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Full Length Research Paper

Antioxidant activity of extract from a brown alga, *Sargassum boveanum*

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Water and ethanol extracts (WE and EE) from the dried sample of brown alga (*Sargassum boveanum*) were prepared and examined for its phenolic compounds. Amount of total phenolic compounds in WE was about 17 ± 0.492 mg catechin equivalent (CE)/g of dry sample, using Folin-Ciocalteu method. The antioxidant activity (AOA) of WE was high at about 90% inhibition of peroxidation of linoleic acid with 7 mg dry sample/ml solvent. The IC_{50} of the WE sample and catechin which was used as the positive control with the hemoglobin catalyzed linoleic acid peroxidation method, were (mg/ml): 3.82 and 0.0713, respectively. The IC_{50} of the WE sample in terms of CE was 18.76 mg CE/g dry sample. The WE sample exhibited noticeable scavenging effects in DPPH free radical scavenging assay. The radical scavenging activity (RSA) was about 94% at 3 mg dry sample/ml solvent. The phenolic constituent appears to be responsible at least in part, for the observed AOA of the algal extract. The results suggest that this alga could be a potential source of natural antioxidant.

Key words: Antioxidant activity, brown algae, *Sargassum boveanum*, polyphenols, radical scavenging activity, linoleic acid peroxidation.

INTRODUCTION

During aerobic life different types of oxygen derivatives such as superoxide anion, singlet state oxygen and hydroxyl radicals along with peroxides and transition metals, are produced. These physiological metabolites have degenerative effects to living cells and DNA, cell membranes, proteins are cellular sites for these degradative types of reactions. The constant risk to oxidation is extended to the biological tissues of different sources from which foods are derived. The derivatives of molecular oxygen are principal cause of qualitative decay of foods. Fat oxidation leads to its rancidity, toxicity, and other destructions of biomolecules present in food systems. Addition of antioxidants to food formulations is an effective way of reducing incidence of lipid oxidation in industrial food production (Decker, 1998). Synthetic antioxidants used as food additives tend to be eliminated and search for antioxidant from natural sources are intensified in recent years (Duh et al., 1992; Osawa and Namiki, 1985). Determination of the efficacy of natural antioxidant is important for evaluation of food protection against oxidative damage, major cause of loss of nutritional value and consequent loss of commercial position of food products in market. By manipulation of food formulations, a new antioxidant could be properly utilized in the industry. As a useful approach, the antioxidant activity (AOA) tests is categorized into two main groups: assays in which by using a lipid substrate, the level of oxidation inhibition is measured and the other tests group includes assays in which capacity of the test system for expressing free radical scavenging activity (RSA) is determined (Sanchez-Moreno, 2002). The mechanism of the peroxidation of linoleic acid catalyzed by hemoglobin is probably via the formation of lipid-heme complex (or ferryl-type heme compound) (Kuo et al., 1999). Formation of the hydroperoxide during the linoleic acid peroxidation is assayed according to a ferricthiocyanate procedure

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after mixing the test solution with ferrous chloride (Kuo et al., 1999; Kuda et al., 2005). Red color appears upon reaction between iron (III) ions and thiocyanate ions while iron (II) ions yield only a colorless solution. These facts are used in detection of iron (III) ions in the presence of iron (II) ions (Nebergall et al., 1972).

Consuming seaweeds as sea vegetables has been a long tradition in the Far East and Pacific while the principal use of seaweeds in Western countries has been as source of thickening and gelling agents for different industrial applications including uses in foods (Jimenez-Escrig and Sanchez-Muniz, 2000; Nagai and Yukimoto, 2003). Moreover, seaweeds are known to contain several compounds having health protective effects (Burtin, 2003). The potential antioxidant compounds in brown algae have identified as fucoxantine in *Hijikia fusidormis* and phylopheophytin in *Eisemia bicyclis* (Kuda et al., 2005a). Polyphenolics are widely distributed in plants and agricultural residues (Asamarai et al., 1996; Amarowicz, 2004; Vahabzadeh et al., 2004). Brown algae (Phaophyceae) like any one of the three groups of seaweeds differ from the other groups with regards to the reserve and cell wall polysaccharides (Jimenez- Escirg and Sanchez-Muniz, 2000). *Sargassum boveanum* is a marine species and its presence in the coastal waters of the Persian Gulf is confirmed (Algae Base, 2007; Sohrabipour and Rabii, 1990). In continuation of our search on brown algae of Persian Gulf for expressing the AOA (Rastian et al., 2007), further works on this subject in *S. boveanum* is reported in the present study. The AOA tests were carried out according to the two assays: the peroxidation of linoleic acid catalyzed by hemoglobin, and the RSA test on basis of using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH).

MATERIALS AND METHOD

Collection of *S. boveanum*

The brown alga *S. boveanum* J. Agardh was collected close to the Bushehr seashore in the northern Persian Gulf between 6 and 12 May 2006. Fresh algal samples were collected in three mornings (the temperature of the water at the collection site was 23 - 26°C), washed several times with distilled water and distributed in a mesh bottom frame (30 × 20 cm) and dried in an oven at 40°C until the moisture content reached to about 13%. The samples were then homogenized with a grinder before the extraction process. *S. boveanum* J. Agardh characterization was confirmed by consulting with the Algae Base (web site: www.algaebase.org) and Sohrabipour and Rabii (1990).

Preparation of water and alcohol extract of the algal samples

The dried samples were weighed (1 g) and after adding of distilled water (100 ml), the samples were autoclaved at 121°C for 15 min. The contribution of this treatment to the microbial removal appears to be important (Kudo et al., 2005a,b). The autoclave treatment was followed by centrifugation (2220 g for 10 min), and then the supernatant as the water extract solution was collected and used in present study (WE). For preparation of the ethanol extract (EE) the dried samples were weighed (4 g), then 50 ml ethanol added to each sample, and the samples were shaken at 37°C for 4 1/2 h. This was followed by centrifugation (2220 g for 10 min), the supernatant as the ethanol extract solution was used in this study.

Total phenolics content

The total phenolic content in the algal extracts prepared as above, was determined by spectrophotometric method based on procedures described by Kuda et al. (2005b). Briefly, 0.4 ml aliquot of the algal extract was transferred into a test tube containing 0.8 ml of the 10% Folin-Ciocalteu-phenol reagent (Sigma). After 3 min, 1.6 ml of the 10% sodium carbonate solution was added. The contents were mixed routinely, using glass rod and left to stand at room temperature for 1 h. Absorbance measurements were recorded at 750 nm using a spectrophotometer (Jasco V-550) while (+) catechin (Sigma) was used for the preparation of the standard curve. Estimation of the phenolics contents were carried out in duplicates and the results were expressed as mg of (+) catechin equivalent (CE)/g dry sample.

Antioxidant activity (AOA) measurement using hemoglobin-induced linoleic acid peroxidation

The antioxidant activity of the algal extract was determined by a spectrophotometric procedure using the hemoglobin catalyzed peroxidation of linoleic acid assay (Kuda et al., 2005b; Fant et al., 2005). The algal extract solution (0.1 ml) was mixed with 0.025 ml linoleic acid (Sigma) - ethanol solution (0.1 M) and 0.075 ml phosphate buffer (0.2 M, pH 7.2). By adding of 0.05 ml hemoglobin (Sigma) solution (0.08%), the linoleic acid peroxidation was started. The reaction mixtures were incubated at 37°C for 1 h, thereafter the reaction was stopped using 5 ml HCl-ethanol solution (0.6%). The thiocyanate method was then used to quantify the peroxidation: 0.02 ml of $FeCl_2$ solution (0.02 M) and 0.01 ml of ammonium thiocyanate (Aldrich) solution (30%) were added to 1.6 ml of the sample solution. Absorbance measurement was obtained at 490 nm. Catechin was used as positive control, IC_{50} as the inhibitory concentration of the WE sample which caused 50% inhibition of the hemoglobin catalyzed peroxidation of linoleic acid was obtained by linear regression method (Figure 1).

Measurement of DPPH (2,2-diphenyl-1-pricrylhydrazyl) radical-scavenging activity (RSA)

The scavenging activity of the algal extracts on the DPPH (radical was determined by a photometry assay based on procedure described elsewhere (Kuda et al., 2005a). The algal extract solution (0.2 ml) was mixed with equal volume of ethanol. 0.001 M DPPH (Fluka)/methanol solution (0.025 ml) was added to the solution and absorbance at 550 nm was measured after 30 min. The RSA was calculated as a percentage of DPPH discoloration using the equation:

$$RSA\% = \left(1 - \frac{A_T}{A_0}\right) \times 100$$

Where A_T is the absorbance of the test solution at 30 min time and A_0 is the absorbance at zero time (initial absorbance).

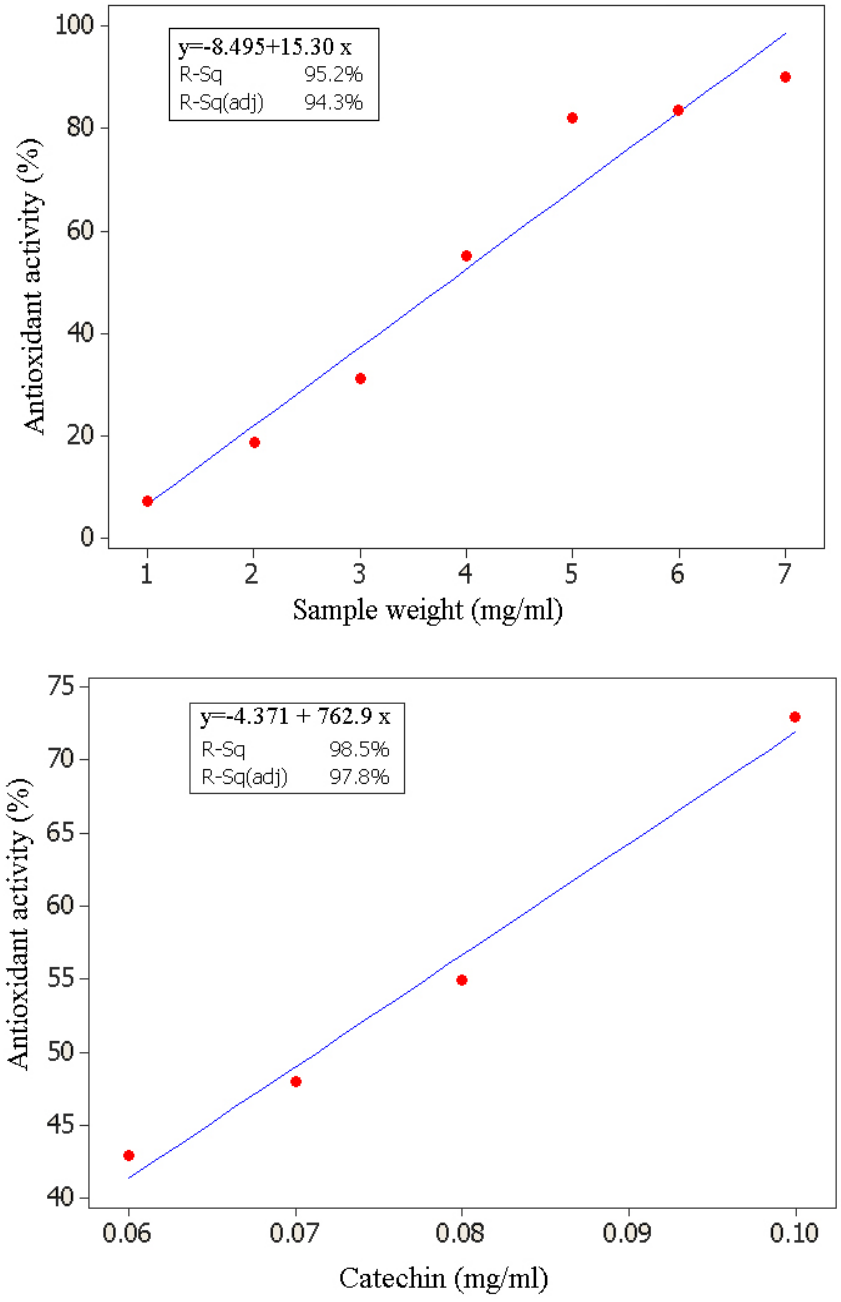

Figure 1. Linear regression relationship between amount of WE sample and its antioxidative activity determined with the hemoglobin catalyzed peroxidation of linoleic acid. Catechin was used as the positive control. The plots were used to obtain IC_{50} for the WE sample and catechine.

Statistical analysis

Analytical values represent means of three independent experiments each with duplicate measurements. The AOA results were compared by one-way analysis of variance (one-way ANOVA) to test for significant differences (Lapin, 1997). Paired t test when needed, was used to assess significant difference between AOA of WE and EE in terms of DPPH scavenging activity (Vining, 1998). Differences among (or between) sample means were reported to be significant when $P < 0.05$.

RESULTS AND DISCUSSION

Total phenolics content for WE and EE samples was (mg CE/g of dry sample) 17 and 0.9, respectively. Chemical assay based on the removal of stable DPPH free radical has been shown to indicate the presence of reducing compounds in terms of hydrogen donation capacity in the test system. Purple color of the freshly prepared DPPH solution fades when an antioxidant compound is present

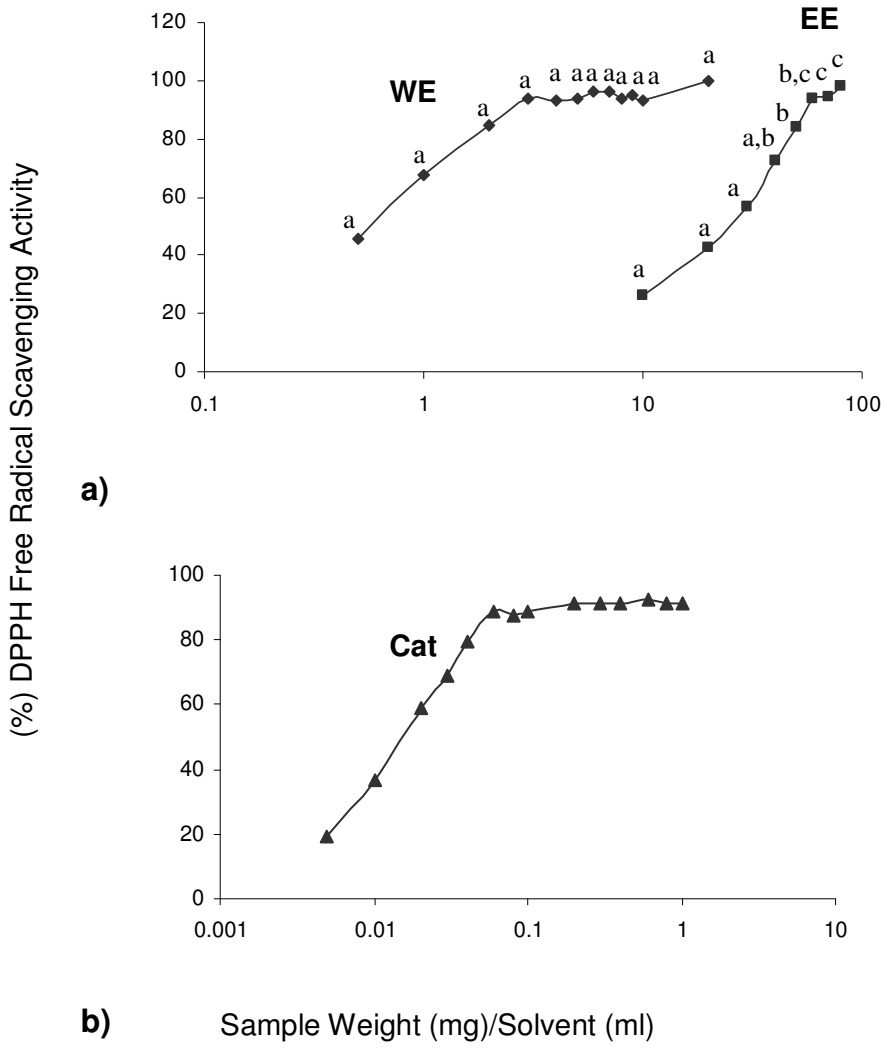

Figure 2. Scavenging effect for water and ethanol extraction of dried *S. boveanum* (WE) and (EE) on DPPH free radicals (A). Scavenging effect of catechin as a positive control (Cat) on DPPH free radicals (B). Each value in the plot is the mean of duplicate analyses. Means at the same data point with different letters (a, b, c) differ significantly at $P<0.05$.

in the test solution, to quench DPPH; the antioxidant molecule provides a hydrogen atom or donates an electron, to DPPH (Amarowicz et al., 2004). Figure 2 shows the results of the DPPH free radical scavenging activity, the absorbance of DPPH solution changed when the algal extracts were added to the solution. WE and EE were both dose dependent and WE scavenged DPPH and reached a plateau at low level of the extract (at less than 5 mg/ml). While the radical scavenging activity of the EE, increased when the amount of the extract added increased (Figure 2A). The RSA of bearberry leaf ethanol extract was about 77% for 0.1 mg extract added to the assay solution, as reported by Amarowicz et al. (2004). In the present study the WE of the algal sample at concentration of 3 mg/ml exhibited about 94% DPPH scavenging activity. The interaction between antioxidant and DPPH is shown to be dependent on the structural conformation of antioxidant (Sanchez-Moreno, 2002). This structural feature of the DPPH test may give the assay, ability to differentiate phenolic compounds among several compounds usually present in natural system capable of expressing AOA. These results show that the WE contained substances which may act as free-radical inhibitor.

The capacity of the compound expressing AOA towards DPPH has also been termed as the antiradical efficiency in which time to reach the plateau of scavenging reaction is determined (Sanchez-Moreno, 2002). This approach in AOA measurement although, is known as an advantage point over other methods in which consideration is based only on the concentration of antioxidant. But lack of similarity between the highly reactive and transient peroxy radicals mainly involved in lipid peroxidation and the DPPH as a long-lived nitrogen con-

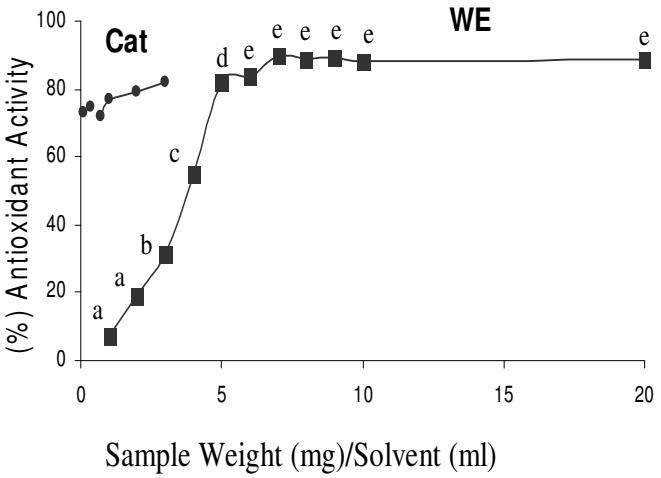

Figure 3. Antioxidant activity for water extraction of dried *S. boveanum* (WA) and catechin (Cat) as a positive control against linoleic acid peroxidation induced by hemoglobin. Each value in the plot is the mean of duplicate analyses. Means at the same data point with different letters (a, b, c, d, e) differ significantly at $P<0.05$.

taining radical, has made the reaction time approach less suitable for rating the antioxidant capacity (Huang et al., 2005). In the present study antiradical efficiency of the algal sample was not obtained. RSA on basis of using the stable radical DPPH has been found to be an easy and accurate method giving results which are reproducible and comparable to the results obtained from other free radical scavenging techniques such as ABTS (2,2-azinobis-(3-ethylbenzolhiazline-6-sulphonate) (Sanchez-Moreno, 2002).

The evaluation of radical scavenging activity effectiveness (elimination of DPPH free radicals) may not necessarily give a whole picture of the AOA since the assay relies on one aspect of AOA. The importance of the solubility characteristics of antioxidant in relation to the site of lipid oxidation should be considered for use of antioxidant in foods (Decker, 1998). Water-soluble free radical scavengers are not effective in lipid emulsions where oxidation occurs in the lipophilic phase (Huang et al., 1996). While water-soluble antioxidants are effective in muscle foods where oxidation occur mainly in the aqueous environment (Decker, 1998). In the present study effectiveness of the radical scavenging activity appeared to be more pronounced in water as compared to that of the sample in ethanol solution. Moreover, method of the Folin-Ciocalteu reagent for determining total phenols actually measures reducing capacity of a sample and this is not reflected in its name total phenolic assay (Huang et al., 2005). In fact, some studies showed there is excellent linear correlation between the total phenols assay by Folin-Ciocalteu reagent and an electron transfer-based AOA assay (ferric ion reducing antioxidant power assay) (Huang et al., 2005). The DPPH assay is believed to involve hydrogen atom transfer, but the findings reported by Foti et al. (2004) suggested the DPPH reaction behaves like an electron transfer reaction (Huang et al., 2005).

In the present study AOA of the algal extract was also measured according to hemoglobin-induced linoleic acid peroxidation method (Kuda et al., 2005b). Figure 3 shows the dose-AOA response curve for the WE. The AOA reached a plateau at about 90% inhibition with a concentration of 7 mg extract/ml and clear AOA of catechin was measured at concentration as low as 0.1 mg/ml (Figure 3). AOA of the dried sample of a brown alga, *Scytosiphon lumentaria* has been found to be highest at 5.5 mg catechin equivalent/ml (Kuda et al., 2005b). The IC_{50} of the WE sample and catechin which was used as the positive control in the present work, using the hemoglobin catalyzed linoleic acid peroxidation method, were (mg/ml): 3.82 and 0.0713, respectively (Figure 1). The IC_{50} of the WE sample in terms of CE was 18.76 mg CE/g dry sample. The IC_{50} of catechin found here (71 ppm), was in the range of the reported values for garlic, tangerine peel and orange peel (68-79 ppm) (Kuo et al., 1999). The IC_{50} of green tea and synthetic antioxidant (BHA) are (ppm): 0.25 and 0.65, respectively (Kuo et al., 1999). AOA of the extracts from a brown alga *S. siliquastrum* was evaluated and on the basis of the results obtained by thin-layer chromatography and UV and IR spectra analyses, phenolic compounds were found to be the major components responsible for this activity (Lim et al., 2002). Based on the different degrees of AOA in various assays used by Lim et al., 2002, the authors concluded that several chemical components might be involved in the AOA of the extract of *S. siliquastrum*. The radical scavenging effects of bearberry leaf extract were 40 and 77% at 0.05 and 0.1 mg level of addition of the extract, respectively (Amarowicz et al., 2004). A direct comparison of the data may not be informative because of the differences exist, but it is useful to see the radical scavenging effect of the algal water extract in the present study was more than 94% at 3 mg level of the extract of dry sample/ml solution.

Sea algae are widely consumed in Japan and there are several seaweeds including brown algae authorized in France for human consumption (Burtin, 2003; Kuda et al., 2005a), moreover, certain alga used in capsule form in Italy as diet integrator (Campanella et al., 2005). These facts support safety of utilization of the algal extract as natural antioxidant. Based on the results obtained from studies on seaweeds, it has been shown that several algal species can prevent oxidative damage by scavenging free radical and hence able to prevent cancer cell formation (Athukorala et al., 2006; Yen and Duh, 1994). Abnormality of cells in human body (cancer cell formation) may directly relate to the presence of free radicals (Prior and Cao, 2000). Use of the algal extract for medical purposes to inhibit peroxidation of lipids in the body could be encouraging. Furthermore, to identify the chemical nature of the compound(s) AOA should be

expressed in WE and EE of the tested alga in the present study, more detailed study is necessary.

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