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In vitro Antioxidant Activity of *Enteromorpha intestinalis* (Linnaeus) Nees

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In this study, antioxidant activity of *Enteromorpha intestinalis* was determined by three different methods. Total phenolic concentration of the extracts was estimated with Folin-Ciocalteu reagent using gallic acid as standard. Free radical scavenging activities were determined based on 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). The copper(II) ion reducing ability of polyphenols is measured by CUPRAC method. Results were compared with standard butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA).

Key Words: Antioxidant, Free radical, *Enteromorpha intestinalis*, DPPH, Folin-Ciocalteu, Cuprac method.

INTRODUCTION

Reactive oxygen species (ROS) are produced in biochemical reactions and physiological processes and cause oxidative stress. In this instance harmful oxidative reactions may occur in organisms. This free radical reactions are the cause of certain human diseases such as cardiovascular disease, cancer. All aerobic organisms have antioxidant defenses including antioxidant enzymes to remove or repair the damaged molecules. However, these natural antioxidant mechanisms can be inefficient and hence dietary intake of antioxidant compounds is very important[1]. Antioxidant compounds play an important role as a health-protecting factor and primary sources of antioxidants are plants. The preservative effect of plant spices and herbs suggests the presence of antioxidative and antimicrobial constituents in their tissues[2]. Dietary antioxidants are helpful in assisting the body to neutralize free radicals. Therefore, it is important to consume a diet high in antioxidants, such as fruits and vegetables, to reduce the harmful effects of oxidative stress. Fruits and vegetables are a rich source of phytochemicals, such as carotenoids, flavonoids and other phenolic compounds. Studies have indicated that these phytochemicals, especially polyphenols, have high free-radical scavenging activity, which helps to reduce the risk of chronic diseases[3]. Phenolic compounds form a major group of phytochemicals found in plants[4]. The antioxidative effect of plants is mainly due to these phenolic components and this effect is mainly due to their redox properties. Marine algae, like other photosynthesizing plants, are exposed to a combination of light and oxygen that leads to the formation of free radicals and other strong oxidizing agents. However,

the absence of oxidative damage in the structural components of macroalgae (*i.e.*, polyunsaturated fatty acids) and their stability to oxidation during storage suggest that their cells have protective antioxidative defence systems[5,6]. In fact, algae have protective enzymes (superoxide dismutase, peroxidase, glutathione reductase, catalase) and antioxidative molecules (phlorotannins, ascorbic acid, tocopherols, carotenoids, phospholipids, chlorophyll related compounds, bromophenols, catechins, mycosporine-like amino acids, polysaccharides, *etc.*) which are similar to those of vascular plants[5,7-9]. *Enteromorpha* is a green algae that is more commonly found in marine environments but can also be found in freshwater environments, it can also survive in temperatures from 30 ºC. It is also widespread in Turkey. It grows brackish water, Marine and Lagunes.

EXPERIMENTAL

Plant material: *Enteromorpha intestinalis* was collected from the Acigöl Lake in Karapinar-Konya between April 2006 and May 2007. Once harvested, macroalgae were stored in plastic bags and placed in ice for transport to the laboratory. Voucher specimens of species was pressed and stored in 4 % formol for identification according to John, Whitton and Brook[10]. Samples were washed thoroughly with fresh water to remove salts, sand and epiphytes and dried.

Preparation of the methanol extracts: The air-dried and finely ground samples were extracted by using the method described elsewhere[11]. Briefly, the sample, weighing about 100 g, was extracted in a Soxhlet apparatus with methanol at 60 ºC for 6 h. The extract was then filtered and concentrated *in vacuo* at 45 ºC. Finally, the extracts were then lyophilized and kept in the dark at 4 ºC until tested.

Total phenolic compound assay: The amounts of phenolics in the plant extracts were determined with Folin-Ciocalteu reagent[12] using the method of to 50 mL of each sample, 2.5 mL of 10 % dilution of Folin-Ciocalteu reagent and 2 mL of Na_2CO_3 (7.5 %, w/v) were added and the resulting mixture was incubated at 45 ºC for 15 min. The absorbance of all samples was measured at 765 nm. Gallic acid was used as a standard for the calibration curve. The total amount of phenolic compounds was calculated and expressed as milligrams of gallic acid equivalent.

Free radical scavenging method: The antioxidant activity of plant extracts was measured in terms of hydrogen donating or radical scavenging ability, using the stable radical, ($DPPH^\bullet$)[13]. A methanolic solution of sample of various concentrations was placed in a cuvette and 4 mL of 6×10^{-5} mol/L methanolic solution of DPPH was added. After 0.5 h incubation period at room temperature, the absorbance was read against a blank at 515 nm. The same procedure was repeated with synthetic antioxidant, BHT and BHA, as positive control and a blank. Inhibition of the free radical $DPPH^\bullet$ in per cent (I %) was calculated as follows:

$$I\ \% = (A_{blank} - A_{sample}/A_{blank}) \times 100$$

where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound. Extract

concentration providing 50 % inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract concentration. Tests were carried out in triplicate and BHT and BHA were used as positive controls.

Cuprac method: The cupric reducing antioxidant capacity of the extract was determined according to the method of Apak *et al.*[14]. To a test tube, 1 mL each of 10 mM Cu(II), 7.5 mM neocuprine and ammonium acetate buffer (1 M, pH 7.0) solutions were added. Extract and standard antioxidant compounds at different concentrations were added to the initial mixture so as to make the final volume 4.1 mL. The tubes were stoppered and after 1 h, the absorbance at 450 nm was recorded against a reagent blank.

RESULTS AND DISCUSSION

Total phenolic compound assay was measured by Folin-Ciocalteu method[12]. The Folin-Ciocalteu reagent assay was used to determine the total phenolics content. Phenolic concentration of *E. intestinalis* was determined as equivalent gallic acid. Total phenolic concentration of *E. intestinalis* was estimated as equivalent gallic acid and has been calculated as 0.327 mg/mL.

Antioxidant activity of plant extracts was studied according to the $DPPH^{\bullet}$ radical scavenging method[13]. The results showed that the decrease in absorbance of the $DPPH^{\bullet}$ radical was due to its reduction by different antioxidants. Absorbance decreases as a result of a colour change from purple to yellow as the radical was scavenged by antioxidant through donation of hydrogen to form the stable DPPH-H. The plant extracts which are placed to the medium at different concentration rates reduce the DPPH concentration through the antioxidant molecules that they contain. The colour lightens as they reduce the DPPH concentration. This change measured as absorbance at spectrophotometer. When the absorbance graph of the plant and DPPH compared to the calibration curve of DPPH, it can be seen easily how much free radicals is reduced in the medium. IC_{50} values are calculated according to this absorbance values. This can be explained as the plant concentration which can remove the half of the free radicals concentration from the medium away. So the less IC_{50} value means more antioxidant effect. IC_{50} value of *E. intestinalis* and standard antioxidant compounds was calculated (Table-1).

It is one of the antioxidant capacity measuring method which depends on the degradation capacity of Cu^{2+} and developed by Apak *et al.*[15]. With this method, which used the *bis*(neocuproin)-copper(II) chloride as a chromogenic redox reactive, either hydrophilic or lipophilic antioxidant capacities can be found. The reaction between the antioxidant compound and neocuproin-copper(II) chloride which is used in CUPRAC method as an oxidation reactive is like that:

$$2n\ Cu(Nc)_2^{2+} + Ar(OH)n \rightarrow 2n\ Cu(Nc)_2^{+} + Ar(=O)_n + 2n\ H^{+}$$

Accordance of experimental findings, combined with the parallellism of the linear calibration curves of each antioxidant compound tested.

TABLE-1
IC_{50} AND TEAC VALUE OF *E. intestinalis* AND
STANDARD ANTIOXIDANT COMPOUND

	IC_{50}	$CUPRAC_{TEAC}$
E. intestinalis	1.304	6.548×10^{-4}
Butylated hydroxy anisole	1.075	9.800×10^{-4}
Butylated hydroxy toluene	1.498	5.780×10^{-4}

There has been an increase in absorbance plus *E. intestinalis* exrtact in trolox. The increase of absorbance showed parallellism with antioxidant capacity. Trolox equivalent antioxidant capacities (TEAC) of BHA, BHT and *E. intestinalis* extract calculated with CUPRAC method (Table-1).

Values showed that antioxidant capacities of BHA is very effective than the others. In this study, antioxidant capacities of *E. intestinalis* was determined by three different method and antioxidant capacity of *E. intestinalis* was compared with synthetic antioxidant compound (BHA and BHT). As a result, it is understood *E. intestinalis* extract has effective antioxidant capacitiy and this result was supported by three different method.

Statistical analysis: All results were obtained in triplicate and data were presented as mean ± standard deviation of three determinations (data were not shown). Statistical analyses were performed using a one-way analysis of variance[16].

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