Asian Journal of Chemistry; Vol. 25, No. 7 (2013), 3573-3578

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2013.13657

In Vitro Antioxidant Activity of Various Extracts of Ginger (*Zingiber officinale* L.) Seed

YESIM YESILOGLU[1,*], HATICE AYDIN[1] and ISMAIL KILIC[2]

[1]Department of Chemistry, Faculty of Science, Trakya University, Edirne, Turkey
[2]Faculty of Education, Trakya University, Edirne, Turkey

*Corresponding author: Fax: +90 284 2358754; Tel: +90 284 2359592; E-mail: yesimyesiloglu@trakya.edu.tr

(*Received*: 27 February 2012; *Accepted*: 4 January 2013) AJC-12651

The methanolic and ethanolic extracts from ginger (*Zingiber officinale* L.) seed (ZOS) were investigated for their antioxidant and radical scavenging activities in eight different assays, namely, total antioxidant activity, reducing power, 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, total flavonoid content, total phenolic compound and metal chelating activities. Both methanolic extract and ethanolic extract of *Zingiber officinale* L. seed exhibited strong total antioxidant activity. These various antioxidant activities were compared with synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene, Trolox, α-tocopherol and ascorbic acid. The methanol extract of *Zingiber officinale* L. seed exhibited greater antioxidant capacity than that of ethanol.

Key Words: *Zingiber officinale* L., Antioxidant activity, Ginger, Free radicals, Scavenging activity.

INTRODUCTION

Lipid peroxidation is one of the major reasons for deterioration of food products during processing and storage. Radicals are known to take part in lipid peroxidation and play an important role in the progression of a great number of pathological disturbances, such as atherosclerosis, brain dysfunction, cancer promotion, heart diseases, immune system decline and neurodegenerative diseases[1]. Antioxidants are the compounds that, when added to food products, act as radical scavengers, prevent the radical chain reactions of oxidation, delay or inhibit the oxidation process and increase shelf life by retarding the process of lipid peroxidation[2].

Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are commonly used as antioxidants in foods to prevent or retard lipid oxidation. However, restrictions on the use of these compounds are being imposed because of their carcinogenicity and some side effects[3]. Thus, evaluation of the antioxidative activity of naturally occurring substances has been the focus of interest in recent years[4].

Consumers are becoming more conscious of the nutritional value and safety of their food and ingredients. The preference for natural foods and food ingredients that are believed to be safer, healthier and less subject to hazards is increasing compared to their synthetic counterparts. Thus, the evaluation of antioxidative activity of naturally occurring substances has beeen focus of interest in recent years[5]. The use of spices in processed foods is becoming of increasing importance in the food industry as an alternative to synthetic antioxidants[6]. Spices are dietary constituents consumed daily to enhance the flavour or taste of human food.

Rhizomes of ginger plants (family Zingiberaceae) have been widely used as spices or condiments[7]. Rhizomes are eaten raw or cooked as vegetables and used for flavouring food. It has been used extensively for headaches, nausea and colds. It has also been suggested for the treatment of various other conditions, including atherosclerosis, migraine headaches, rheumatoid arthritis, high cholesterol, ulcers, depression and impotence[8]. In addition to these medicinal uses, ginger continues to be valued around the world as an important cooking spice and is believed to help the common cold, flu-like symptoms and even painful menstrual periods. Due to these properties, it has gained considerable attention as a botanical dietary supplement in the USA and Europe in recent years. The phenolic substances present in herb and spices have cancer chemopreventive activities, both in *in vitro* as well as *in vivo* animal models[9]. These agents are believed to suppress the transformative, hyperproliferative and inflammatory processes that initiate carcinogenesis, as well as the later steps of carcinogenesis, namely angiogenesis and metastasis. Some phenolic substances present in ginger, generally, possess strong anti-inflammatory and antioxidative properties and exert substantial anticarcinogenic and antimutagenic activities[10].

Past studies on the antioxidant properties of ginger species were confined to rhizomes[11]. Skin-lightening cosmeceutical products were recently developed from rhizomes of gingers[12]. However, there is no information about methanol extract and ethanol extract of *Zingiber officinale* L. seed.

The aim of the present study was to determine the antioxidant activity by using different antioxidant tests including total antioxidant activity by ferric thiocyanate method, reducing power, free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, total flavonoid content, total phenolic compound and metal chelating activities.

EXPERIMENTAL

Linoleic acid, α-tocopherol, ethylenediaminetetraacetic acid (EDTA), potassium persulfate, nicotinamide adenine dinucleotide (NADH), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), nitroblue tetrazolium (NBT), phenazine methosulfate (PMS), 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 3-(2-pyridyl)-5,6-*bis*(4-phenyl-sulfonic acid)-1,2,4-triazine (ferrozine) were obtained from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany).

Ammonium thiocyanate, ferrous chloride, polyoxyethylenesorbitan monolaurate (Tween-20), trichloroacetic acid were purchased from Merck. All other chemicals used were in analytical grade and obtained from either Sigma-Aldrich or Merck.

Plant material and extraction procedures: *Zingiber officinale* L. seed was obtained from a spice seller at Tekirdag City (Tekirdag, Turkey). Seeds were kept at ambient temperature (26 ± 2 ºC) overnight before the analyses were performed. For solvent extraction, 25 g sample was put into a fine powder in a mill and was mixed with 500 mL solvent (methanol or ethanol). The residue was re-extracted until extraction solvents became colorless. The obtained extracts were filtered over Whatman No. 1 paper and the filtrate was collected, then solvent was removed by a rotary evaporator (Buchi R-200, Switzerland) at 40 ºC to obtain dry extract. Both extracts were placed in a plastic bottle and then stored at -20 ºC until used.

Total antioxidant activity determination: The total antioxidant activity of *Zingiber officinale* L. seed was determined according to the thiocyanate method[13]. For stock solutions, 10 mg solvent extract of *Zingiber officinale* L. seed was dissolved in 10 mL solvent. Then, the solution, which contains the same concentration of *Zingiber officinale* L. seed extracts or standard samples (150 µg/mL) in 2.5 mL of potassium phosphate buffer (0.04 M, pH 7) was added to 2.5 mL of linoleic acid emulsion in potassium phosphate buffer (0.04 M, pH 7). Fifty millilitres linoleic acid emulsion contained 175 µg Tween-20, 155 µL linoleic acid and 0.04 M potassium phosphate buffer (pH 7.0). On the other hand, 5 mL control was composed of 2.5 mL linoleic acid emulsion and 2.5 mL, 0.04 M potassium phosphate buffer (pH 7). The mixed solution (5 mL) was incubated at 37 ºC in a glass flask. At regular intervals during incubation, a 0.1 mL aliquot of the mixture was diluted with 3.7 mL of solvent (ethanol or methanol), followed by the addition of 0.1 mL of 30 % ammonium thiocyanate and 0.1 mL of 20 mM ferrous chloride in 3.5 % hydrochloric acid. The peroxide level was determined by reading the absorbance at 500 nm in a spectrophotometer (Shimadzu UV-1601, Japan). This step was repeated every 10 h until the control reached its maximum absorbance value. Therefore, high absorbance indicates high linoleic acid emulsion oxidation. All data on total antioxidant activities are the average of triplicate experiments. The per cent inhibition of lipid peroxidation in linoleic acid emulsion was calculated by the following equation:

$$\text{Inhibition of lipid peroxidation (\%)} = \left[\frac{(A_0 - A_1)}{A_0}\right] \times 100$$

A_0 is the absorbance of the control, A_1 is the absorbance of the sample.

Reducing power: The reducing power of *Zingiber officinale* L. seed extracts was determined by the method of Oyaizu[14]. Different concentrations of *Zingiber officinale* L. seed extracts (50-250 µg/mL) in 1 mL of distilled water were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 mL, 1 %). The mixture was incubated at 50 ºC for 20 min. Aliquots (2.5 mL) of trichloroacetic acid (10 %) were added to the mixture, which was then centrifuged for 10 min at 1500 × g (Hettich, Rotina 38 R, Germany). The upper layer of the 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 in a spectrophotometer. Increased absorbance of the reaction mixture indicates increased reducing power.

Superoxide anion scavenging activity: Measurement of superoxide anion scavenging activity of *Zingiber officinale* L. seed extracts was based on the method described by Liu *et al.*[15], with slight modification. Superoxide radicals are generated in PMS-NADH systems by oxidation of NADH and assayed by the reduction of nitroblue tetrazolium (NBT). In this experiments, the superoxide radicals were generated in 3 mL of *Tris*-HCl buffer (16 mM, pH 8.0) containing 1 mL of NBT (50 µM) solution, 1 mL NADH (78 µM) solution and sample solution of *Zingiber officinale* L. seed extracts (from 50-250 µg/mL) in water. The reaction started by adding 1 mL of phenazine methosulphate (PMS) solution (10 µM) to the mixture. The reaction mixture was incubated at 25 ºC for 5 min and the absorbance at 560 nm was measured against blank samples. Decreased absorbance of the reaction mixture indicates increased superoxide anion scavenging activity. All data are an average of triplicate analyses. The percentage inhibition of superoxide anion generation was calculated using the following formula:

$$\text{Inhibition (\%)} = \left[\frac{(A_0 - A_1)}{A_0}\right] \times 100$$

A_0 is the absorbance of the control. A_1 is the absorbance of the sample.

Free radical scavenging activity: The free radical scavenging activity of *Zingiber officinale* L. seed extracts was measured by the 1,1-diphenyl-2-picryl-hydrazil ($DPPH^{\bullet}$) method proposed by Shimada *et al.*[16]. Briefly, 0.1 mM solution of $DPPH^{\bullet}$ in ethanol (or methanol) was prepared and 1 mL of this solution was added to 3 mL of *Zingiber officinale* L. seed extracts solution at different concentrations (50-250 µg/mL). After 0.5 h, the absorbance was measured at 517 nm. Lower

absorbance of the reaction mixture indicates higher free radical scavenging activity. The capacity to scavenge the DPPH$^{\bullet}$ radical was calculated using the following equation:

$$\text{DPPH}^{\bullet}\text{ scavenging effect (\%)} = \left[\frac{(A_0 - A_1)}{A_0}\right] \times 100$$

A_0 is the absorbance of the control. A_1 is the absorbance of the sample.

Metal chelating activity on ferrous ions (Fe^{2+}): The ferrous ions (Fe^{2+}) chelating activities of both *Zingiber officinale* L. seed extracts and standards were investigated according to the method of Dinis *et al.*[17]. Briefly, extracts (50-250 µg/mL) were added to a solution of 2 mM $FeCl_2$ (0.05 mL). The reaction was initiated by the addition of 5 mM 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. All test and analyses were run in triplicate and averaged. The percentage of inhibition of ferrozine-Fe^{2+} complex formation was calculated using the formula given bellow:

$$\text{Metal chelating effect (\%)} = \left[\frac{(A_0 - A_1)}{A_0}\right] \times 100$$

A_0 is the absorbance of the control. A_1 is the absorbance of the sample.

Scavenging of hydrogen peroxide: The hydrogen peroxide scavenging ability of the *Zingiber officinale* L. seed extracts was determined according to the method of Ruch *et al.*[18]. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Extracts (50-250 µg/mL) in distilled water were added to a hydrogen peroxide solution (0.6 mL, 40 mM). Absorbance of hydrogen peroxide at 230 nm was determined 10 min later against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging of both *Zingiber officinale* L. seed extracts and standard compounds was calculated:

$$H_2O_2\text{ scavenging activity (\%)} = \left[\frac{(A_0 - A_1)}{A_0}\right] \times 100$$

A_0 is the absorbance of the control. A_1 is the absorbance of the sample.

Determination of total phenolic compounds: Total soluble phenolics in the *Zingiber officinale* L. seed extracts were determined with Folin-Ciocalteu reagent according to the method of Slinkard and Singleton[19] using gallic acid as a standard phenolic compound. Briefly, 1 mL of extract solution containing 1 mg exracts in a volumetric flask was diluted with distilled water (46 mL). 1 mL of Folin-Ciocalteu reagent was added and the content of the flask mixed thoroughly. After 3 min, 3 mL of Na_2CO_3 (2 %) were added and the mixture was allowed to stand for 2 h with intermittent shaking. The absorbance was measured at 760 nm. Results were expressed as milligrams of total phenolics (gallic acid equivalents) per gram dry weight (mg GAE/g DW). The calibration equation for gallic acid was $y = 0.0011x - 0.0022$ ($R^2 = 0.9992$).

Total flavonoid contents: Total flavonoid content was determined by a colorimetric method described by Wang *et al.*[20], with minor modification. An aliquot of 10 mL of appropriate dilution of each extract was added to volumetric flask containing 1 mL of 5 % (w/v) sodium nitrite and placed for 6 min, followed by reaction with 1 mL of (10 %) (w/v) aluminum nitrate to form a flavonoid-aluminum complex. After 6 min, 10 mL of 4.3 % (w/v) NaOH was added and the total was made up to 25 mL with distilled water. After 15 min at room temperature, the final solution was mixed well again and the absorbance was measured against a blank at 510 nm with a UV-1601 UV/VIS recording spectrophotometer (Shimadzu UV-1601, Japan). The total flavonoids were expressed as milligrams of pyrocatechol equivalents (PE) per gram of dry weight (mg PE/g DW). The calibration equation for pyrocatechol was $y = 0.0024x + 0.0244$ ($R^2 = 0.9985$).

Statistical analysis: All data are the average of triplicate analyses. The data were recorded as mean ± standard deviation and analyzed by SPSS (version 11.5 for Windows 98, SPSS Inc.). One-way analysis of variance was performed by ANOVA procedures. Significant differences between means were determined by Duncan's Multiple Range tests. p values < 0.05 were regarded as significant and p values < 0.01 were significant.

RESULTS AND DISCUSSION

Total antioxidant activity by ferric thiocyanate method measures the amount of peroxide produced during the initial stages of oxidation and peroxide is the primary product of oxidation. The effect of 150 µg/mL concentration of methanolic and ethanolic extracts on lipid peroxidation of linoleic acid emulsion are shown in Fig. 1 and were found as 79.4 and 82.3 %, respectively. Otherwise, α-tocopherol, ascorbic acid, BHA and BHT exhibited 45, 50.3, 34.3 and 42.2 % inhibition, respectively, on peroxidation of linoleic acid emulsion at the same concentration. The results clearly showed that both *Zingiber officinale* L. seed extracts had stronger total antioxidant activity than α-tocopherol, ascorbic acid, BHA and BHT at the same concentration (150 µg/mL).

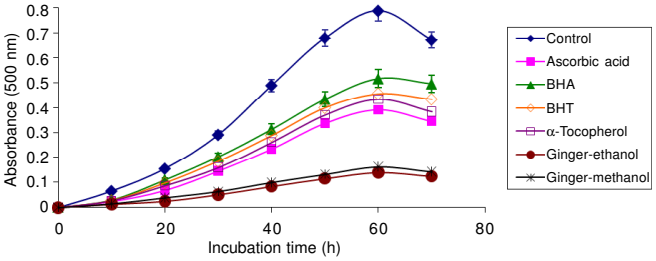

Fig. 1. Total antioxidant activities of extracts of *Zingiber officinale* L. seed, ascorbic acid, α-tocopherol, BHA and BHT at the same concentration (150 µg/mL) as determined by the thiocyanate method. Results are average of triplicate experiments

Fig. 2 shows the reductive capabilities of *Zingiber officinale* L. seed extracts compared with BHA, BHT, α-tocopherol and ascorbic acid. For the measurements of the reductive ability, we investigated the Fe^{3+}-Fe^{2+} transformation in the presence of *Zingiber officinale* L. seed extracts using the method of Oyaizu[14]. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity[21]. The antioxidant activity of putative antioxidants have been attributed to various mechanisms, among which are prevention

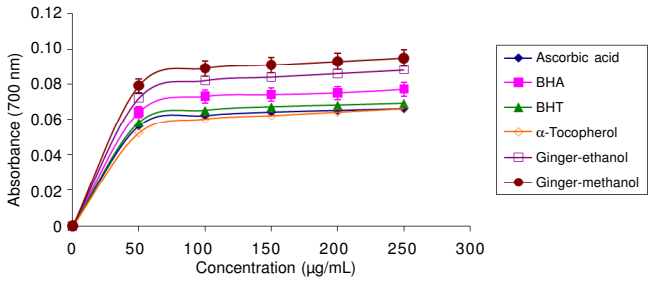

Fig. 2. Total reductive potential of different concentrations (50 and 250 µg/mL) of extracts of *Zingiber officinale* L. seed, ascorbic acid, α-tocopherol, BHA and BHT using spectrophotometric detection of Fe^{3+}-Fe^{2+} transformation

of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging. Like the antioxidant activity, the reducing power of *Zingiber officinale* L. seed extracts increased with increasing concentrations of sample. Reducing power of methanol and ethanol extracts of *Zingiber officinale* L. seed and standard compounds exhibited the following order: methanol extract of *Zingiber officinale* L. seed > ethanolic extract of ZOS > BHA > BHT > ascorbic acid > α-tocopherol.

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 indicates the consumption of superoxide anion in the reaction mixture. Fig. 3 shows the % inhibition of superoxide radical generation by 50, 100, 150, 200 and 250 µg/ml of methanol and ethanol extracts of *Zingiber officinale* L. seed and comparison with same concentrations of BHA, BHT and α-tocopherol. Both extracts of *Zingiber officinale* L. seed exhibited higher superoxide radical scavenging activity than α-tocopherol. As seen in Fig. 3, the percentage inhibition of superoxide generation by 250 µg/mL concentration of methanol and ethanol extracts of *Zingiber officinale* L. seed was found as 30.6 and 28.6 % and lower than that same doses of BHA and BHT (44.9 and 34.6 %), respectively. Superoxide radical scavenging activity of those samples showed the following order: BHA > BHT > methanol extract of ZOS > ethanol extract of *Zingiber officinale* L. seed > α-tocopherol.

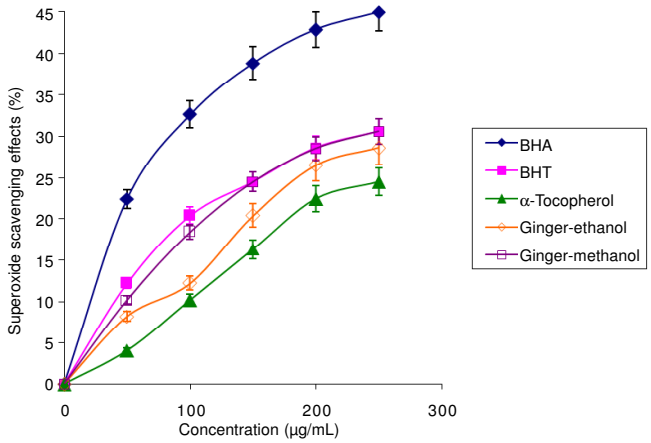

Fig. 3. Superoxide anion radical scavenging activity of different concentrations of extracts of *Zingiber officinale* L. seed, α-tocopherol, BHA and BHT by the PMS-NADH-NBT method

DPPH• is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule[22]. The reduction capability of DPPH• radicals was determined by the decrease in its absorbance at 517 nm, which is induced by antioxidants. Hence, DPPH• is often used as a substrate to evaluate antioxidative activity of antioxidants[23]. Fig. 4 illustrates a significant decrease in the concentration of DPPH• radical due to the scavenging ability of the extracts of *Zingiber officinale* L. seed and standards. We used BHA, BHT and α-tocopherol as standards. The scavenging effect of methanol and ethanol extracts of *Zingiber officinale* L. seed and standards on the DPPH• radical decreased in that order: BHA > α-tocopherol > BHT > ethanolic extract of *Zingiber officinale* L. seed > methanolic extract of *Zingiber officinale* L. seed, which were 52.5, 50.7, 49.8, 39.6 and 36.1 %, respectively, at the concentration of 250 µg/mL. EC_{50} values were found to be ± 3.96 and ± 3.61 µg/mL for ethanolic extract of *Zingiber officinale* L. seed and methanolic extract of *Zingiber officinale* L. seed, respectively. A higher DPPH radical scavenging activity is associated with a lower EC_{50} value.

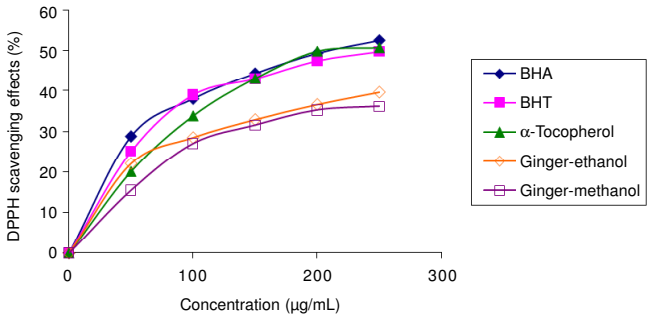

Fig. 4. Free radical scavenging activity of extracts of *Zingiber officinale* L. seed, α-tocopherol, BHA and BHT by 1,1-diphenyl-2-picrylhydrazyl radicals

The chelating of ferrous ions by extracts of *Zingiber officinale* L. seed was estimated by the method of Dinis *et al.*[17]. Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of chelating agents, the complex formation is disrupted, resulting in a decrease in the red colour of the complex. Measurement of colour reduction therefore allows estimating the metal chelating activity of the coexisting chelator[24]. In this assay both extracts of *Zingiber officinale* L. seed and standard compounds are interfered with the formation of ferrous and ferrozine complex, suggesting that they have chelating activity and are able to capture ferrous ion before ferrozine.

EDTA is a strong metal chelating. Hence it is used as standard metal chelating agent in this study. Ferrous ion chelating activities of both extracts of *Zingiber officinale* L. seed, BHA, BHT, ascorbic acid, α-tocopherol and EDTA are shown in Fig. 5. The absorbance of Fe^{2+}-ferrozine complex was linearly decreased dose dependently (from 50, 100, 150, 200 and 250 µg/mL). The percentages of metal scavenging capacity of 250 µg/mL concentration of methanol and ethanol extracts of *Zingiber officinale* L. seed, BHA, BHT, ascorbic acid, α-tocopherol and EDTA were found as 22.3, 30.2, 49.1, 42.8, 34, 39.4 and 54.9 %, respectively. The metal scavenging effect of both extracts of *Zingiber officinale* L. seed and standards decreased in the order of EDTA > BHA > BHT > α-tocopherol > ascorbic acid

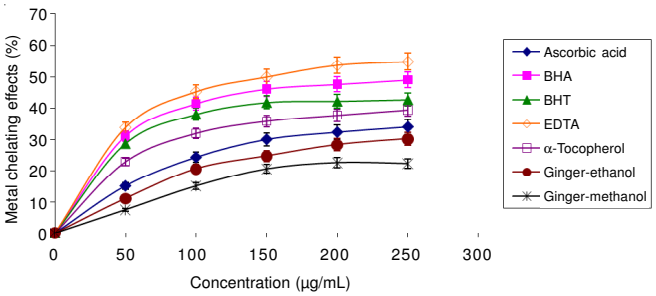

Fig. 5. Metal chelating effect of different concentrations of extracts of *Zingiber officinale* L. seed, ascorbic acid, α-tocopherol, EDTA, BHA and BHT on ferrous ions

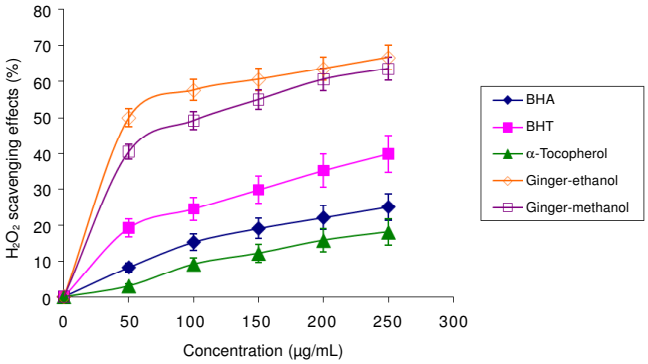

Fig. 6. Hydrogen peroxide scavenging activity of different conccentration of extracts of ZOS, α-tocopherol, BHA and BHT

> ethanolic extract of *Zingiber officinale* L. seed > methanolic extract of *Zingiber officinale* L. seed.

Metal chelating capacity was significant, since it reduced the concentration of the catalysing transition metal in lipid peroxidation[24]. 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 stabilizing the oxidized form of the metal ion. The data obtained from Fig. 5 reveal that both extracts of *Zingiber officinale* L. seed demonstrate a marked capacity for iron binding, suggesting that their action as peroxidation protector may be related to its iron binding capacity.

The ability of the both extracts of *Zingiber officinale* L. seed to scavenge hydrogen peroxide was determined according to the method of Ruch *et al.*[18]. The scavenging ability of methanol and ethanol extracts of *Zingiber officinale* L. seed on hydrogen peroxide is shown in Fig. 6 and compared with that of BHA, BHT and α-tocopherol as standards. Extracts of *Zingiber officinale* L. seed were capable of scavenging hydrogen peroxide in a concentration-dependent manner. Of methanol and ethanol extracts 250 µg/mL of *Zingiber officinale* L. seed exhibited 63.6 and 66.7 % scavenging activity on hydrogen peroxide, respectively. In the other hand, BHA, BHT and α-tocopherol exhibited 25, 39.9 and 18 % hydrogen peroxide scavenging activity at the same dose. These results showed that both *Zingiber officinale* L. seed extracts had effective hydrogen peroxide scavenging activity. The hydrogen peroxide scavenging effect of 250 µg/mL concentration of the both extracts of *Zingiber officinale* L. seed and standards decreased in the order of ethanol extract of *Zingiber officinale* L. seed > methanol extract of *Zingiber officinale* L. seed > BHT > BHA > α-tocopherol. Hydrogen peroxide itself is not very reactive, but it can sometimes be toxic to cell because it may give rise to hydroxyl radical in the cell[25]. Thus, removing H_2O_2 as well as O_2-radical is important for protection of biological systems.

Scavenging of H_2O_2 by both *Zingiber officinale* L. seed extracts may be attributed to their phenolics, which could donate electrons to H_2O_2, thus neutralizing it to water[26]. The differences in H_2O_2 scavenging capacities may be attributed to the structural features of their active components, which determine their electron donating abilities[27].

Total phenol and total flavonoid contents of extracts of *Zingiber officinale* L. seed were evaluated (Table-1). Phenols are very important plant constituents because of their radical scavenging ability due to their hydroxyl groups[28]. In methanol and ethanol extracts of *Zingiber officinale* L. seed, 462.9 ± 10.9 and 400.2 ± 10.1 mg gallic acid equivalent of phenols was detected. These results suggest that the higher levels of antioxidant activity were due to the presence of phenolic components. The same relationship was also observed between phenolics and antioxidant activity in rosehip extracts[29]. It is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans when ingested up to 1 g daily from a diet rich in fruits and vegetables[30]. Phenolic compounds from spices are known to be good natural antioxidants. However, the activity of synthetic antioxidants was often observed to be higher than that of natural antioxidants[31]. Phenolic compounds, at certain concentrations, markedly slowed down the rate of conjugated diene formation. The interests of phenolics are increasing in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food[32].

TABLE-1
EXTRACTION YIELDS, AND CONTENTS OF TOTAL PHENOLS, TOTAL FLAVONOIDS IN GINGER SEED EXTRACTS

Extracts	Extraction yield (%)	Total phenols (mg GAE/g extract)	Total flavonoids (mg PE/g extract)
Methanol extract	41.88	462.9 ± 10.9	286.5 ± 3.5
Ethanol extract	44.12	400.2 ± 10.1	268.2 ± 3.1

This study exhibited that methanolic extract had the higher total flavonoid content (286.5 ± 3.5 mg PE/g extract) while ethanol extract had the lower value (268.2 ± 3.1 mg PE/g extract), using the standard curve of pyrocatechol ($R^2 = 0.9985$). These amounts were comparable with results described in the literature for other extracts of plant products[33]. The rich-flavonoid plants could be a good source of antioxidants that would help to increase the overall antioxidant capacity of an organism and protect it against lipid peroxidation[34]. Phenolic acids and flavonoids have been reported to be the main phytochemicals responsible for the antioxidant capacity of fruits and vegetables. Plant derived polyphenols display characteristic inhibitory patterns toward the oxidative reaction *in vitro* and *in vivo*[35].

ACKNOWLEDGEMENTS

This work was supported by Research Fund of Trakya University, Edirne-Turkey (Project number: TUBAP-2010-03).

REFERENCES

1. E. Czinner, K. Hagymasi, A. Blazovics, A. Kery, E. Szoke and E. Lemberkovics, *J. Ethnopharmacol.*, **77**, 31 (2001).
2. I.S. Young and J.V. Woodside, *J. Clin. Pathol.*, **54**, 176 (2001).
3. Y.S. Velioglu, G. Mazza, L. Gao and B.D. Oomah, *J. Agric. Food Chem.*, **46**, 4113 (1998).
4. M.E. Narhaizan, S.H. Fong, I. Arnin and L.Y. Chew, *Food Chem.*, **122**, 1055 (2010).
5. S.C. Liu, J.T. Lin, C.K. Wang, H.Y. Chen and D.J. Yang, *Food Chem.*, **114**, 577 (2009).
6. P.K. Saxena, *Trends Food Sci. Technol*., **20**, 596 (2009).
7. A.A. Shati and F.G. Elsaid, *Food Chem. Toxicol*., **47**, 1945 (2009).
8. M. Mueller, S. Hobiger and A. Jungbaver, *Food Chem.*, **122**, 987 (2010).
9. E.G. Mejia, M.V. Ramirez-Mares and S. Puangpraphant, *Brain Behav. Immun.*, **23**, 721 (2009).
10. M. Khader, N. Bresgen and P.M. Eckl, *J. Ethnopharmacol.*, **127**, 319 (2010).
11. I.-M. Chung, M. Ali and A. Ahmad, *Asian J. Chem.*, **17**, 1915 (2005); I.-M. Chung, N. Praveen, S.-J. Kim and A. Ahmad, *Asian J. Chem*., **24**, 831 (2012).
12. E.W.C. Chan, Y.Y. Lim, L.F. Wong, S. Lianto, S.K. Wong, K.K. Lim, C.E. Joe and T.Y. Lim, *Food Chem.*, **109**, 477 (2008).
13. H. Mitsuda, K. Yuasumoto and K. Iwami, *Eiyo to Shokury*, **19**, 210 (1996).
14. M. Oyaizu, *Japan. J. Nutr.*, **44**, 307 (1986).
15. Q. Liu, G. Zhu and P. Huang, *Zhongguo Zhong Yao Za Zhi*, **161**, 50 (1991).
16. K. Shimada, K. Fujikawa, K. Yahara and T. Nakamura, *J. Agric. Food Chem.*, **40**, 945 (1992).
17. T.C.P. Dinis, V.M.C. Madeira and L.M. Almeida, *Arch. Biochem. Biophys*., **315**, 161 (1994).
18. R.J. Ruch, S.J. Cheng and J.F. Klaunig, *Carcinogenesis*, **10**, 1003 (1989).
19. K. Slinkard and V.L. Singleton, *Am. J. Enol. Viticult*., **28**, 49 (1977).
20. J. Wang, X. Yuan, Z. Jin, Y. Tian and H. Song, *Food Chem.*, **104**, 242 (2007).
21. S. Meir, J. Kanner, B. Akin and S.P. Hadas, *J. Agric. Food Chem*., **43**, 1813 (1995).
22. J.R. Soares, T.C.P. Dins, A.P. Cunha and L.M. Ameida, *Free Radic. Res.*, **26**, 469 (1997).
23. P.D. Duh, Y.Y. Tu and G.C. Yen, *Lebens. Wissen. Technol.*, **32**, 269 (1999).
24. F. Yamaguchi, T. Ariga, Y. Yoshimira and H. Nakazawa, *J. Agric. Food Chem.*, **48**, 180 (2000).
25. B. Halliwell, In eds.: O.I. Aruoma and B. Halliwell: The Biological Toxicity of Free Radicals and Other Reactive Species, Free Radicals and Food Additives, London: Taylor and Francis (1991).
26. B. Halliwell and J.M.C. Gutteridge, Free Radical in Biology and Medicine, Oxford University Press, Oxford, UK (1985).
27. M. Wettasinghe and F. Shahidi, *Food Chem.*, **70**, 17 (2000).
28. T. Hatano, R. Edamatsu, A. Mori, Y. Fujita and E. Yasuhara, *Chem. Pharm. Bull.*, **37**, 2016 (1980).
29. X. Gao, M. Ohlander, N. Jeppsson, L. Bjork and V. Trajkovski, *J. Agric. Food Chem.*, **48**, 1485 (2000).
30. M. Tanaka, C.W. Kuei, Y. Nagashima and T. Taguchi, *Nippon Suisan Gakkaishi*, **54**, 1409 (1998).
31. M.B. Ningappa, R. Dinesha and L. Srinivas, *Food Chem.*, **106**, 720 (2007).
32. W. Aneta, O. Jan and C. Renata, *Food Chem.*, **105**, 940 (2007).
33. R. Manian, N. Anusuya, P. Siddhuraju and S. Manian, *Food Chem*., **107**, 1000 (2008).
34. F. Sharififiar, G. Dehgn-Nudeh and M. Mirtajaldini, *Food Chem.*, **112**, 885 (2009).
35. S. Bahramikia, A. Ardestani and R. Yazdanparast, *Food Chem.*, **115**, 37 (2009).