SCREENING OF ANTIOXIDANT PROPERTIES OF SEVEN UMBELLIFERAE FRUITS FROM IRAN

BAHMAN NICKAVAR* AND FARIDEH AL-SADAT ABOLHASANI

Department of Pharmacognosy, School of Pharmacy,
Shahid Beheshti University (M.C.), PO Box: 14155-6153, Tehran, Iran

ABSTRACT

Antioxidative activities (IC_{50}) of ethanol extracts from seven Umbelliferae fruits (*Bunium persicum*, *Coriandrum sativum*, *Cuminum cyminum*, *Foeniculum vulgare*, *Heracleum persicum*, *Pimpinella anisum* and *Trachyspermum copticum*) have been studied by the DPPH (2,2'-diphenyl-1-picrylhydrazyl) radical scavenging test. All the studied extracts showed antioxidant capability and *P. anisum* extract exhibited the strongest activity. The scavenging activity of the extracts in decreasing order was: *P. anisum* > *T. copticum* > *C. cyminum* > *F. vulgare* ≥ *B. persicum* ≥ *C. sativum* > *H. persicum*. The extracts were also investigated regarding their total flavonoid contents by the $AlCl_3$ technique. The decreasing order of the flavonoid content of the extracts was: *C. cyminum* > *T. copticum* > *P. anisum* ≥ *H. persicum* ≥ *B. persicum* ≥ *F. vulgare* ≥ *C. sativum*. However, a favorable correlation was not found between the antioxidant activity and the total flavonoid content of the extracts. As well, the most active extract (i.e. *P. anisum*) was partitioned with *n*-hexane, chloroform and ethyl acetate to yield three organic fractions together with the remaining aqueous fraction. The antioxidative activities (IP%) and flavonoid contents of the fractions were also determined. The ethyl acetate fraction exhibited the highest activity and content. A positive correlation was found between the antioxidant potency and flavonoid content of the fractions.

Keywords: Umbelliferae, antioxidant activity, free radical scavenging activity, DPPH assay, flavonoid content.

INTRODUCTION

The role of free radicals and other reactive species has been implicated in the causation of several diseases. These molecules can induce changes in different biological tissues and cell biomolecules such as lipids, proteins, DNA or RNA (Galvez *et al*., 2005; Nakiboglu *et al*., 2007). Free radicals can also affect food quality, reducing its nutritional content and promoting the development of food deterioration (Puertas *et al*., 2005; Ozgen *et al*., 2006). Oxidative stress involves in the pathogenesis of certain human diseases like cancer, atherosclerosis, inflammatory diseases and neuro-degenerative processes (Orhan *et al*., 2003; Kukic *et al*., 2006; Mathew and Abraham, 2006). Therefore, antioxidant substances are required for the protection against the oxidizing agents. Many synthetic antioxidants have been used in the food industries, but recent publications have mentioned the disadvantages of them and their possible toxic properties for human and animal health (Wangensteen *et al*., 2004; Tepe *et al*., 2006). Then, the development of alternative antioxidants from natural origin has attracted considerable attention and there is an increasing interest in the investigation of naturally occurring antioxidants from plants (Mensor *et al*., 2001; Argolo *et al*., 2004; Satyanarayana *et al*., 2004; Kiselova *et al*., 2006).

Herbs have used in many domains including medicine, nutrition, flavoring, beverages, fragrances, cosmetics and other industrial purposes and a wide variety of the flora have been extensively evaluated for their antioxidant activities (Satyanarayana *et al*., 2004; Miliauskas *et al*., 2004; Nakiboglu *et al*., 2007). A part of medicinal herbs properties is attributed to the antioxidant activities of their constituents including a wide range of polyphenols and vitamins A, E and C (Kiselova *et al*., 2006). Polyphenols are commonly found in plants and they have been reported to have multiple biological effects including antioxidant activity. They constitute one of the most numerous and widely distributed extremely heterogeneous groups of the substances in the plant kingdom. A wide range of polyphenols from different subgroups are found in various tissues of plants (Balasundram *et al*., 2005; Tepe *et al*., 2006; Kukic *et al*., 2006).

Generally, spices are dietary constituents consumed daily to enhance the flavor or taste of the human food. A group of well known plants which are used as spices and condiments belong to the Umbelliferae family (Satyanarayana *et al*., 2004). The plants such as wild caraway [*Bunium persicum* (Boiss.) Fedtsch.], coriander (*Coriandrum sativum* L.), cumin (*Cuminum cyminum* L.), fennel (*Foeniculum vulgare* Miller), cow parsnip (*Heracleum persicum* Desf. Ex Fischer), anise (*Pimpinella anisum* L.), bishop's weed [*Trachyspermum copticum* (L.) Link.] are usually to improved either the

* *Corresponding author:* Tel.: +98-21-88773521, Fax: +98-21-88795008, e-mail: bnickavar@yahoo.com

flavor or taste and make the food more palatable. The plants have been previously studied in different studies concerning their chemical composition, pharmacological properties and therapeutic uses (Evans, 1996; Blumental, 2000; Barnes *et al*., 2002; Amin, 2005). Nevertheless, the literature data on their antioxidant activities are scarce and frequently scattered through out the papers. The data available are often different to compare because of the differences in the methods between each study so, the results are not directly comparable. Thus, a need exists to directly compare the antioxidant activities of these species using a similar approach. The present study is a part of an investigation on the antioxidant properties and the contribution of polyphenols to the antioxidant activities of Iranian food and medicinal plants. In this paper we screen the extracts of seven Umbelliferae fruits for their antioxidant activities and compare to the flavonoid contents in the extracts.

MATERIALS AND METHODS

Plant materials and chemicals

All the chemicals were of analytical grade. Solvents and rutin were purchased from Merck (Darmstadt, Germany). 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical was purchased from Sigma Chemical Co. (St. Louis, MO, USA).

The dried fruits from following species were studied: *Bunium persicum*, *Coriandrum sativum*, *Cuminum cyminum*, *Foeniculum vulgare*, *Heracleum persicum*, *Pimpinella anisum* and *Trachyspermum copticum*. They were purchased from a local market in Tehran, Iran. The plants were identified at the Herbarium of Department of Pharmacognosy, School of Pharmacy, Shahid Beheshti University (M.C.) where the voucher specimens were preserved.

Extraction and fractionation procedure

The dried and powdered fruits (100 g) were extracted with 90% ethanol for 48 h. The extracts were filtered and concentrated under reduced pressure at 40°C.

Crude extract of *Pimpinella anisum* was dissolved in a mixture of ethanol:water (20:80) at room temperature and partitioned successively with *n*-hexane, chloroform and ethyl acetate. An aqueous final fraction was also obtained. The fractions were concentrated under reduced pressure at 40°C.

DPPH radical scavenging test

The antioxidant capacities of the extracts (IC_{50}) were estimated and compared to rutin using the stable DPPH radical (Nickavar *et al*., 2006). Briefly, a 0.3 mM solution of DPPH$^{\bullet}$ solution in 90% ethanol was prepared and then 1 mL of this solution was mixed with 2.5 mL of each sample (crude extract) at concentrations of 1200, 900, 600, 450, 300, 225, 150, 75, 37.5, 18.75 µg/mL in 90% ethanol. After 30 min incubation in the dark, the decrease in the solution absorbance was measured at 518 nm in a Shimadzu Multispect-1501 spectrophotometer (Kyoto, Japan). Rutin at various concentrations (50, 25, 10, 5, 2.5, 1.25 µg/mL in 90% ethanol) was used as a standard. The DPPH radical scavenging activity (RSD) was calculated using the following formula:

$$RSD\% = [A_{control} - (A_{sample} - A_{blank})]/A_{control} \times 100$$

90% ethanol (1 mL) plus plant extract solution (2.5 mL) was used as a blank. DPPH$^{\bullet}$ solution (1 mL) plus 90% ethanol (2.5 mL) was used as a control.

The RSD% was plotted against the sample concentrations and a logarithmic regression curve was established in order to calculated the IC_{50} value (µg/mL) which is the concentration of the extract that inhibited DPPH$^{\bullet}$ by 50%. The antioxidant activities of the fractions [Inhibition percentage (IP%)] were also estimated according to the above procedure. IP% is the inhibition percentage of DPPH radical at a fixed concentration of each fraction (150 µg/mL).

Determination of the total flavonoid content

The total flavonoid content (TFC) in each extract and fraction was determined using $AlCl_3$ reagent (Nickavar *et al*., 2006). Briefly, 2.5 mL of each sample (and/or rutin as the standard), previously dissolved in 90% ethanol, was mixed with 2.5 mL of a 2% $AlCl_3$ solution in 90% ethanol. After 40 min, the absorbance of the yellow color produced was measured at 415 nm with a Shimadzu Multispect-1501 spectrophotometer. The TFC [as µg rutin equivalents (RE) / mg of sample] for each sample was calculated on the basis of a linear calibration curve (absorbance versus concentration) obtained using rutin. The plot was found to be linear across the range assayed (50-12.5 µg/mL, $r^2 > 0.99$).

Statistical analysis

All the experiments were done in triplicate. The IC_{50} values were presented by their respective 95% confidence limits. The TFC (µg/mg) and IP% for each sample was shown as mean ± SEM. A one-way analysis of variance (ANOVA) followed by Tukey's post test was used for comparison between the extracts and fractions. A difference was considered statistically significant when $p < 0.05$. All the statistical analyses were accomplished using the computer software GraphPad Prism 3.02 for Windows (GraphPad Software, San Diego, CA, USA).

RESULTS AND DISCUSSION

The DPPH test is a very convenient method for screening small antioxidant molecules (such as flavonoids) because the reaction intensity can be analyzed by the

Table 1: IC_{50} values of DPPH$^{\bullet}$ scavenging activities and TFCs of the studied Umbelliferae fruits

Plant species	IC_{50} (µg/mL)[1,3]	TFC (µg/mg)[2,3]
Bunium persicum	208.40 (196.80-220.60)^{a}	20.21±0.84^{f,i,j,k}
Coriandrum sativum	222.40 (206.80-239.20)^{a,f}	9.81±0.54^{e,h}
Cuminum cyminum	149.90 (141.40-158.90)^{b}	56.92±2.80^{d}
Foeniculum vulgare	199.20 (183.40-216.40)^{a,f}	16.27±0.33^{c,g,h,k}
Heracleum persicum	294.00 (277.10-311.80)^{c}	22.23±1.078^{b,g,j}
Pimpinella anisum	109.80 (104.10-115.80)^{d}	26.89±1.39^{b,i}
Trachyspermum copticum	126.40 (118.50-134.70)^{e}	40.18±1.39^{a}

*Note: The IC_{50} value of the positive control, rutin, was measured as 8.31 (7.70-8.96) µg/mL.
[1]The IC_{50} values are presented with their respective 95% confidence limits.
[2]The TFC values are mean ± SEM of three determinations.
[3]Letters (*a*-*k*) denote homogenous subsets at $p < 0.05$ (one way ANOVA followed by Tuckey's post test).

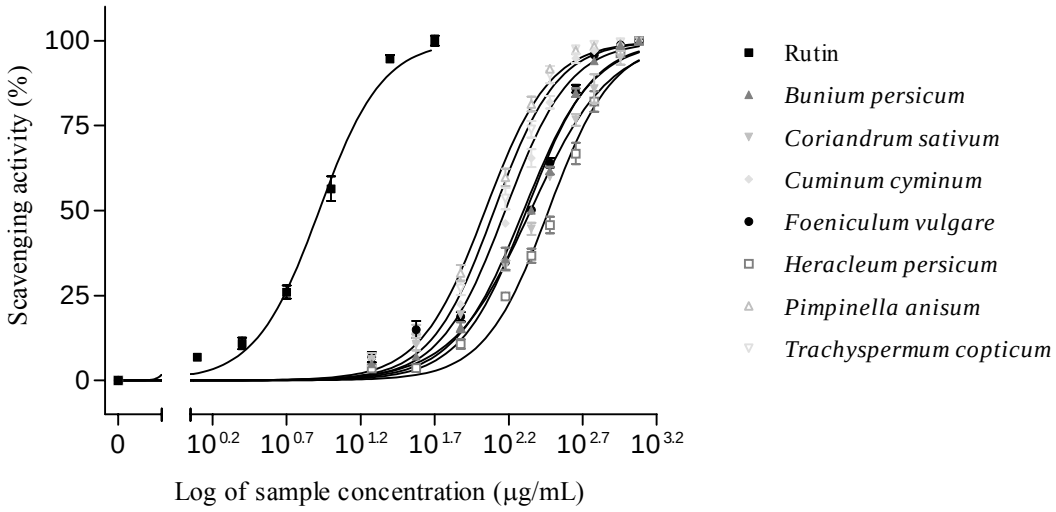

Fig. 1: Radical scavenging activities of the studied Umbelliferae fruits extracts using DPPH$^{\bullet}$. Each point represents the mean of three experiments, and the vertical bars represent the SEM.

spectrophotometric method (Puertas *et al*., 2005; Soler-Rivas *et al*., 2000). Therefore, in this study the selected plants were screened for their antioxidant activities by this method and their IC_{50} and IP% values were calculated for further comparison.

All the extracts exhibited a dose dependent antioxidant activity (fig. 1). When the IC_{50} values (with 95% confidence intervals) were compared for the seven extracts tested, *P. anisum* [IC_{50}=109.80 (104.10-115.80) µg/mL] showed the highest scavenging activity. The extract with the weakest scavenging potency was *H. persicum* [IC_{50}=294.00 (277.10-311.80) µg/mL] which had significantly lower activity than the other extracts. Therefore, the scavenging activity of the extracts in decreasing order was: *P. anisum* > *T. copticum* > *C. cyminum* > *F. vulgare* ≥ *B. persicum* ≥ *C. sativum* > *H. persicum* (table 1).

Because the extracts studied were obtained with ethanol, first approach to the characterization of each extract was to determine the amount of the TFC by the $AlCl_3$ assay. The extract of *C. cyminum* (56.92±2.80 µg/mg) was the richest in the TFC, whereas *C. sativum* (9.81±0.54 µg/mg) had the lowest content. The order obtained in relation with the TFC for the extracts was: *C. cyminum* > *T. copticum* > *P. anisum* ≥ *H. persicum* ≥ *B. persicum* ≥ *F. vulgare* ≥ *C. sativum* (table 1 and fig. 2).

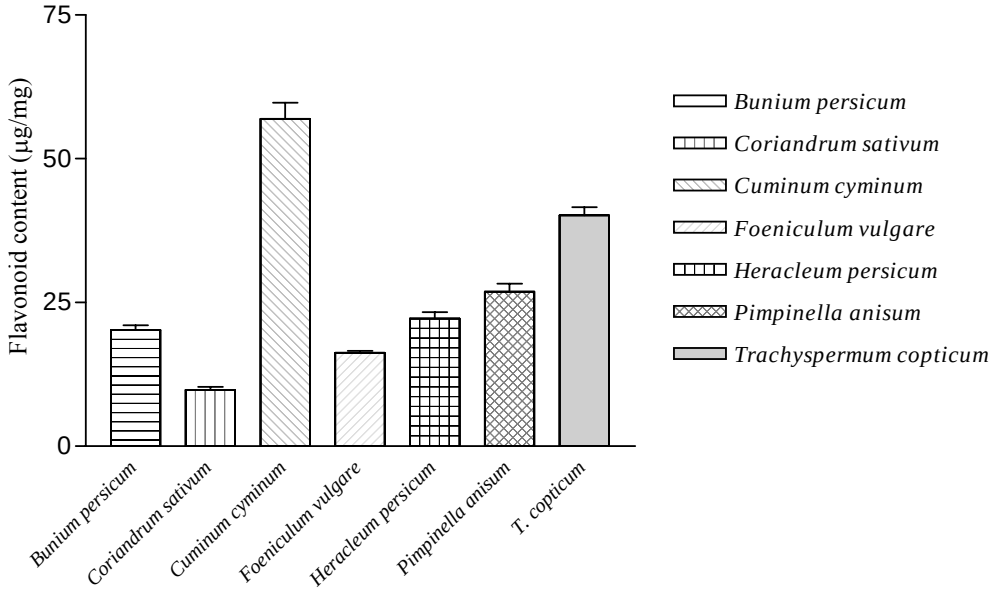

Fig. 2: TFCs of the studied Umbelliferae extracts. Data are represented as mean ± SEM (n=3). Vertical bars represent the SEM.

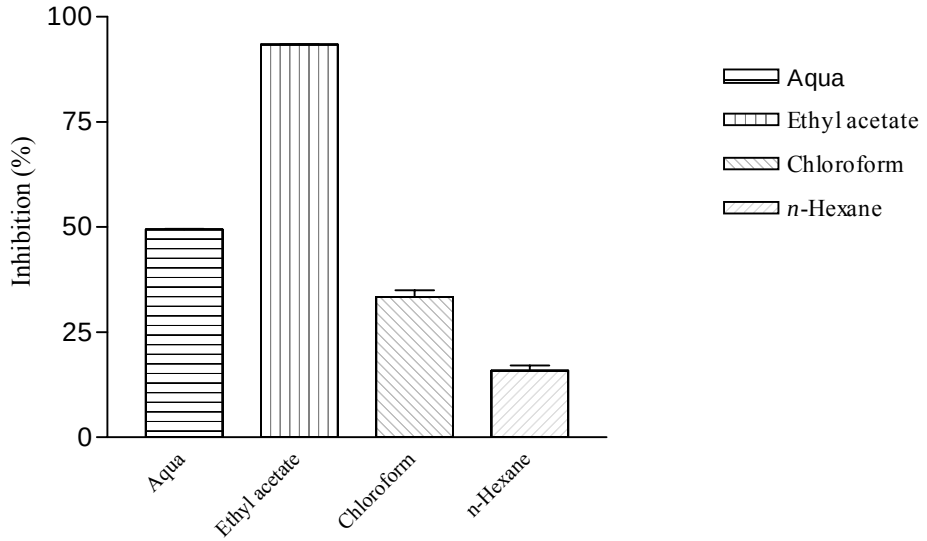

Fig. 3: IP% of DPPH radicals by different fractions obtained from *P. anisum*. Data are represented as mean ± SEM (n=3). Vertical bars represent the SEM.

With the aim of establishing a quantitative relationship between the antioxidant potency (expressed as $1/IC_{50}$) and the TFC a correlation study was carried out. However, among the extracts tested, there is no simple correlation between TFCs and antioxidant capacities. The lack of correlation is in agreement with other literature (Heinonen *et al*., 1998; Matthaus, 2002). It is known that only flavonoids with a certain structure can act as the proton donating and show the antioxidant and radical scavenging activity. On the other hand, the extracts are very complex mixtures of many different compounds with distinct activities (Mensor *et al*., 2001; Hou *et al*., 2003).

Among the seven plants, *P. anisum* had more potent antioxidant activity than the other samples. Therefore, to obtain useful information about the antioxidant activity and flavonoid content of this plant, its ethanol extract was partitioned successively with *n*-hexane, chloroform and ethyl acetate and all the fractions were the subject to the $DPPH^{\bullet}$ and $AlCl_3$ assays.

According to the IP% values displayed in table 2 and fig. 3, the inhibition percentage of DPPH radical is very high for the ethyl acetate fraction (IP%=93.39±0.14) compared with the other fractions. The inhibition activity of the fractions in decreasing order was: ethyl acetate fraction > aqueous fraction > chloroform fraction > *n*-hexane fraction.

For further specification of the fractions, their TFCs were determined. Table 2 present the TFC for each fraction. When the TFC for each fraction was compared with the others, it could be observed that the ethyl acetate fraction was significantly higher (105.99±2.85 µg/mg). The other fraction, that is, the aqueous fraction, showed the next highest TFC. However, chloroform and *n*-hexane fractions were poor in flavonoids.

Table 2: IP% of DPPH radicals by different fractions obtained from *P. anisum* and TFCs of the fractions.

Fractions	IP (%)[1,2]	TFC (µg/mg)[1,2]
Aqua	49.40±0.22[a]	14.07±4.63[a]
Ethyl acetate	93.39±0.14[b]	105.99±2.85[b]
Chloroform	33.41±1.53[c]	0.00±0.00[c]
n-Hexane	15.87±1.18[d]	0.00±0.00[c]

[1]Data are represented as mean ± SEM (n=3).
[2]Letters (*a*-*d*) denote homogenous subsets at $p < 0.05$ (one way ANOVA followed by Tuckey's post test).

After these analyses on the fractions, the relationship between the IPs% and the TFCs was studied. As indicated in fig. 4, a positive correlation ($r^2 \geq 0.90$) was found. This correlation suggests that flavonoids may be responsible for a significant part of antioxidant effects of *P. anisum*.

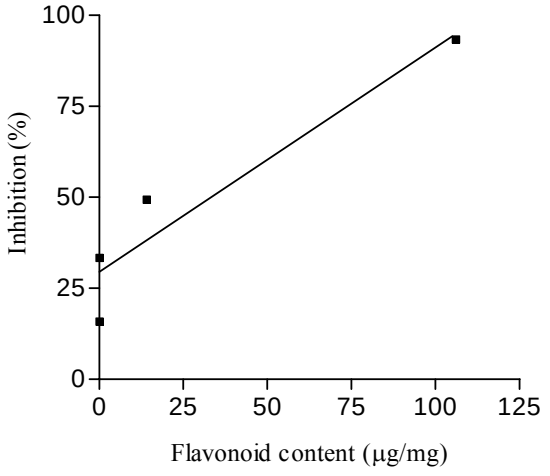

Fig. 4: Correlation between IP% of DPPH radicals by different fractions obtained from *P. anisum* and TFCs (µg/mg) of the studied fractions ($r^2 \geq 0.90$).

CONCLUSSION

The present study indicated the antioxidant activities of the ethanol extracts of the selected Umbelliferae fruits. Previous investigations on these Umbelliferae fruits demonstrated the presence of terpenoids and flavonoids (Evans, 1996; Blumental, 2000; Barnes *et al*., 2002). Therefore, the presence of these compounds in the extracts might be responsible for their observed antioxidant activities. On the other hand, the results of this study show that the selected Umbelliferae fruits, especially *P. anisum*, can serve as natural sources to develop the antioxidants and free radical scavengers and they could be considered as useful sources of materials for human health and as food preservatives.

REFERENCES

Amin G (2005). Popular Medicinal Plants of Iran. Vice-chancellorship of Research, Tehran University of Medical Sciences, Tehran, pp.54, 112, 154 168, 170, 172, 230.

Argolo ACC, Sant'Ana AEG, Pletsch M and Coelho LCBB (2004). Antioxidant activity of leaf extracts from *Bauhinia monandra*. *Bioresource Technol*., **95**: 229-233.

Balasundram N, Ai TY, Sambanthamurthi R, Sundram K and Samman S (2005). Antioxidant properties of palm fruit extracts. *Asia. Pac. J. Clin. Nut.*, **4**: 319-324.

Barnes J, Anderson LA and Phillipson JD (2002). Herbal Medicines: A Guide for Healthcare Professionals. Pharmaceutical Press, London, pp.51-54.

Blumental M (2000). Herbal Medicines: Expanded Commission E Monographs. Integrative Medicine Communications, Newton, MA, 75-77, 124-129.

Evans WC (1996). Trease and Evans' Pharmacognosy. WB Saunders Company Ltd., London, pp.262-264, 453-458, 480.

Galvez M, Martin-Cordero C, Houghton PJ and Ayuso MJ (2005). Antioxidant activity of methanol extracts obtained from *Plantago* species. *J. Agric. Food Chem.*, **53**: 1927-1933.

Heinonen IM, Lehtonen PJ and Hopia AI (1998). Antioxidant activity of berry and fruit wines and liquors. *J. Agric. Food Chem.*, **46**: 25-31.

Hou WC, Lin RD, Cheng KT, Hung YT, Cho CH, Chen CH, Hwang SY and Lee MH (2003). Free radical scavenging activity of Taiwanese native plants. *Phytomedicine*, **10**: 170-175.

Kiselova Y, Ivanova D, Chervenkov T, Gerova D, Galunska B and Yankova T (2006). Correlation between the in vitro antioxidant activity and polyphenol content of aqueous extracts from Bulgarian herbs. *Phytother. Res.*, **20**: 961-965.

Kukic J, Petrovic S and Niketic M (2006). Antioxidant activity of four endemic *Stachys* taxa. *Biol. Pharm. Bull.*, **29**: 725-729.

Mathew S and Abraham TE (2006). In vitro antioxidant activity and scavenging effects of *Cinnamomum verum* leaf extract assayed by different methodological. *Food Chem. Toxicol.*, **44**: 198-206.

Matthaus B (2002). Antioxidant activity of extracts obtained from residues of different oil seeds. *J. Agric. Food Chem.*, **50**: 3444-3452.

Mensor LL, Menezes FS, Leitao GG, Reis AS, dos Santos TC, Coube CS and Leitao SG (2001). Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytother. Res.*, **15**: 127-130.

Miliauskas G, Venskutonis PR and van Beek TA (2004). Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chem.*, **85**: 231-237.

Nakiboglu M, Urek RO, Kayali HA and Tarhan L (2007). Antioxidant capacities of endemic *Sideritis sipylea* and *Origanum sipyleum* from Turkey. *Food Chem.*, **104**: 630-635.

Nickavar B, Kamalinejad M, Haj-Yahya M and Shafaghi B (2006). Comparison of the free radical scavenging activity of six Iranian *Achillea* species. *Pharm. Biol.*, **44**: 208-212.

Orhan I, Aydin A, Colkesen A, Sener B and Isimer AI (2003). Free radical scavenging activities of some edible fruit seeds. *Pharm. Biol.*, **41**: 163-165.

Ozgen U, Mavi A, Terzi Z, Yildirim A, Coskun M and Houghton PJ (2006). Antioxidant properties of some medicinal Lamiaceae (Labiatae) species. *Pharm. Biol.*, **44**: 107-112.

Puertas MA, Mesa AM and Saez JA (2005). In vitro radical scavenging activity of two Columbian Magnoliaceae. *Naturwissenschaften*, **92**: 381-384.

Satyanarayana S, Sushruta K, Sarma GS, Srinivas N and Subba Raju GV (2004). Antioxidant activity of the aqueous extracts of spicy food additives: evaluation and comparison with ascorbic acid in vitro system. *J. Herb. Pharmacother.*, **4**: 1-10.

Soler-Rivas C, Espin JC and Wichers HJ (2000). An easy and fast test to compare total free radical scavenger capacity of foodstuffs. *Phytochem. Analysis*, **11**: 1-9.

Tepe B, Sokmen M, Akpulat HA and Sokmen A (2006). Screening of the antioxidant potentials of six *Salvia* species from Turkey. *Food Chem.*, **95**: 200-204.

Wangensteen H, Samuelsen AB and Malterud KE (2004). Antioxidant activity in extracts from coriander. *Food Chem.*, **88**: 293-297.