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Antioxidant and Radical Scavenging Activities of Capsules of Caper (*Capparis spinosa*)

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There is more and more interest in caper (*Capparis spinosa*) in recent years as it has different areas to be applied especially within the food and cosmetic world. This study evaluated the *in vitro* antioxidant activity of water and ethanol extracts of capsules of caper (*Capparis spinosa*). The antioxidant properties of the caper were evaluated *in vitro* by antioxidant assays such as 2-azino-*bis*(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical scavenging, 1,1-diphenyl-2-picryl-hydrazyl free radical (DPPH$^{\bullet}$) scavenging, total antioxidant activity by ferric thiocyanate method, total reductive capability using the potassium ferricyanide reduction method, superoxide anion radical scavenging and metal chelating activities. α-Tocopherol, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) were used as the reference antioxidant compounds. The concentration of 10, 30 and 50 μg/mL of water and ethanol extracts of caper showed 95.9, 96.4, 97.0, 97.04, 97.4, 94.8 % inhibition on peroxidation of linoleic acid emulsion, respectively. On the other hand, 30 μg/mL of standard antioxidant such as α-tocopherol indicated an inhibition of 88.1 % on peroxidation of linoleic acid emulsion. In addition capper is an effective ABTS$^{\bullet}$ + scavenging, DPPH$^{\bullet}$ scavenging, superoxide anion radical scavenging, total reducing power and metal chelating on ferrous ions activities.

Key Words: Caper, *Capparis spinosa*, Antioxidant activity, Metal Chelating, Reducing power, Radical scavenging.

INTRODUCTION

Caper (*Capparis spinosa*) is said to be native of the Mediterranean basin, but its range stretches from the Atlantic coasts of the Canary Islands and Morocco to the Black Sea to the Crimea and eastward to the Caspian Sea and into Iran. Capers probably were originated from dry regions in west or central Asia[1]. Capers contain considerable amounts of the antioxidant bioflavonoid rutin. This peculiarity gained extra advantage for use in the food industry. Capers are said to reduce flatulence

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and be antirheumatic in effect. In Ayurvedic medicine, capers (Capers = Himsra) are recorded as hepatic stimulants and protectors and also improve liver function. Capers have been reported used in arteriosclerosis, as diuretics, kidney disinfectants, vermifuges and tonics. In addition to this, infusions and decoctions from caper root bark have been traditionally used for dropsy, anemia, arthritis and gout. Caper extracts and pulps have been used in cosmetics[2].

The harmful intervention of free radicals in normal metabolic processes leading to pathologic changes is a consequence of their interaction with various biological compounds inside and outside cells. To protect biomolecules against the attack of free radicals and/or to suppress the resultant damage, numerous natural and synthetic free radical scavengers and antioxidants have been developed and studied. Among them, flavonoids, natural polyphenolic compounds, have attracted significant interest[3]. Actually, the beneficial clinical and curative effects of flavonoids in the treatment of virus infection, inflammation, diabetes mellitus, headache, *etc*. in humans have long been shown, but they are "to a large extent based on empirism since this praxis is much older than the science of chemistry"[4]. However, at present, many studies show the importance of the antiradical activity of flavonoids. Thus, it has been shown that flavonoids are the effective inhibitors of lipid peroxidation, oxygen radical overproduction by inflammatory cells, free radical-mediated cytotoxicity and chromosome damage[5-11].

Flavonoids (or bioflavonoids) are a group of about 4000 naturally occurring compounds that are ubiquitous in all vascular plants[4]. They are pigments responsible for the autumnal burst of hues and the many shades of yellow, orange and red in flowers[12]. They are also important for the normal growth, development and defense of plants[13]. Flavonoids, important constituents of the human diet are found in fruits (in citrus fruit they may represent up to 1 % of fresh material) and vegetables. Beverages like red wine, tea, coffee and beer also contain large amounts of flavonoids: on the average, the daily diet contains *ca.* 1 g of flavonoids per day[14]. Flavonoids are also found in several medicinal plants and herbal remedies containing flavonoids have been used in folk medicine around the world.

The interest in polyphenolic antioxidants has increased remarkably in the last decade because of their elevated capacity in scavenging free radicals associated with various diseases. This property has been evidenced by a large number of tests measuring the antioxidant activity *in vitro*[15-19]. *In vivo*, the antioxidant protection attributed to the polyphenols can be checked through the level of biomarkers, such as malondialdehyde, which is associated with lipid peroxidation[20] or 8-oxo-7,8-dihydroguanine, which indicates oxidative damage to the DNA bases[21]. The protective capacity of polyphenols is also supported by a number of studies indicating an effect of dietary polyphenols on coronary heart disease (CHD)[22] cancer[23,24], gene regulation[25] and neurodegenerative diseases[26].

However, there is no information about antioxidant activity of aqueous extract of caper (*Capparis spinosa*). In present investigation, the antioxidant effects of

Caper (*Capparis spinosa*) and to compare their antioxidant effects with those commonly used as food antioxidants, such as BHT, BHA and α-tocopherol are described. In addition to this, the components responsible for the antioxidative ability of caper (*Capparis spinosa*), are not reported. Hence, it is suggested that further work could be performed on the isolation and identification of the antioxidative components in caper (*Capparis spinosa*).

Capsules composes a part of eatable of caper the present study investigated the antioxidant properties of caper (*Capparis spinosa*) in order to evaluate its medicinal value and to point to an easily accessible source of natural antioxidants that could be used as a possible food supplement or in the pharmaceutical, cosmetic and perfume industries.

EXPERIMENTAL

Ammonium thiocyanate was purchased from E. Merck. Ferrous chloride, polyoxyethylenesorbitan monolaurate (Tween-20), α-tocopherol, 1,1-diphenyl-2-picrylhydrazyl (DPPH$^{\bullet}$), 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 obtained from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). Muller Hinton agar was also obtained from Oxoid Ltd. (Basingstoke, Hampshire, England, CM337). All other chemicals used were of analytical grade and were obtained from Sigma (Sigma-Aldrich GmbH, Sternheim, USA).

Plant material and extraction: Capers (*Capparis spinosa*) were collected from the land of Erdemli/Mersin in July. A voucher specimen was deposited in the Herbarium of Faculty of Biology, Atatürk University. For water extraction, 25 g sample was put into a fine powder in a mill and was mixed with 500 mL boiling water by magnetic stirrer for 15 min. Then, the extract was filtered over Whatman No. 1 paper. The filtrates were frozen and lyophilized in lyophilizator at 5 μm-Hg pressure at -50 °C (Labconco, Freezone 1L). For ethanol extraction 25 g sample was put into a fine powder in a mill and was mixed with 500 mL ethanol. The residue was re-extracted until extraction solvents became colourless. The obtained extracts were filtered over Whatman No. 1 paper and the filtrate was collected and then ethanol was removed by a rotary evaporator (RE 100 Bibby, Stone, Staffordshire England, ST15 OSA) at 50 °C to obtain dry extract. Both extracts were placed in a plastic bottle and then stored at -20 °C until used.

Total antioxidant activity determination: The antioxidant activity of caper was determined according to the thiocyanate method[27]. For stock solutions, 10 mg of each caper extracts was dissolved in 10 mL water. Then, the solution, which contains different concentration of stock caper solution or standards samples (15, 30 and 50 μg/mL) in 2.5 mL of potassium phosphate buffer (0.04 M, pH 7.0), was added to 2.5 mL of linoleic acid emulsion in potassium phosphate buffer (0.04 M, pH 7.0). Fifty mL 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, 5.0 mL control was composed of 2.5 mL linoleic acid emulsion and 2.5 mL, 0.04 M potassium phosphate buffer (pH 7.0). The mixed solution (5 mL) was incubated at 37 °C in a glass flask. The peroxide level was determined by reading the absorbance at 500 nm in a spectrophotometer (8500 II, Bio-Crom Gmb, Zurich, Switzerland) after reaction with $FeCl_2$ and thiocyanate at intervals during incubation. During the linoleic acid oxidation, peroxides are formed, which oxidize Fe^{2+} to Fe^{3+}. The latter ions form a complex with SCN^- and this complex has a maximum absorbance at 500 nm. Therefore, high absorbance indicates high linoleic acid oxidation. The solutions without added extracts were used as blank samples. All data on total antioxidant activity are the average of duplicate analyses. The % inhibition of lipid peroxidation was calculated by following equation:

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

where A_o is the absorbance of the control reaction and A_1 is the absorbance in the presence of the sample of caper extracts[28].

Reducing power: The reducing power of caper extracts was determined by the method of Oyaizu[29]. Different concentrations of caper extracts (2.7-13.4 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 °C for 20 min. Aliquots (2.5 mL) of trichloroacetic acid (10 %) were added to the mixture, which was then centrifugated for 10 min at 1036×g (MSE Mistral 2000, UK). 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 in a spectrophotometer. Increased absorbance of the reaction mixture indicates increased reducing power.

Superoxide anion scavenging activity: Measurement of superoxide anion scavenging activity of caper extracts was based on the method described by Liu *et al.*[30] 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 caper extract of 10 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 °C for 5 min and the absorbance at 560 nm was measured against blank samples. L-Ascorbic acid was used as a control. Decreased absorbance of the reaction mixture indicates increased superoxide anion scavenging activity. The percentage inhibition of superoxide anion generation was calculated using the following formula:

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

where A_o is the absorbance of the control and A_1 is the absorbance of caper extracts or standards[31].

Free radical scavenging activity: The free radical scavenging activity of caper extracts was measured by the 1,1-diphenyl-2-picryl-hydrazil ($DPPH^{\bullet}$) method proposed by Blois[32]. 0.1 mM solution of $DPPH^{\bullet}$ in ethanol was prepared and 1 mL of this solution was added to 3 mL of caper extracts solution in water at different concentrations of 10 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 $DPPH^{\bullet}$ concentration in the reaction medium was calculated from the following calibration curve, determined by linear regression (R^2: 0.9545):

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

The capability to scavenge the $DPPH^{\bullet}$ radical was calculated using the following equation:

$$DPPH^{\bullet}\ \text{Scavenging effect}\ (\%) = [(A_o - A_1 / A_o) \times 100]$$

where A_o is the absorbance of the control reaction and A_1 is the absorbance in the presence of the sample of caper extracts.

Ferrous ions chelating activity: The chelation of ferrous ions by the caper extracts and standards was estimated by the method of Dinis *et al.*[33]. The extracts of 10 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 kept 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:

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

where A_o is the absorbance of the control and A_1 is the absorbance in the presence of the sample of caper extracts or standards. The control does not contain $FeCl_2$ and ferrozine, complex formation molecules.

Scavenging of hydrogen peroxide: The ability of the caper extracts to scavenge hydrogen peroxide was determined according to the method of Ruch *et al.*[34]. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Hydrogen peroxide concentration was determined spectrophotometrically measuring absorption with extinction coefficient for H_2O_2 of 81 M^{-1} cm^{-1}. Extracts (12.5-62.5 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 caper extracts and standard compounds was calculated:

$$\%\ \text{Scavanged}\ [H_2O_2] = [(A_o - A_1) / A_o] \times 100$$

where A_o is the absorbance of the control and A_1 is the absorbance in the presence of the sample of caper extracts or standards.

Determination of total phenolic compounds: Total soluble phenolics in the caper extracts were determined with Folin-Ciocalteu reagent according to the method of Slinkard and Singleton[35] using gallic acid as a standard phenolic compound. 1.0 mL of extract solution containing 1.0 g extracts in a volumetric flask was diluted with distilled water (46 mL). One mL of Folin-Ciocalteau reagent was added and the content of the flask mixed thoroughly. After 3 min, 3 mL of Na_2CO_3 (2 %) was added and 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 caper extracts was determined as microgram of gallic acid equivalent using an equation obtained from the standard gallic acid graph:

$$\text{Absorbance} = 0.0008 \times \text{Gallic acid } (\mu g)$$

Statistical analysis: Experimental results were mentioned as mean ± SD of three parallel measurements. P values < 0.05 were regarded as significant and P values < 0.01 as very significant.

RESULTS AND DISCUSSION

Total antioxidant activity of caper extracts was determined by the thiocyanate method. Both caper extracts exhibited effective antioxidant activity at all concentrations. The effects of various amounts of water and ethanol extracts of caper (from 15 to 50 µg/mL) on peroxidation of linoleic acid emulsion are shown in Figs. 1 and 2. The antioxidant activity of both caper extracts increased with increasing concentration. The different concentration of water and ethanol extracts (15, 30 and 50 µg/mL) of caper showed higher antioxidant activities than that of 30 µg/mL concentration of α-tocopherol. The percentage of inhibition in linoleic acid system was 95.9, 96.4, 97.0, 97.04, 97.4, 94.8 respectively and greater than that of 30 µg/mL of α-tocopherol (88.1 %).

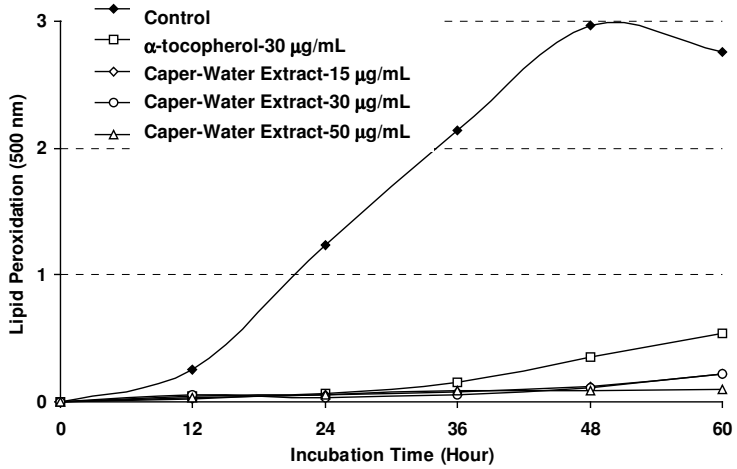

Fig. 1. Antioxidant activity of different concentrations of water extracts of caper and α-tocopherol in the linoleic acid emulsion caper (*Capparis spinosa*)

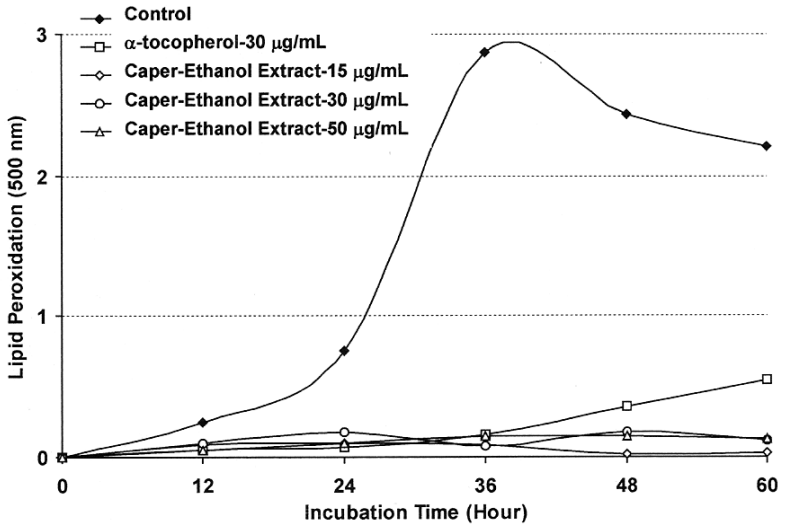

Fig. 2. Antioxidant activity of different concentrations of ethanol extracts of caper and α-tocopherol in the linoleic acid emulsion caper (*Capparis spinosa*)

Fig. 3 shows the reductive capabilities of caper extracts compared with BHA, BHT and α-tocopherol. For the measurements of the reductive ability, we investigated the Fe^{3+}–Fe^{2+} transformation in the presence of caper extracts using the method of Oyaizu[29]. Like the antioxidant activity, the reducing power of caper extracts increased with increasing amount of sample. All of the amounts of both caper extracts showed higher activities than control and these differences were statistically significant ($P < 0.01$). Reducing power of water and ethanol extracts of caper and standard compounds exhibited the following order: BHA > BHT > α-tocopherol > water extract of caper > ethanol extract of caper.

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. 4 shows the % inhibition of superoxide radical generation by 10 μg/mL of water and ethanol extracts of caper and comparison with same concentrations of BHA, BHT and α-tocopherol. Both extracts of caper had strong superoxide radical scavenging activity and exhibited higher superoxide radical scavenging activity than BHT and α-tocopherol. The results were found statistically significant ($P < 0.05$). As seen in Fig. 4, the percentage inhibition of superoxide generation by 10 μg/mL concentration of BHA, BHT, α-tocopherol, water and ethanol extracts of caper was found as 75.6, 47.5, 75.6, 27.3 and 25.7, respectively. Superoxide radical scavenging activity of those samples showed the following order: α-tocopherol > BHA > BHT > water extract of caper > ethanol extract of caper.

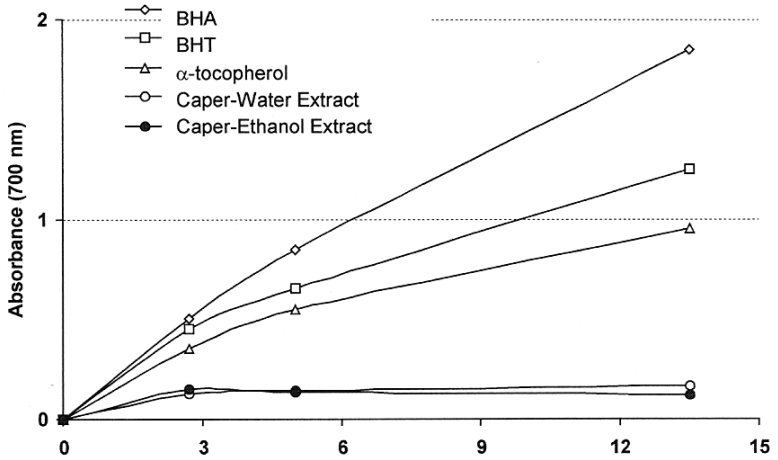

Fig. 3. Reducing power of water and ethanol extracts of caper, BHA, BHT and α-tocopherol. (Spectrophotometric detection of the Fe^{3+}–Fe^{2+} transformation, caper (*Capparis spinosa*), BHA: Butylated hydroxyanisole, BHT: Butylated hydroxytoluene)

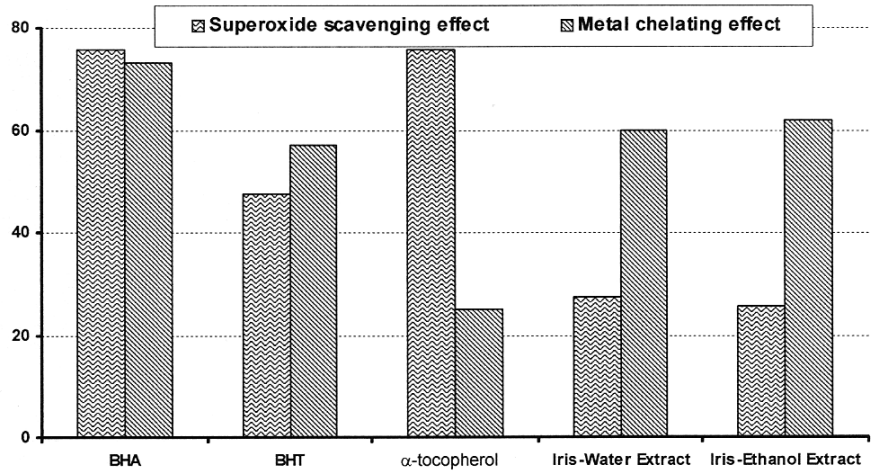

Fig. 4. Superoxide anion radical scavenging activity of water and ethanol extracts of caper, BHA, BHT and α-tocopherol by the PMS-NADH-NBT method caper (*Capparis spinosa*), BHA: butylated hydroxyanisole, BHT: butylated hydroxytoluene)

The chelation of ferrous ions by the extracts of caper was estimated by the method of Dinis *et al.*[33].

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[36]. As shown in Fig. 4, the formation of the Fe^{2+}-ferrozine complex is not complete in the presence of water and ethanol extracts of caper, indicating that both extracts of caper chelate with the iron. The absorbance of Fe^{2+}-ferrozine complex was linearly decreased dose dependently 10 µg/mL. The difference between both extracts of caper and the control was statistically significant ($P < 0.05$). The percentages of metal scavenging capacity of 10 µg/mL concentration of water and ethanol extracts of caper, BHA, BHT and α-tocopherol, were found as 88.98, 79.1, 72.5, 61.5 and 66.96 %, respectively. The metal scavenging effect of both extracts of caper and standards decreased in the order of BHA > caper ethanol extract > caper water extrct > BHT > α-tocopherol.

Ferrous ion chelating capacity was significant, since it reduced the concentration of the catalyzing transition metal in lipid peroxidation[28]. 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[37]. The data obtained from Fig. 4 reveal that both extracts of caper demonstrate a marked capacity for iron binding, suggesting that their action as peroxidation protector may be related to its iron binding capacity.

$DPPH^{\bullet}$ is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule[38]. The reduction capability of $DPPH^{\bullet}$ radicals was determined by the decrease as shown by its absorbance at 517 nm, which is induced by antioxidants. Hence, $DPPH^{\bullet}$ is often used as a substrate to evaluate antioxidative activity of antioxidants[28]. Fig. 5 illustrates a significant ($P < 0.05$) decrease in the concentration of DPPH radical due to the scavenging ability of the extracts of caper and standards. In present studies BHA, BHT and α-tocopherol were used as standards. The scavenging effect of water and ethanol extracts of caper and standards on the DPPH radical decreased in that order: Caper ethanol extract > caper water extract > BHT > α-tocopherol > BHA which were 91.1, 89.9, 87.4, 79.7 and 69.8 %, respectively, at the concentration of 50 µg/mL. These results indicates that both caper extracts have a noticeable effect on scavenging free radical. Free radical scavenging activity also increased with increasing concentration.

The ability of the both extracts of caper to scavenge hydrogen peroxide was determined according to the method of Ruch *et al.*[34]. The scavenging ability of water and ethanol extracts of caper on hydrogen peroxide is shown in Fig. 6 and compared with that of BHA, BHT and α-tocopherol as standards. Caper extracts were capable of scavenging hydrogen peroxide in a concentration-dependent manner. Of water and ethanol extracts 10 µg/mL of caper exhibited 76.5 and 72.6 % scavenging activity on hydrogen peroxide, respectively. The BHA, BHT and α-tocopherol exhibited 37.5, 86 and 57 % hydrogen peroxide scavenging activity at the same dose. These results showed that both caper extracts had stronger hydrogen peroxide scavenging activity. These values are close to that of BHA, but lower than that of BHT and α-tocopherol. There was statistically significant correlation between those values and control ($P < 0.05$). The hydrogen peroxide scavenging effect of 10 µg/mL

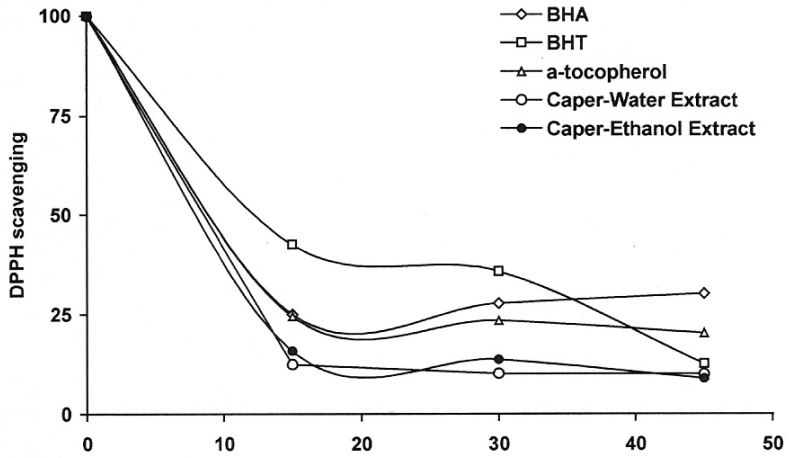

Fig. 5. Free radical scavenging activity of water and ethanol extracts of caper, BHA, BHT and α-tocopherol. Caper (*Capparis spinosa*), BHA: butylated hydroxyanisole, BHT: Butylated hydroxytoluene)

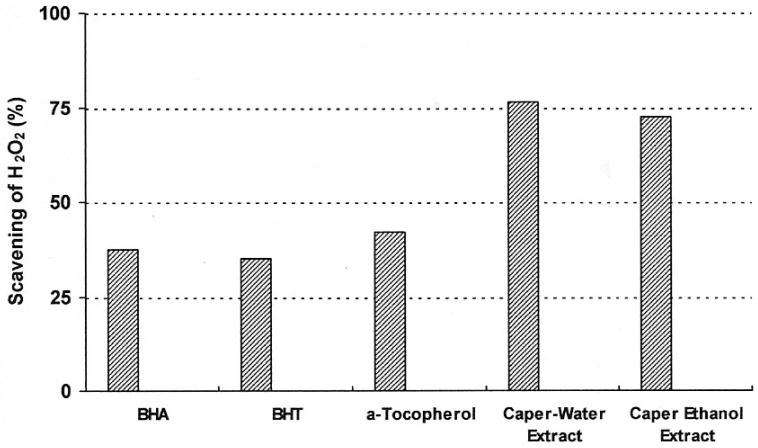

Fig. 6. Hydrogen peroxide scavenging activities of water and ethanol extracts of caper, BHA, BHT and α-tocopherol. caper (*Capparis spinosa*), BHA: butylated hydroxyanisole, BHT: butylated hydroxytoluene)

concentration of the both extracts of caper and standards decreased in the order of water extract of caper > ethanol extract of caper > α-tocopherol > BHA > BHT. 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 cells[39]. Thus, removing H_2O_2 as well as $O_2^{\bullet-}$ is very important for protection of food systems.

Phenols are very important plant constituents because of their radical scavenging ability due to their hydroxyl groups. In water and ethanol extracts of caper (1 mg), 65.2 and 76.8 μg gallic acid equivalent of phenols was detected. There was no relationship between total phenols and total antioxidant activity in caper extracts. The phenolic compounds may contribute directly to the antioxidative action[28]. 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 caper in fruits and vegetables.

Both extracts of caper showed strong antioxidant activity, reducing power, DPPH radical and superoxide anion scavenging, hydrogen peroxide scavenging and metal chelating activities when compared with different standards such as BHA, BHT and α-tocopherol.

The results of this study show that the extract of caper can be used as an easily accessible source of natural antioxidants and as a possible food supplement or in the pharmaceutical and cosmetic industry.

REFERENCES

1. R.E. Bond, *The Herbalist*, **56**, 77 (1990).
2. G. Ancora and L. Cuozzo, In 28th Conv. Ann. Ital. Gen. Agr. Bracciano, Italy, p. 82 (1984).
3. L.G. Korkina and I.B. Afanas'ev, in ed.: M. Sies, Antioxidant and Chelating Properties of Flavonoids, Antioxidants in Disease Mechanisms and Therapy: Advanced Pharmacology, San Diego: Academic Press, Vol. 38, p. 151 (1997).
4. B. Havsteen, *Biochem. Pharmacol.*, **32**, 1141 (1983).
5. I. Maridonneau-Parini, P. Braquet and R.P. Garay, *Pharmacol. Res. Commun.*, **18**, 61 (1986).
6. J. Robak and R.J. Gryglewski, *Biochem. Pharmacol.*, **37**, 837 (1988).
7. I.B. Afanas'ev, A.I. Dorozhko, A.V. Brodskii, V.A. Kostyuk and A.I. Potapovitch, *Biochem. Pharmacol.*, **38**, 1763 (1989).
8. I.B. Afanas'ev, E.A. Ostrachovitch, N.E. Abramova and L.G. Korkina, *Biochem. Pharmacol.*, **50**, 627 (1995).
9. A. Negre-Salvayre, A. Affany, C. Hariton and R. Salvayre, *Pharmacology*, **42**, 262 (1991).
10. B. Limasset, C. le Doucen, J.C. Dore, T. Ojasoo, M. Damon and A.C. de Paulet, *Biochem. Pharmacol.*, **46**, 1257 (1993).
11. I. Morel, G. Lescoat, P. Cogrel, O. Sergent, N. Pasdeloup, P. Cillard and J. Cillard, *Biochem. Pharmacol.*, **45**, 13 (1993).
12. C.F. Timberlake and B.S. Henry, *Endeavour*, **10**, 31 (1986).
13. J.B. Harborn,e, in eds.: V. Cody, E. Middleton and J.B. Harborne, Nature, Distribution and Function of Plant Flavonoids, In: Plant Flavonoids in Biology and Medicine: Biochemical, Pharmacological and Structure Activity Relationships, New York: Liss., p. 25 (1986).
14. J. Kuhnau, *World Rev. Nutr. Diet.*, **24**, 117 (1976).
15. G. Cao and R.L. Prior, *Method Enzymol.*, **299**, 50 (1999).
16. O. Hirayama, M. Takagi, K. Hukumoto and S. Katoh, *Anal. Biochem.*, **247**, 237 (1997).
17. B. Ou, M. Hampsch-Woodill and R.L. Prior, *J. Agric. Food Chem.*, **49**, 4619 (2001).
18. R. Re, N. Pelligrini, A. Proteggente, A. Pannala, M. Yang and C.A. Rice-Evans, *Free Radical Bio. Med.*, **26**, 1231 (1999).
19. K. Robards, P.D. Prenzler, G. Tucker, P. Swatsitang and W. Glover, *Food Chem.*, **66**, 401 (1999).
20. F. Nielsen, B.B. Mikkelsen, J.B. Nielsen, H.R. Andersen and P. Grandjean, *Clin. Chem.*, **43**, 1209 (1997).

21. J. Cadet, T. Douki, D. Gasparutto and J.L. Ravanat, *Mutat. Res./Fund. Mol.*, **M531**, 5 (2003).
22. J.H. Weisburger, *Food Chem. Toxicol.*, **37**, 943 (1999).
23. G.G. Duthie, S.J. Duthie and J.A.M. Kyle, *Nutr. Res. Rev.*, **13**, 79 (2000).
24. C.S. Yang, J.M. Landau, M.T. Huang and H.L. Newmark, *Annu. Rev. Nutr.*, **21**, 381 (2001).
25. M.C.W. Myhrstad, H. Carlsen, O. Nordstrom, R. Blomhoff and J.O. Moskaug, *Free Radical Bio. Med.*, **32**, 386 (2002).
26. A.Y. Sun and Y.M. Chen, *J. Biomed. Sci.*, **5**, 401 (1998).
27. H. Mitsuda, K. Yuasumoto and K. Iwami, *Eiyo to Shokuryo*, **19**, 210 (1996).
28. P.D. Duh, Y.Y. Tu and G.C. Yen, *Lebnesm. Wiss. Technol.*, **32**, 269 (1999).
29. M. Oyaizu, *Japan J. Nutr.*, **44**, 307 (1986).
30. F. Liu, V.E.C. Ooi and S.T. Chang, *Life Sci.*, **60**, 763 (1997).
31. X.Y. Ye, H.X. Wang, F. Liu and T.B. Ng, *Int. J. Biochem. Cell Biol.*, **32**, 235 (2000).
32. M.S. Blois, *Nature*, **26**, 1199 (1958).
33. T.C.P. Dinis, V.M.C. Madeira and L.M. Almeida, *Arch. Biochem. Biophys.*, **315**, 161 (1994).
34. R.J. Ruch, S.J. Cheng and J.E. Klaunig, *Carcinogenesis*, **10**, 1003 (1989).
35. K. Slinkard and V.L. Singleton, *Am. J. Enol. Viticult.*, **28**, 49 (1977).
36. F. Yamaguchi, *J. Agric. Food Chem.*, **48**, 2320 (2000).
37. M.H. Gordon, in ed.: B.J.F. Hudson, The Mechanism of the Antioxidant Action *in vitro*, Food Antioxidants, London: Elsevier, pp. 1 (1990).
38. J.R. Soares, T.C.P. Dinis, A.P. Cunha and L.M. Almeida, *Free Rad. Res.*, **26**, 469 (1997).
39. B. Halliwell, *Drugs*, **42**, 569 (1991).

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