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Determination of in vitro antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts

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Abstract

In this study, the antioxidant activity of water and ethanol extracts of fennel (*Foeniculum vulgare*) seed (FS) was evaluated by various antioxidant assay, including total antioxidant, free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, metal chelating activities and reducing power. Those various antioxidant activities were compared to standard antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and α-tocopherol. The water and ethanol extracts of FS seeds showed strong antioxidant activity. 100 μg of water and ethanol extracts exhibited 99.1% and 77.5% inhibition of peroxidation in linoleic acid system, respectively, and greater than the same dose of α-tocopherol (36.1%). The both extracts of FS have effective reducing power, free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, and metal chelating activities. This antioxidant property depends on concentration and increasing with increased amount of sample. In addition, total phenolic compounds in the water and ethanol extracts of fennel seeds were determined as gallic acid equivalents. The results obtained in the present study indicated that the fennel (*F. vulgare*) seed is a potential source of natural antioxidant. Although, the tests presented here show the usefulness of FS extracts as in vitro antioxidants it still needs to be that this extracts show their activity in emulsions, biological systems, health implications or dry foods.

Keywords: Fennel; *Foeniculum vulgare*; Antioxidant activity; In vitro; Seed

1. Introduction

Reactive oxygen species (ROS) including free radicals such as superoxide anion radicals ($O_2^{\bullet -}$), hydroxyl radicals (OH•), singlet oxygen ($^1O_2$) and non-free-radical species such as hydrogen peroxide (H_2O_2) are various forms of activated oxygen and often generated by oxidation product of biological reactions or exogenous factors (Ceruitti 1991; Yıldırım, Mavi, & Kara, 2001; Gülçin, Oktay, Kufrevioglu, & Aslan, 2002b). ROS have aroused significant interest among scientists in the past decade. Their broad range of effects in biological and medicinal systems has drawn on the attention of many experimental works (Büyükokuroglu, Gülçin, Oktay, & Kufrevioglu, 2001; Gülçin, Büyükokuroglu, Oktay, & Kufrevioglu, 2002a). In living organism, various ROS can form by different ways. Normal aerobic respiration stimulates polymorphonuclear leukocytes and macrophages, and peroxisomes appear to be the main endogenous sources of most of the oxidants produced by cells. Exogenous sources of ROS include tobacco smoke, certain pollutants, organic solvents, and pesticides (Halliwell and Gutteridge, 1989; Davies 1994; Robinson, Maxwell, & Thorpe, 1997). ROS can cause lipid peroxidation in foods, which leads to the deterioration of the food (Miller, Diplock, & Rice-Evans, 1995; Sasaki, Ohta, & Decker, 1996). In addition, it is well known that ROS induce some oxidative damage to biomolecules like lipids, nucleic acids, proteins, amines, deoxyribonucleic acid and carbohydrates. Its damage causes ageing, cancer, and other many diseases (Kehrer, 1993; Aruoma, 1994). As a result of this, ROS have been implicated in more than 100 diseases, including malaria, acquired immunodeficiency syndrome, heart disease, stroke, arteriosclerosis, diabetes, and cancer (Tanizawa et al., 1992; Hertog,

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Feskens, Hollman, Katan, & Kromhout, 1993; Duh, 1998; Alho & Leinonen, 1999; Yıldırım, Mavi, Oktay, Kara, Algur, & Bilaloglu, 2000).

ROS are continuously produced during normal physiologic events, and removed by antioxidant defence mechanisms (Halliwell, Gutteridge, & Cross, 1992). There is a balance between generation of ROS and antioxidant system in organisms. In pathological condition, ROS are overproduced and result in lipid peroxidation and oxidative stress. The imbalance between ROS and antioxidant defence mechanisms leads to oxidative modification in cellular membrane or intracellular molecules (El-Habit, Saada, & Azab, 2000). Various endogenous antioxidant defence mechanisms play an important role in the elimination of ROS and lipid peroxides, and therefore, protect the cells against toxic effects of ROS and lipid peroxides (Halliwell, 1991; Halliwell et al., 1992; El-Habit et al., 2000).

Many antioxidant compounds, naturally occurring from plant sources, have been identified as free radical or active oxygen scavengers (Yen & Duh, 1994; Duh, 1998). Recently, interest has increased considerably in finding naturally occurring antioxidant for use in foods or medicinal materials to replace synthetic antioxidants, which are being restricted due to their side effects such as carcinogenecity (Ito, Fukushima, Hasegawa, Shibata, & Ogiso, 1983; Zheng & Wang, 2001). Natural antioxidants can protect the human body from free radicals and retard the progress of many chronic diseases as well as retard lipid oxidative rancidity in foods (Pryor, 1991; Kinsella, Frankel, German, & Kanner, 1993; Lai, Chou, & Chao, 2001).

Fennel (*Foeniculum vulgare*) is a plant belonging to the Umbelliferae (Apiaceae) family, known and used by humans since antiquity. It was cultivated in every country surrounding the Mediterranean Sea because of its flavour (Muckensturm, Foechterlen, Reduron, Danton, & Hildenbrand, 1997). The renewed interest in natural product rather than synthetic agents has again focused attention on plants as a source of flavouring compounds (Yaylayan, 1991). Fennel is an aromatic edible plant whose seed are used for savoury formulations, sauces, liqueurs, confectionery, etc. (Guilled & Manzanons, 1996). Due to unique and preferred flavour and aroma, the swollen bases of fennel are freshly consumed in salad or cooked as kitchen vegetable (Baytop, 1999; Atta-Aly, 2001).

The therapeutic effects and culinary utilisation of fennel were so large that it was exported from country to country for centuries (Puelo, 1980). Today, all culture of fennel has given rise to a great complexity, and simple observation of this plant's morphological characteristics is not sufficient to classify (Jansen, 1981). The major constituents of fennel essential oil such as anethole and limonene are also used some medicinal purposes and essence in cosmetics and perfumes industry (Stuart, 1982; Marotti et al., 1993).

Much work has recently studied the yield and composition of fennel seed (Verghese, 1988; Arslan, Bayrak, & Akgul, 1989; Lawrence, 1989, 1992; Betts, 1992; Cavaleiro, Roque, & Cunha, 1993; Piccaglia & Marotti, 1993; Katsiotis, 1988). In addition, influence of spices on protein utilisation (Pradeep & Geervani, 1994), microbiological survey (Satchell, Bruce, Allen, Andrews, & Gerber, 1989), effects of fungal metabolites on the germination (Sharma & Sharma, 1983), the effect of fertiliser treatments on yield (Abdallah, El-Gengaihi, & Sedrak, 1978), and immune reactivity (Schwartz, Jones, Rojas, Squillace, & Yunginger, 1997) of fennel seed was investigated. Also, the effect of fennel seed extract on the genital organs of male and female rats is performed (Malini et al., 1985).

Fennel is very common in northern Anatolia region (Baytop, 1999). In the Turkish folk medicine, this plant especially its seeds have been used as tranquilliser, tonic and soporific drug (Asımgal, 1997; Baytop, 1999).

The chemical composition of the essential oil obtained from various parts of the bitter Turkish fennel plant has been reported (Akgül & Bayrak, 1988). The vascular effect of aqueous extract of fennel leaves was tested using pentobarbital-anaesthetised rats. The aqueous extract of fennel inhibited the hypotensive effect in a dose-related manner (Abdulghani & Amin, 1988). Ruberto et al. demonstrated that the oils of fennel had in vivo antioxidant capacity (Ruberto, Baratta, Deans, & Dorman, 2000), but there is no information about in vitro antioxidant activity of water or ethanol extracts of fennel seeds. However, from a toxicological point of view, ethanol and water, as solvent, are safer than acetone, methanol, and other organic solvent, and therefore more suitable for food industry. Thus, water and ethanol extracts are used in the following study. The purpose of present study was to evaluate the antioxidant activity of the water and ethanol extracts of fennel seeds and to elucidate their antioxidative actions.

2. Materials and methods

2.1. Chemicals

Ammonium thiocyanate was purchased from E. Merck. Ferrous chloride, polyoxyethylenesorbitan monolaurate (Tween-20), α-tocopherol, 1,1-diphenyl-2-picryl-hydrazyl (DPPH•), 3-(2-Pyridyl)-5,6-bis (4-phenyl-sulfonic acid)-1,2,4-triazine (Ferrozine), nicotinamide adenine dinucleotide (NADH), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and trichloracetic acid (TCA) were purchased from Sigma Chemical Co. All others unlabelled chemicals and reagents were analytical grade.

2.2. Plant material and extraction

Fennel (*F. vulgare*) seeds (FS) were obtained from a local market at Erzurum, Turkey. For water extraction, 25 g sample of FS ground into a fine powder in a mill and was mixed with 500 ml boiling water by magnetic stirrer during 15 min. Then the extract was filtered over Whatman No.1 paper. The filtrates were frozen and lyophilised in a lyophilisator at 5 μm Hg pressure and at −50°C (Labconco, Freezone 1L). For ethanol extraction, 25 g powder of FS was mixed five times with 100 ml ethanol. Extraction continued until the extraction solvents became colourless (total solvent volume is 500 ml). The obtained extracts were filtered over Whatman No.1 paper and the filtrate was collected, then ethanol was removed by a rotary evaporator at 50°C.

2.3. Total antioxidant activity determination

Total antioxidant activity of both FS extracts was determined according to the thiocyanate method (Mitsuda, Yuasumoto, & Iwami, 1996). Ten milligrams of FS extracts was dissolved in 10 ml water. One milligram of FS extracts in 1 ml of water was added to linoleic acid in potassium phosphate buffer (2.5 ml, 0.04 mol/L, pH 7.0). Fifty millilitre linoleic acid emulsion consisting of 175 μg Tween-20, 155 μl linoleic acid, and 0.04 mol/L potassium phosphate buffer (pH 7.0). On the other hand, 5.0 ml control consisting of 2.5 ml linoleic acid emulsion and 2.5 ml potassium phosphate buffer (0.04 mol/L, pH 7.0). The mixed solution was incubated at 37°C in a glass flask. The peroxide value was determined by reading the absorbance at 500 nm, after reaction with $FeCl_2$ and thiocyanate at several intervals during incubation. The solutions without added extracts used as blank samples. All data are the average of triplicate analyses. The inhibition of lipid peroxidation in percent was calculated by following equation:

$$\% \text{ Inhibition} = 100 - [(A_1/A_0) \times 100],$$

where A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of the FS extracts sample (Duh, Tu, & Yen, 1999).

2.4. Reducing power

The reducing power of FS extracts was determined according to the method of Oyaizu (1986). Different amounts of FS extracts (50–250 μg) in 1 ml of distilled water was mixed with phosphate buffer (2.5 ml, 0.2 mol/L, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm (MSE Mistral 2000, UK) for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

2.5. Superoxide anion scavenging activity

Measurement of superoxide anion scavenging activity of water and ethanol extracts of FS was done based on the method described by Liu, Ooi, and Chang (1997) with slight modification. One millilitre of nitroblue tetrazolium (NBT) solution (156 μmol/L NBT in 100 mmol/L phosphate buffer, pH 7.4) 1 ml NADH solution (468 μmol/L in 100 mmol/L phosphate buffer, pH 7.4) and 0.1 ml of sample solution of FS extracts in water were mixed. The reaction started by adding 100 μl of phenazine methosulphate (PMS) solution (60 μmol/L PMS in 100 mmol/L phosphate buffer, pH 7.4) to the mixture. The reaction mixture was incubated at 25°C for 5 min, and the absorbance at 560 nm was measured against blank samples. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. The percentage inhibition of superoxide anion generation was calculated using the following formula:

$$\% \text{ Inhibition} = [(A_0 - A_1)/A_0] \times 100,$$

where A_0 was the absorbance of the control, and A_1 was the absorbance of the FS extracts and standards (Ye, Wang, Liu, & Ng, 2000).

2.6. Free radical scavenging activity

The free radical scavenging activity of water and ethanol extracts of FS was measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH•) using the method of Shimada, Fujikawa, Yahara, and Nakamura (1992). The 0.1 mmol/L solution of DPPH• in ethanol was prepared and 1 ml of this solution was added 3 ml of FS extracts solution in water at different concentrations (50–250 μg). After 30 min absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The DPPH• concentration in the reaction medium was calculated from the following calibration curve, determined by linear regression (R^2 : 0.9545):

$$\text{Absorbance} = 0.0036 \times [\text{DPPH}\bullet].$$

2.7. Metal chelating activity

The chelating of ferrous ions by the both FS extracts was estimated by the method of Dinis, Madeira, and Almeida (1994). Briefly the extracts samples (50–250 μg/μl) were added to a solution of 2 mmol/L $FeCl_2$

(0.05 ml). The reaction was initiated by the addition of 5 mmol/L ferrozine (0.2 ml) and the mixture was shaken vigorously and left standing at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm. The percentage of inhibition of ferrozine–Fe^{2+} complex formation was given below formula

$$\%\text{Inhibition} = [(A_0 - A_1)/A_0] \times 100,$$

where A_0 was the absorbance of the control, and A_1 was the absorbance in the presence of the sample of FS extracts and standards.

2.8. Scavenging of hydrogen peroxide

The ability of both FS extracts to scavenge hydrogen peroxide was determined according to the method of Ruch, Cheng, and Klaunig (1989). A solution of hydrogen peroxide (2 mmol/L) was prepared in phosphate buffer (pH 7.4). Hydrogen peroxide concentration was determined spectrophotometrically from absorption at 230 nm with molar absorbtivity 81 $mol/L^{-1}/cm$. Extracts samples (50–250 μg/μl) in distilled water were added to a hydrogen peroxide solution (0.6 ml). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing in phosphate buffer without hydrogen peroxide. The percentage of scavenging of hydrogen peroxide of both FS extracts and standard compounds

$$\%\,\text{Scavenged } H_2O_2 = [(A_0 - A_1)/A_0] \times 100,$$

where A_0 was the absorbance of the control, and A_1 was the absorbance in the presence of the sample of FS extracts and standards.

2.9. Determination of total phenolic compounds

Total soluble phenolics in the FS extracts were determined with Folin-Ciocalteu reagent according to the method of Slinkard and Singleton (1977) using gallic acid as a standard phenolic compound. Briefly, 1 ml of extract solution contains 1000 μg extract was mixed with 45 ml distilled water. One millilitre of Folin-Ciocalteu reagent was added and the content of the flask mixed thoroughly. After 3 min 3 ml of Na_2CO_3 (2%) was added then the mixture was allowed to stand for 2 h with intermittent shaking. The absorbance was measured at 760 nm. The concentration of total phenolic compounds in the both FS extracts determined as microgram of gallic acid equivalent by using an equation that was obtained from standard gallic acid graph. The equation is given below

$$\text{Absorbance} = 0.0008 \times \text{gallic acid (μg)}.$$

2.10. Statistical analysis

Experimental results were mean ±S.D. of five parallel measurements. P values <0.05 were regarded as significant and P values <0.01 very significant.

3. Results and discussion

3.1. Total antioxidant activity

Total antioxidant activity of ethanol and water extracts of FS was determined by the thiocyanate method. Table 1 shows the extraction yields and gallic acid equivalents. In addition, Table 2 shows inhibition of lipid peroxidation in percent of water and ethanol extracts of FS, α-tocopherol, BHA and BHT. Ethanol and water extracts of FS exhibited effective antioxidant activity at all concentrations. The effects of various amounts of water and ethanol extracts of FS (50–250 μg) on peroxidation of linoleic acid emulsion are shown in Figs. 1 and 2. The antioxidant activity of ethanol and water extracts of FS increased with increasing concentration. The all of doses of water and ethanol extracts of FS showed higher antioxidant activities than that 100 μg of α-tocopherol. As seen Table 2, the percentage of inhibition of 50, 100 and 250 μg doses of water extracts of FS on peroxidation in linoleic acid system was 83.9, 91.6, 96.7 and 50, 100 and 250 μg doses of ethanol extracts was 65.5%, 78.6%, and 91.8%, respectively. The values of both extracts were greater than that 100 μg

Table 1
Yield and gallic acid equivalents (GAE) of water and ethanol extracts of FS [FS: fennel (*F. vulgare*) seeds]

Solvent	Yield (g/100 g)	GAE (μg)
Water	16.20	21.25
Ethanol	10.95	90.00

Table 2
Inhibition of lipid peroxidation in percent of water and ethanol extracts of FS, α-tocopherol, BHA, and BHT [FS: Fennel (*F. vulgare*) seeds]

Sample	Inhibition of lipid peroxidation (%)[a]
Water extract	91.6
Ethanol extract	98.6
α-Tocopherol	36.9
BHA	94.3
BHT	97.8

[a] The antioxidant activity of the samples (100 μg) was determined by the thiocyanate method. The peroxide values were determined by reading the absorbance at 500 nm after reaction with $FeCl_2$ and thiocyanate.

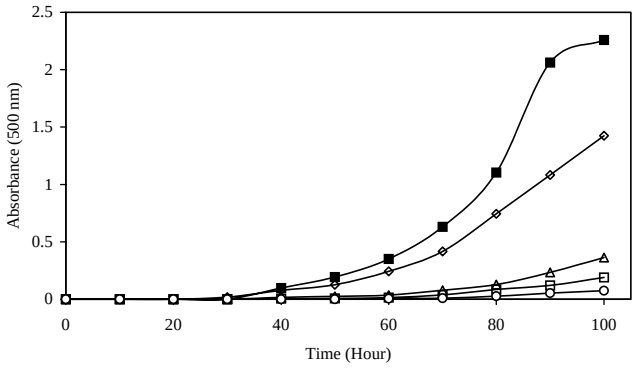

Fig. 1. Antioxidant activity of different doses of water extracts of FS and α-tocopherol in the linoleic acid emulsion was determined by the thiocyanate method. ■, control; ◇, α-tocopherol; △, water extract of fennel (50 μg); □, water extract of fennel (100 μg); ○, water extract of fennel (250 μg).

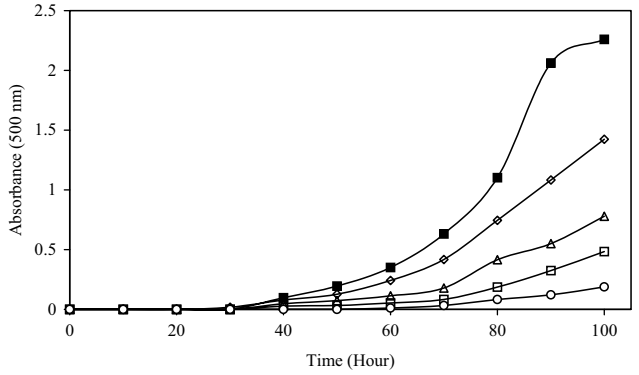

Fig. 2. Antioxidant activity of different doses of ethanol extracts of FS and α-tocopherol in the linoleic acid emulsion was determined by the thiocyanate method. ■, control; ◇, α-tocopherol; △, ethanol extract of fennel (50 μg); □, ethanol extract of fennel (100 μg); ○, ethanol extract of fennel (250 μg).

of α-tocopherol (36.9%), but lower than that same dose of BHA and BHT (94.3% and 97.8%).

3.2. Reducing power

Fig. 3 shows the reductive capabilities of samples FS extracts compared to BHA, BHT and α-tocopherol. For the measurements of the reductive ability, we investigated the Fe^{3+}–Fe^{2+} transformation in the presence of FS extracts samples using the method of Oyaizu (1986). The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Meir, Kanner, Akiri, & Hadas, 1995). However the antioxidant activity of antioxidants have been attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging (Diplock, 1997; Yıldırım et al., 2000). Like the antioxidant activity, the reducing power of

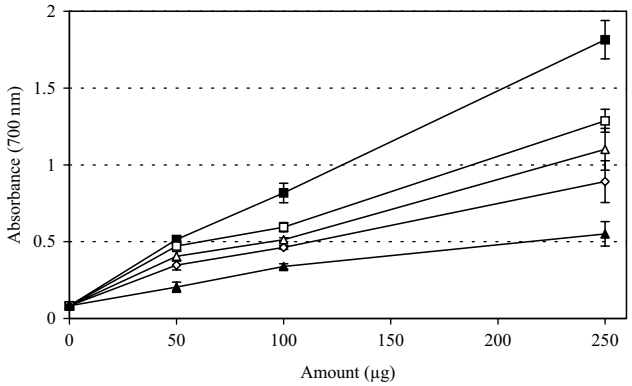

Fig. 3. Reducing power of water and ethanol extracts of FS, BHA, BHT, and α-tocopherol. Results are mean ± S.D. of five parallel measurements. P value <0.01 when compared to control. (Spectrophotometric detection of the Fe^{+3}–Fe^{+2} transformation.) [FS: fennel (*F. vulgare*) seed; BHA: buthylated hydroxyanisole; BHT: Buthylated hydroxytoluene]. ■, BHA; □, BHT; ◇, α-tocopherol; △, water extract of fennel; ▲, ethanol extract of fennel.

both FS extracts increased with increasing amount of sample. All of the amounts of FS extracts showed higher activities than control and these differences were statistically very significant ($P<0.01$). Reducing power of water and ethanol extracts of FS and standard compounds followed the order: BHA > BHT > water extract of FS > α-tocopherol > ethanol extract of FS.

3.3. Superoxide anion scavenging activity

In the PMS/NADH-NBT system, superoxide anion derived from dissolved oxygen by PMS/NADH coupling reaction reduces NBT. The decrease of absorbance at 560 nm with antioxidants thus indicates the consumption of superoxide anion in the reaction mixture. Fig. 4 shows the % inhibition of superoxide radical generation of 50, 100, and 250 μg of water and ethanol extracts of FS and comparison with same doses of BHA, BHT, and α-tocopherol. The both extracts of FS have strong superoxide radical scavenging activity and exhibited higher superoxide radical scavenging activity than BHT and α-tocopherol. The results were found statistically significant ($P<0.05$). The percentage inhibition of superoxide generation by 250 μg doses of BHA, water and ethanol extracts of FS was found as 98.7, 96.6 and 94.0% and greater than that same doses of BHT and α-tocopherol (88.3 and 80.5%), respectively. Superoxide radical scavenging activity of those samples followed the order: BHA > water extract of FS > ethanol extract of FS > BHT > α-tocopherol.

3.4. Free radical scavenging activity

DPPH• is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic

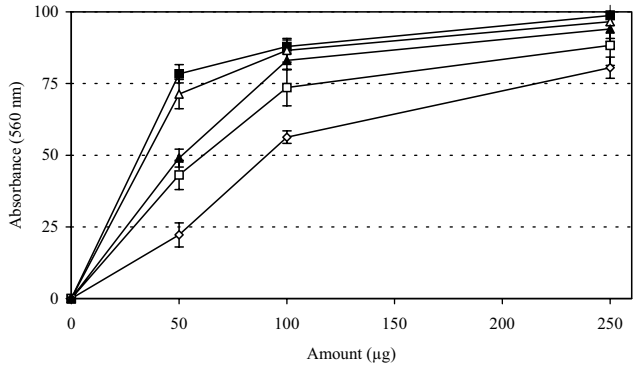

Fig. 4. Superoxide anion radical scavenging activity of water and ethanol extracts of FS, BHA, BHT, and α-tocopherol by the PMS/NADH-NBT method. Results are mean ± S.D. of five parallel measurements. P value <0.05 when compared to control. [FS: Fennel (*F. vulgare*) seed; BHA: buthylated hydroxyanisole; BHT: buthylated hydroxytoluene]. ■, BHA; □, BHT; ◇, α-tocopherol; △, water extract of fennel; ▲, ethanol extract of fennel.

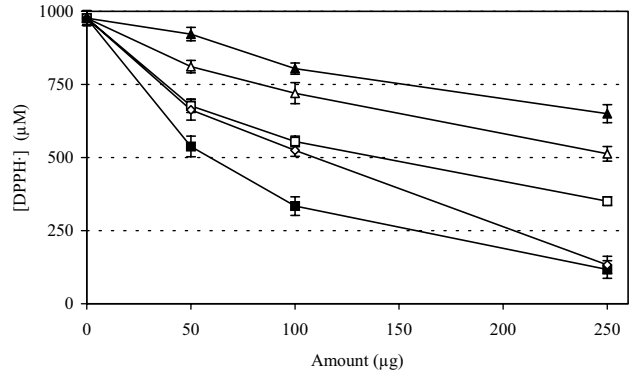

Fig. 5. Free radical scavenging activity of water and ethanol extracts of FS, BHA, BHT, and α-tocopherol by 1,1-diphenyl-2-picrylhydrazyl radicals. Results are mean ± S.D. of five parallel measurements. P value <0.05 when compared to control. [FS: fennel (*F. vulgare*) seed; BHA: buthylated hydroxyanisole; BHT: buthylated hydroxytoluene]. ■, BHA; □, BHT; ◇, α-tocopherol; △, water extract of fennel; ▲, ethanol extract of fennel.

molecule (Soares, Dins, Cunha, & Ameida, 1997). The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm induced by antioxidants. Hence, DPPH• is usually used as a substrate to evaluate antioxidative activity of antioxidants (Oyaizu, 1986). Fig. 5 illustrates a significant ($P<0.05$) decrease the concentration of DPPH radical due to the scavenging ability of soluble solids in the both extracts of FS and standards. We used BHA, BHT and α-tocopherol as standards. The scavenging effect of water and ethanol extracts of FS and standards on the DPPH radical decreased in the order of BHA > α-tocopherol > BHT > water extract > ethanol extract and were 88.02%, 86.38%, 64.07%, 47.49% and 36.46% at the dose of 250 µg, respectively. These results indicated that the both FS extracts have a noticeable effect on scavenging free radical. Free radical scavenging activity also increased with increasing concentration. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH}\bullet\text{ scavenging effect}(\%) = [(A_0 - A_1/A_0) \times 100],$$

where A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of the sample of FS extracts.

3.5. Metal chelating activity

The chelating of ferrous ions by the extracts of FS was estimated by the method of Dinis et al. (1994). Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of other chelating agents, the complex formation is disrupted with the result that the red colour of the complex is decreased. Measurement of the rate of colour reduction therefore allows estimation of the chelating activity of the coexisting chelator (Yamaguchi, Ariga, Yoshimira, & Nakazawa, 2000). In this assay the both extracts of FS and standard compounds interfered with the formation of ferrous and ferrozine complex, suggesting that it has chelating activity and captures ferrous ion before ferrozine.

As shown in Fig. 6, the formation of the ferrozine–Fe^{2+} complex is not complete in the presence of water and ethanol extracts of FS, indicating that both extracts of FS chelate the iron. The absorbance of Fe^{2+}–ferrozine complex was linearly decreased dose dependently (from 50 to 250 µg). The difference between both extracts of FS and the control was statistically significant ($P<0.05$). The percentage of metal scavenging capacity of 250 µg doses of water and ethanol extracts of FS, α-tocopherol, BHA, and BHT were found as 30.7%, 26.5%, 43.0%, 74.8% and 40.6%, respectively. The metal scavenging effect of the both extracts of FS and standards decreased in the order of BHA > α-tocopherol > BHT > water extract > ethanol extract of FS.

Metal chelating capacity was significant since they reduced the concentration of the catalysing transition metal in lipid peroxidation (Duh et al., 1999). It was reported that chelating agents, which form σ-bonds with a metal, are effective as secondary antioxidants because they reduce the redox potential thereby stabilising the oxidised form of the metal ion (Gordon, 1990). The data obtained from Fig. 6 reveal that the both extracts of FS demonstrate an effective capacity for iron binding, suggesting that its action as peroxidation protector may be related to its iron binding capacity.

3.6. Scavenging of hydrogen peroxide

The ability of the both extracts of FS to scavenge hydrogen peroxide was determined according to the

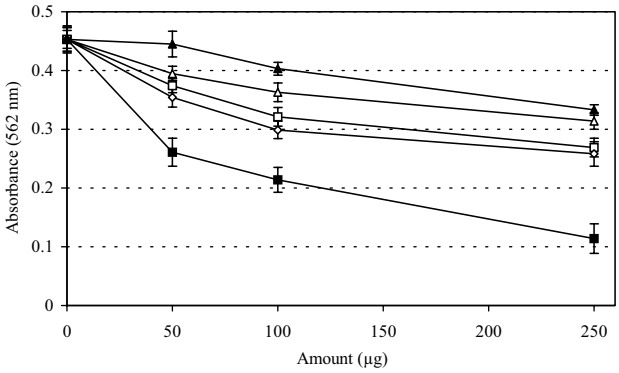

Fig. 6. Metal chelating effect of different amount of water and ethanol extracts of FS, BHA, BHT, and α-tocopherol on ferrous ions. Each value is expressed as mean ± S.D. of five parallel measurements. P value <0.05 when compared to control. [FS: fennel (*F. vulgare*) seed; BHA: buthylated hydroxyanisole; BHT: buthylated hydroxytoluene]. ■, BHA; □, BHT; ◇, α-tocopherol; △, water extract of fennel; ▲, ethanol extract of fennel.

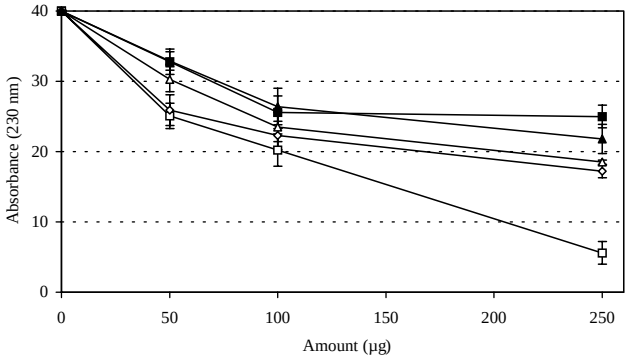

Fig. 7. Hydrogen peroxide scavenging activities of water and ethanol extracts of FS, BHA, BHT, and α-tocopherol. Results are mean ± S.D. of five parallel measurements. P value <0.05 when compared to control. [FS: fennel (*F. vulgare*) seed; BHA: buthylated hydroxyanisole; BHT: buthylated hydroxytoluene]. ■, BHA; □, BHT; ◇, α-tocopherol; △, water extract of fennel; ▲, ethanol extract of fennel.

method of Ruch et al. (1989). The scavenging ability of water and ethanol extracts of FS on hydrogen peroxide is shown Fig. 7 and compared with BHA, BHT and α-tocopherol as standards. The both FS extracts were capable of scavenging hydrogen peroxide in an amount-dependent manner. 250 µg of water and ethanol extracts of FS exhibited 53.8% and 45.5% scavenging activity on hydrogen peroxide, respectively. In the other hand, at the same dose, BHA, BHT, and α-tocopherol exhibited 37.5%, 86%, and 57% hydrogen peroxide scavenging activity. These results showed that the both FS extracts had stronger hydrogen peroxide scavenging activity. Those values close to BHA, but lower than that BHT and α-tocopherol. There was statistically significant correlation between those values and control ($P<0.05$). The hydrogen peroxide scavenging effect of 250 µg of the both extracts of FS and standards decreased in the order of BHT > α-tocopherol > water extract of FS > BHA > ethanol extract of FS. Hydrogen peroxide itself is not very reactive, but it can sometimes be toxic to cell because of it may give rise to hydroxyl radical in the cells (Halliwell, 1991). Thus, the removing of H_2O_2 is very important for antioxidant defence in cell or food systems.

3.7. The total phenolic compounds

The phenolic compounds are very important plant constituents because of their scavenging ability due to their hydroxyl groups (Hatano, Edamatsu, Mori, Fujita, & Yasuhara, 1989). It was determined that there were 21, 25 and 90.0 µg gallic acid equivalent of phenolic compounds in the 1 mg of the water and ethanol extracts of FS, respectively (Table 1). These results indicate that there is no correlation between antioxidant activity and total phenolic content. However, different results were reported on this aspect; some authors found correlation between phenolic content and antioxidant activity (Yen, Duh, & Tsai, 1993; Yang, Lin, & Mau, 2002), whereas the others found no such relationship, since other compounds are responsible for the antioxidant activity (Bocco, Cuvelier, Richard, & Berset, 1998; Maillard & Berset, 1995; Heinonen, Lehtonen, & Hopia, 1998; Kahkonen et al., 1999). The phenolic compounds may contribute directly to antioxidative action (Duh et al., 1999). It is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when up to 1.0 g daily ingested from a diet rich in fruits and vegetables (Tanaka, Kuei, Nagashima, & Taguchi, 1998).

As a conclusion, the water and ethanol extracts of FS showed strong antioxidant activity, reducing power, DPPH radical, superoxide anion scavenging, hydrogen peroxide scavenging, and metal chelating activities when compared to standards such as BHA, BHT, and α-tocopherol. The results of this study show that the water and ethanol extract of FS can be used as easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical industry. It can be used in stabilising food against oxidative deterioration. However, the polyphenolic compounds or other components responsible for the antioxidant activity of water and ethanol extracts of FS are already unknown. Therefore, it is suggested that further work could be performed on the isolation and identification of the antioxidant components in FS.

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