalteu's method as described by Taga et al.[10] 100 μL aliquot of 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 gallic acid equivalent per gram of fresh tissue.

Total Antioxidant Capacity (Water and Lipid-Soluble)

For the determination of lipid-soluble antioxidant capacity, 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. Sample 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 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.[11]

For the determination of water-soluble antioxidant capacity samples of water extracts (200 μL) were supplemented with 1 ml 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.[11]

Vitamin (E, C, and A) Contents

Vitamin E content was determined by using a method described by Prieto et al.[11] 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 measured at 695 nm. Vitamin E content was expressed as α-tocopherol equivalents per 100 gram of fresh tissue.

Ascorbic acid concentrations were determined by the titrimetic method as Association of Official Analytical Chemists (AOAC) no 967.21 using 2,6-dichlorophenol indophenol as a titrant.[12] For the determination of vitamin A, the samples were extracted with hexane. Vitamin A content was determined and calculated by using a method described by Rutkowski et al.[13]

Determination of Total Soluble Carbohydrate and Total Protein

Total Soluble Carbohydrate was assayed by anthrone-sulphuric acid method which involved extraction with 15% trichloroacetic acid.[14] 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.[15]

Statistical Analysis

Three samples were prepared for each experiment. The data were presented as mean ± standard deviation.

RESULTS AND DISCUSSION

Total Phenolic Content

Using the Folin Ciocalteu method phenolic content of *G. bursa-pastoris* was investigated and expressed as mg gallic acid/g (Table 1). Phenolic compounds are 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.[16] Therefore, a number of studies have focused on the biological activities

Table 1 The bioactive molecules and antioxidant capacity of *Gracilaria bursa-pastoris.* (mean ± SD), (n=3).

	Gracilaria bursa-pastoris
CALT (μmol α-tocopherol/g)	254.56 ± 11.61
CAHT (μmol L-ascorbic acid/g)	587.25 ± 62.13
Vitamin E (mg α-tocopherol/100g)	57.0 ± 38.0
Vitamin A (μM)	1.31 ± 0.36
Vitamin C (mg L-ascorbic acid/100g)	21.6 ± 8.0
Total phenol (mg gallic acid/g)	0.35 ± 0.05
Total protein (%)	41.5 ± 4.95
Total carbohydrate (%)	35.49 ± 5.27

CAHT, Water soluble antioxidant capacity; CALT, Lipid soluble antioxidant capacity.

of phenolic compounds. In this study, the red algae *G. bursa-pastoris* was found to contain total phenolic compound of 0.35 mg Gallic acid/g. The total phenolic content in the *G. bursa-pastoris* was higher than the amount in the *Codium fragile* (0.27 mg gallic acid/g) and *Gracilaria gracilis* (0.10 mg gallic acid/g).[17] Phenolic compounds are widely distributed in seaweeds and are known to exhibit higher antioxidative activities and chemopreventive agents.[18] According to this result, it is clearly indicated that *G. bursa-pastoris* have a high antioxidant activity.

Total Antioxidant Capacity (Water and Lipid-Soluble)

Total water-soluble and lipid-soluble antioxidant capacity of *G. bursa-pastoris* is presented on Table 1. Kumaran and Karunakaran[19] have reported total antioxidant activity is between the range of 245 to 376 mg ascorbic acid equivalent/g in higher plant extracts. In addition, Mohamed et al.[20] observed the highest levels of water-soluble antioxidant capacity of 277.7 μmol L-ascorbic acid/g in wheat germ and the highest level of lipid-soluble antioxidant capacity of 118.5 μmol α-tocopherol/g in chilly pepper seeds. Total lipid soluble (254.56 μmol α-tocopherol/g = 110 mg α-tocopherol/g) and water soluble (587.25 μmol L-ascorbic acid/g = 103 mg L-ascorbic acid/g) antioxidant capacity of *G. bursa-pastoris* was found nearly 2 times higher than that of these observations. The positive correlation between polyphenolic content of algae and its antioxidant activity is well documented.[21] In this study *G. bursa-pastoris* showed an excessive antioxidant capacity. Therefore, this could be as a result of its high polyphenolic content and characteristic pigments.

Vitamin (E, C and A) Contents

Seaweeds are an important unconventional source of liposoluble and hydrosoluble vitamins (such as; riboflavin, thiamine, β-carotene and tocopherols), commonly consumed as fresh or dried in many coastal areas. Vitamin E is the major liposoluble antioxidant responsible for protecting the polyunsaturated fatty acids in membranes against lipid peroxidation, free radicals and singlet oxygen species.[22] α-tocopherol is the most common form of Vitamin E present in nature and it is the most biologically active form.

Ortiz et al.[23] found that 258.0 mg/kg α-tocopherol in *Durvillaea antarctica* stem. On the other hand, Ching and Mohamed[24] investigated α-tocopherol content in 62 edible tropical plants. In their study, the highest α-tocopherol content was found to be 42.68 mg α-tocopherol/100 g fresh weight in *Sauropus androgynus*. According to this study, *G. bursa-pastoris* showed an excessively high value (57 mg α-tocopherol/100 g FW) when compared above results.

Ascorbic acid, also referred as L-Ascorbic acid or Vitamin C, is a water-soluble vitamin and it is mainly used in therapy as anti-infections in cells.[25] The vitamin C content in *G. bursa-pastoris* is 21.6 mg ascorbic acid/100 g and this value is higher than some of the consumable vegetables such as; banana (9.83 mg A.A/100 g), tomato (12.16 mg A.A/100 g), green cabbage (8.55 mg A.A/100 g), spinach (14.22 mg A.A/100 g) [26] On the other hand, the mean ascorbic acid content found in this study is in agreement with the value reported for *Gracilaria changgi* (28.5 mg A.A/100 g).[27] Another antioxidant molecule vitamin A was found to be 1.31 μM in *G. bursa-pastoris.*

Total Carbohydrate Content

Marine plants characteristically contain sulfated polysaccharides that are not found 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, *G. bursa-pastoris* showed high carbohydrate content (Table 1). However, Ortiz et al.[23] found that carbohydrate content of *U. lactuca* was 61.5% which is higher than the observed values in this study (35.49%).

Total Protein Content

Total protein content of *G. bursa-pastoris* is illustrated in Table 1. The mean protein content found in this study is roughly 2 folds higher than the values reported for various macroalgae.[23] *G. bursa-pastoris* showed a high protein content similar to traditional high protein seaweed sources, which makes them a healthy food for human or animal nutrition.

CONCLUSION

G. bursa-pastoris, a red algae also found in Turkey, was analyzed for its biologically active components, antioxidant capacity and phenolic compounds. The antioxidants are essentially needed in body system and they are considered to be important in human defense system. It is well known that the marine algae have got phenolic compounds and antioxidant activity. However, this is the first report of a large scale investigation on the bioactive components of *G. bursa-pastoris*. The results obtained in this study shows that *G. bursa-pastoris* can be utilized as a source of natural antioxidant compounds. This finding clearly demonstrated that *G. bursa-pastoris* can be used in the development of therapeutic products. Moreover, *G. bursa-pastoris* is an easily accessible source of natural antioxidant molecules can be a possible food supplement or can be used in pharmaceutical applications.

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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. Ahn, C.B.; Jeon, Y.J.; Kang, D.S.; Shin, T.S.; Jung, B.M. Free radical scavenging activity of enzymatic extracts from a brown seaweed *Scytosiphon lomentaria* by electron spin resonance spectrometry. Food Research International **2004**, *37*, 253–258.
6. Kloareg, B.; Quatrano, R.S. Structure of the cell walls of marine algae and ecophysiological function of the matrix polysaccharides. Oceanography and Marine Biology, An Annual Review **1988**, *26*, 259–315.
7. Safer, A.M.; Al-Nughamish. Hepatotoxicity induced by the antioxidant food additive butylated hydroxytoluene (BHT) in rats: an electron microscopical study. Histology and Histopathology **1999**, *14*, 391–406.
8. Kabouche, A.; Kabouche, Z.; Öztürk, M.; Kolak, U.; Topçu, G. Antioxidant abietane diterpenoids from *Salvia barrelieri*. Food Chemistry **2007**, *102*, 1281–1287.
9. Matsukawa, R.; Dubinsky, Z.; Kishimoto, E.; Masaki, K.; Masuda, Y.; Takeuchi, T.; Chihara, M.; Yamamoto, Y.; Niki, E.; Karube, I. A comparison of screening methods for antioxidant activity in seaweeds. Applied Phycology **1997**, *9*, 29–35.
10. 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.
11. 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.
12. AOAC. Association of Official Analytical Chemistry. Official Methods of analysis, 16th ed.; AOAC: Arlington, VA, USA. 1995.
13. 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.
14. 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.
15. Bradford, M. A rapid and sensitive method for the quantification of micrograms quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry **1976**, *72*, 248–254.
16. 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.

17. 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.
18. Bravo, L. Polyphenols: chemistry, dietary source, metabolism, and nutritional significance. Nutrition Reviews **1998**, *56*, 317–333.
19. Kumaran, A.; Karunakaran, R.J. In vitro antioxidant properties of methanol extracts of five Phillanthus species from India. LWT Food Science and Technology **2007**, *40*, 344–352.
20. 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.
21. 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.
22. 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.
23. 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.
24. Ching, L.S.; Mohamed, S. Alpha-Tocopherol Content in 62 Edible Tropical Plants. Journal of Agricultural Food Chemistry **2001**, *49*, 3101–3105.
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. Melo, E.A.; Lima, V.L.A.G.; Maciel, M.I.S.; Caetano, A.C.S.; Leal, F.L.L. Polyphenol, ascorbic acid and total carotenoid contents in common fruits and vegetables. Brazilian Journal of Food Technology **2006**, *9*, 89–94.
27. Norziah, M.H.; Ching, C.Y. Nutritional composition of edible seaweed *Gracilaria changgi*. Food Chemistry **2000**, *68*, 69–76.
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.