ANTIOXIDANT AND FREE RADICAL SCAVENGING ACTIVITY OF *H. OFFICINALIS L. VAR. ANGUSTIFOLIUS, V. ODORATA, B. HYRCANA* AND *C. SPECIOSUM*

MOHAMMAD ALI EBRAHIMZADEH[a]*, SEYED MOHAMMAD NABAVI[a,b], SEYED FAZEL NABAVI[a], FATEMEH BAHRAMIAN[c] AND AHMAD REZA BEKHRADNIA[a]

[a]*Pharmaceutical Sciences Research Center, School of Pharmacy, Mazandaran University of Medical Sciences, 48189, Sari, Iran*

[b]*Department of Biology, University of Mazandaran, Babolsar, Iran.*

[c]*Student Research Committee, Mazandaran University of Medical Sciences, Sari, Iran.*

ABSTRACT

Extracts of 4 medicinal and aromatic plants were investigated for their antioxidant potency employing six various established in vitro system: *H. officinalis L. var. angustifolius* aerial parts, *C. speciosum* flowers, *V. odorata* and *B. hyrcana* leaves.With regard to IC_{50} values (µg/ ml), the order in DPPH radical-scavenging were *CS* (585.6) > *HO* (311) > *VO* (245.1) > and *BH* (113.1). Effectiveness in reducing powers were high and in a descending order of *HO* > *CS* > *BH* > *VO* (at the concentrations of 25-800 µg/ ml). IC_{50} for Fe^{2+} chelating ability were 188, 750 and 980 µg/ ml for *VO*, *CS* and *HO*, respectively. *BH* extract has shown only 38% inhibition at 800 µg/ ml. The extracts showed weak nitric oxide-scavenging activity. All extracts exhibited very low and moderate concentration-dependent antioxidant activity in FTC methods. IC_{50} for scavenging of H_2O_2 were 169 for *BH*, 175 for *CS*, 640 for *VO* and 663 µg/ ml for *HO*. The content of total phenolic compounds and flavonoids were measured in plant extracts. The data obtained in the in vitro models clearly establish the antioxidant potency of all extracts.

Keywords: Antioxidant activity, DPPH, *H. officinalis*, *C. speciosum*, *V. odorata*, *B. hyrcana*.

INTRODUCTION

Free radicals are created when cells use oxygen to generate energy. These by-products are generally reactive oxygen species (ROS) such as super oxide anion, hydroxyl radical and hydrogen peroxide that result from the cellular redox process. At low or moderate concentrations, ROS exert beneficial effects on cellular responses and immune function but at high levels, free radicals and oxidants generate oxidative stress, a deleterious process that can damage cell structures, including lipids, proteins, and DNA (Pham-Huy *et al.,* 2008). Oxidative stress plays a major part in the development of chronic and degenerative ailments such as cancer, autoimmune disorders, rheumatoid arthritis, cataract, aging, cardiovascular and neurodegenerative diseases, (Willcox *et al.,* 2004; Pham-Huy *et al.,* 2008). The human body has several mechanisms to counteract oxidative stress by producing antioxidants, which are either naturally produced in situ, or externally supplied through foods and/or supplements. These antioxidants act as free radical scavengers by preventing and repairing damages caused by ROS, and therefore can enhance the immune defense and lower the risk of cancer and degenerative diseases (Pham-Huy *et al.,* 2008). In recent years, there is an increasing interest in finding antioxidant phytochemicals, because they can inhibit the propagation of free radical reactions, protect the human body from diseases (Terao and Piskula, 1997). The most effective components seem to be flavonoids and phenolic compounds of many plant raw materials, particularly in herbs, seeds, and fruits. Their metal-chelating capabilities and radical- scavenging properties have enabled phenolic compounds to be thought of as effective free radical scavengers and inhibitors of lipid peroxidation (Terao and Piskula, 1997).

Hyssop, *Hyssopus officinalis* (Lamiaceae) is one of the most important pharmaceutical herbs that extensively cultivated in central and south European countries such as Russia, Spain, France and Italy (Omidbaigi, 2000). Despite having a bitter taste, it is used as a food flavor and in sauce formulations (Kazazi *et al.,* 2007). It is used in tea blends for cough relief, for antispasmodic effects, and to relieve catarrh (Khazaie *et al.,* 2008). Hyssop possesses antifungal and anti-bacterial properties (Omidbaigi, 2000), insecticidal (Pavela, 2004), antiplatelet (Tognolini *et al.,* 2006) and α-Glucosidase inhibitory activities (Matsuura *et al.,* 2004). Chemical composition of essential oil of hyssop has been reported (Kerrola *et al.,* 1994). *Viola odorata L.* (Violaceae) is used in alternative medicine mainly for respiratory ailments. Flowers are edible and used as food additives. Root contains violine which is emetic. Also, it is used as a laxative and to treat digestive disorders. It is used externally to reduce swelling (www.altnature). Also

Corresponding author: Ph: +98-151-3543081-3, Fax: +98-151-3543084, e-mail zadeh20@yahoo.com

antifungal (Amin *et al.,* 2002) and antipyretic activities have been reported (Khattak *et al.,* 1985). Some compounds were separated and identified from volatile components of violet leaves (Cu *et al.,* 1992). Some Cyclotide also identified (Ireland *et al.,* 2006). Previous chemical studies on various species of genus *Buxus* have resulted in the isolation of over 80 new steroidal alkaloids (Meshkatalsadat *et al.,* 2006) *Buxus hyrcana* is abundant in Iran and previously, over 10 new steroidal alkaloids have been reported from this plant (Choudhary *et al.,* 2006; Meshkatalsadat *et al.,* 2006; Babar *et al.,* 2006). *Buxus* alkaloids exhibit various biological activities such as anti-HIV and acetylcholinesterase inhibitory activities (Atta-ur-Rahman *et al.,* 1998; Durant *et al.,* 1998; Atta-ur-Rahman and Choudhary, 1999; Babar *et al.,* 2006). Antioxidative activity has been reported by thiobarbituric acid in *B. hyrcana* (Souri *et al.,* 2004). *Colchicum speciosum* (Colchicaceae) is used for its cholinergic activity (Basova *et al.,* 2006) and antileukemia (Kupchan *et al.,* 1973). Determination of colchicine has been reported in *Colchicum species* by many researchers (Churadze *et al.,* 1976; Alali *et al.,* 2007). Yet very little information is available about antioxidative activity of these plants.

In this study, we examined the antioxidant activity of *Hyssopus officinalis*, *Viola odorata*, *Buxus hyrcana* and *Colchicum speciosum* employing six in vitro assay systems, i.e., DPPH and nitric oxide radical scavenging, reducing power, scavenging of hydrogen peroxide, linoleic acid and iron ion chelating power, in order to understand the usefulness of this plant as a foodstuff as well as in medicine.

MATERIALS AND METHODS

Chemicals

Ferrozine, Linoleic acid, trichloroacetic acid (TCA), 1,1-diphenyl-2-picryl hydrazyl (DPPH), potassium ferricyanide were purchased from Sigma Chemicals Co. (USA). Gallic acid, quercetin, Butylated hydroxyanisole (BHA), Vitamin C, sulfanilamide, N-(1-naphthyl) ethylenediamine dihydrochloride, EDTA and ferric chloride were purchased from Merck (Germany). All other chemicals were of analytical grade or purer.

Plant material

H. officinalis L. var. angustifolius aerial parts, *C. speciosum* flowers, *V. odorata* and *B. hyrcana* leaves and were collected from Mazandaran forest and identified by Dr. Bahman Eslami. A voucher (No. 975-979) has been deposited in the Sari School of Pharmacy herbarium. Materials dried at room temperature and coarsely ground before extraction. Each part was extracted at room temperature by percolation method using methanol. The resulting extract was concentrated over a rotary vacuum until a crude solid extract was obtained, which was then freeze-dried for complete solvent removal.

Determination of total flavonoid content

Colorimetric aluminum chloride method was used for flavonoid determination (Nabavi *et al.,* 2008a; Ebrahimzaded *et al.,* 2009a, b). Briefly, 0.5 ml solution of each plant extracts in methanol were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water, and left at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415 nm with a double beam Perkin Elmer UV/Visible spectrophotometer (USA). Total flavonoid contents were calculated as quercetin from a calibration curve. The calibration curve was prepared by preparing quercetin solutions at concentrations 12.5 to 100 mg/ ml in methanol.

Determination of total phenol content

Total phenolic compound contents were determined by the Folin-Ciocalteau method (Ebrahimzaded *et al.,* 2008a, b; Nabavi *et al.,* 2008a).The extract samples (0.5 ml of different dilutions) were mixed with Folin Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) for 5 min and aqueous Na_2CO_3 (4 ml, 1 M) were then added. The mixture was allowed to stand for 15 min and the phenols were determined by colorimetric method at 765 nm. The standard curve was prepared by 0, 50, 100, 150, 200, and 250 mg/ ml solutions of Gallic acid in methanol: water (50:50, v/v). Total phenol values are expressed in terms of Gallic acid equivalent (mg/ g of dry mass), which is a common reference compound.

DPPH radical-scavenging activity

The stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for determination of free radical-scavenging activity of the extracts (Ebrahimzadeh *et al.,* 2009a,b; Ghasemi et al., 2009). Different concentrations of each extracts were added, at an equal volume, to methanolic solution of DPPH (100 μM). After 15 min at room temperature, the absorbance was recorded at 517 nm. The experiment was repeated for three times. Vitamine C, BHA and quercetin were used as standard controls. IC_{50} values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals.

Reducing power determination

The reducing power of extracts was determined according to the method of Yen and Chen (1995). Different amounts of each extracts (25-800 μg/ ml) in 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. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture to stop the reaction, which was then centrifuged at 3000 rpm 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. Vitamine C was used as positive control.

Assay of nitric oxide-scavenging activity

The procedure is based on the principle that, sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions. For the experiment, sodium nitroprusside (10 mM), in phosphate-buffered saline, was mixed with different concentrations of each extracts dissolved in water and incubated at room temperature for 150 min. After the incubation period, 0.5 ml of Griess reagent was added. The absorbance of the chromophore formed was read at 546 nm. Quercetin was used as positive control (Ebrahimzadeh *et al.,* 2009d).

Metal chelating activity

The chelating of ferrous ions by extracts was estimated by our recently published paper (Ebrahimzadeh *et al.,* 2008a). Briefly, the extract (0.2-3.2 mg/ml) was 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), 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 inhibition of ferrozine- Fe^{2+} complex formation was calculated as:

$$[(A_0 - A_s)/A_s] \times 100$$

Where A_0 was the absorbance of the control, and A_s was the absorbance of the extract or Na_2EDTA (positive control).

Determination of Antioxidant Activity by the FTC (Ferric thiocyanate) Method

The inhibitory capacity of extracts was tested against oxidation of linoleic acid by FTC method (Ebrahimzadeh *et al.,* 2009b,c). Twenty mg/ ml of samples dissolved in 4 ml of 95% (w/v) ethanol were mixed with linoleic acid (2.51%, v/v) in 99.5% (w/v) ethanol (4.1 ml), 0.05 M phosphate buffer pH 7.0 (8 ml), and distilled water (3.9 ml) and kept in screw cap containers at 40°C in the dark. To 0.1 ml of this solution was then added 9.7 ml of 75% (v/v) ethanol and 0.1 ml of 30% (w/v) ammonium thiocyanate. Precisely 3 min after the addition of 0.1 ml of 20 mM ferrous chloride in 3.5% (v/v) hydrochloric acid to the reaction mixture, the absorbance at 500 nm of the resulting red solution was measured, and it was measured again every 24 h until the day when the absorbance of the control reached the maximum value. The percent inhibition of linoleic acid peroxidation was calculated as: (%) inhibition = 100 - [(absorbance increase of sample/absorbance increase of control) × 100].

All tests were run in duplicate, and analyses of all samples were run in triplicate and averaged. Vitamin C and BHA used as positive control.

Scavenging of hydrogen peroxide

The ability of the extracts to scavenge hydrogen peroxide was determined according to our recently published papers (Nabavi *et al.,* 2008a and 2009a). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). The concentration of hydrogen peroxide was determined by absorption at 230 nm using a spectrophotometer. Extracts (0.1-1 mg/ ml) in distilled water were added to a hydrogen peroxide solution (0.6 ml, 40 mM). The absorbance of hydrogen peroxide at 230 nm was determined after ten minutes against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging by the extracts and standard compounds was calculated as follows: % Scavenged $[H_2O_2] = [(A_o - A_1)/A_o] \times 100$ where A_o was the absorbance of the control and A_1 was the absorbance in the presence of the sample of extract and standard (Nabavi *et al.,* 2008a,b).

STATISTICAL ANALYSIS

Experimental results are expressed as means ± SD. All measurements were replicated three times. The data were analyzed by an analysis of variance ($p<0.05$) and the

Table 1: Total phenol and flavonoids contents, DPPH and NO scavenging activities of *H. officinalis L. var. angustifolius*, *V. odorata*, *B. hyrcana* and *C. speciosum*.

Sample	Total phenol contents (mg/ g)	Flavonoid contents (mg/ g)	DPPH radical scavenging, IC_{50} (µg/ ml)[a]	NO-scavenging IC_{50} (µg/ ml)[b]
H. officinalis L. var. angustifolius aerial parts	90.0 ± 1.2	30.6 ± 2.1	311 ± 14.5	275
V. odorata leaves	35.4 ± 0.9	22.8 ± 1.0	245.1 ± 9.6	34[c] (%)
B. hyrcana leaves	55.8 ± 2.3	48.2 ± 2.4	113.1 ± 8.9	27[c] (%)
C. speciosum flowers	48.4 ± 2.1	39.0 ± 1.6	585.6 ± 21.2	248

[a] IC_{50} for BHA, Vitamin C and Quercetin were 53.96 ± 3.1, 5.05 ± 0.1 and 5.28 ± 0.2 µg ml^{-1}, respectively.
[b] IC_{50} for Quercetin was 17 ± 0.9 µg/ ml, [c] Percentage of inhibition at 800 µg/ ml

means separated by Duncan's multiple range tests (by InStat3 software). The IC_{50} values were calculated from linear regression analysis.

RESULTS AND DISCUSSION

Total phenol and flavonoid contents

Total phenol compounds, as determined by Folin Ciocalteu method, are reported as Gallic acid equivalents by reference to standard curve ($y = 0.0063x$, $r^2 = 0.987$). The total flavonoid contents are reported as mg quercetin equivalent/g of extract powder, by reference to standard curve ($y = 0.0067x + 0.0132$, $r^2 = 0.999$) (table 1). It was noted that *H. officinalis* aerial parts and *B. hyrcana* leaves extracts had significant higher total phenol and flavonoids contents than did other extracts, respectively. Phenols and polyphenolic compounds, such as flavonoids, are widely found in food products derived from plant sources, and they have been shown to possess significant antioxidant activities (Nabavi et al., 2009a). The high amount of phenols and flavonoids in extracts may explain their high antioxidative activities.

DPPH radical-scavenging activity

The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples (Ebrahimzadeh *et al.,* 2008b). DPPH is a stable nitrogen-centered free radical the color of which changes from violet to yellow upon reduction by either the process of hydrogen- or electron- donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers (Dehpour *et al.,* 2009). It was found that the radical-scavenging activities of all the extracts increased with increasing concentration. IC_{50} for DPPH radical-scavenging activity were reported in Table 1. Usually, higher total phenol and flavonoids contents lead to better DPPH-scavenging activity (Ebrahimzadeh *et al.,* 2009a-c). *B. hyrcana* leaves with high level of phenolic contents and highest amount of flavonoids showed the best activity (113.1 ± 8.9) µg/ ml.

Reducing power

Fe (III) reduction is often used as an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant action (Nabavi *et al.,* 2009a). In the reducing power assay, the presence of antioxidants in the samples would result in the reducing of Fe^{3+} to Fe^{2+} by donating an electron. Amount of Fe^{2+} complex can be then be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Increasing absorbance at 700 nm indicates an increase in reductive ability. Fig. 1 shows dose-response curves for the reducing powers of the extract. It was found that the reducing powers of all the extracts also increased with the increase of their concentrations. All extracts had shown good reducing power that was comparable with Vitamin C ($p>0.05$). The *H. officinalis* aerial parts had shown better reducing power than Vitamin C ($p< 0.05$).

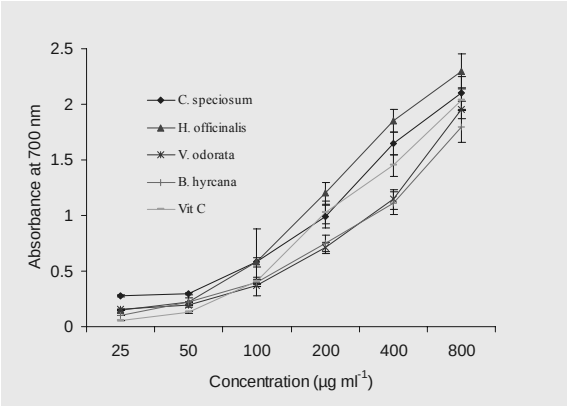

Fig. 1: Reducing power of *H. officinalis L. var. angustifolius* aerial parts, *C. speciosum* flowers, *V. odorata* and *B. hyrcana* leaves. Vitamin C used as control.

Assay of nitric oxide-scavenging activity

The extracts showed weak nitric oxide-scavenging activity between 0.1 and 800 µg/ ml. The % inhibition was increased with increasing concentration of the extract. The *C. speciosum* flowers extract had shown better reducing power than other extracts (table 1). However, activity of quercetin was very more pronounced than that of our extracts. In addition to reactive oxygen species, nitric oxide is also implicated in inflammation, cancer and other pathological conditions (Nabavi *et al.,* 2008a,b).

Fe^{2+} chelating ability

Bivalent transition metal ions play an important role as catalysts of oxidative processes, leading to the formation of hydroxyl radicals and hydro peroxide decomposition reactions via Fenton chemistry (Halliwell, 1997). The transition metal, iron, is capable of generating free radicals from peroxides and may be implicated in human cardiovascular disease (Halliwell and Gutteridge, 1990). Because Fe^{2+} causes the production of oxyradicals and lipid peroxidation, minimizing its concentration affords protection against oxidative damage. The chelating of ferrous ions by the extract was estimated by our recently published paper (Ebrahimzadeh *et al.,* 2008a). In the presence of other chelating agents, the Ferrozine complex formation is disrupted with the result that the red color of the complexes decreases. The absorbance of Fe^{2+}-ferrozine complex was decreased dose-dependently, i.e. the activity was increased on increasing concentration from 50 to 1600 µg/ml. It was reported that chelating agents are effective as secondary antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion (Gordon, 1990). IC_{50} for Fe^{2+} chelating ability were 188, 750 and 980 µg/ ml for *V. odorata*, *C. speciosum* and *H. officinalis*, respectively. *B. hyrcana* extract has shown only 38% inhibition at 800

µg/ml. EDTA showed very strong activity (IC_{50} = 18 µg/ ml).

FTC method

Membrane lipids are rich in unsaturated fatty acids that are most susceptible to oxidative processes. Specially, linoleic acid and arachidonic acid are targets of lipid per oxidation (Nabavi *et al.,* 2009a). Fig. 2 shows the time-course plots for the antioxidative activity of the plants extract using the FTC method. All extracts exhibited very low and moderate concentration-dependent antioxidant activity. There were significant differences ($p < 0.001$) among plants extracts and Vitamin C or BHA at different incubation times. It suggests that peroxidation inhibition have not any role in antioxidant activity of our four extracts and other mechanism may evolve.

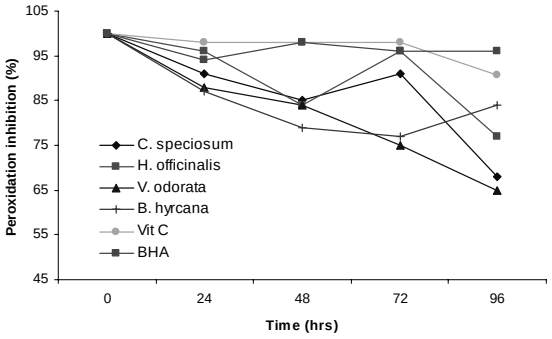

Fig. 2: Antioxidant activity of *H. officinalis L. var. angustifolius* aerial parts, *C. speciosum* flowers, *V. odorata* and *B. hyrcana* leaves in FTC method at different incubation times (0.2 mg/ ml). Vitamin C and BHA used as controls (0.1 mg/ ml).

Hydrogen Peroxide Scavenging

Scavenging of H_2O_2 by extracts may be attributed to their phenolics, which can donate electrons to H_2O_2, thus neutralizing it to water (Nabavi *et al.,* 2008b; Ebrahimzadeh *et al.,* 2009d).The ability of the extracts to effectively scavenge hydrogen peroxide, determined according to the method of Ruch (Nabavi *et al.,* 2008), where they are compared with that of Quercetin as standard. The extracts were capable of scavenging hydrogen peroxide in a concentration-dependent manner. IC_{50} for scavenging of H_2O_2 were 169 ± 7.25 for *B. hyrcana* leaves, 175 ± 6.95 for *C. speciosum*, 640 ± 11.67 for *V. odorata* leaves and 663 ± 9.38 µg/ ml for *H. officinalis* aerial parts, respectively. The IC_{50} values for vitamin C and quercetin were 21.4 ± 0.12 and 52.0 ± 3.11 (g/ ml, respectively. Although hydrogen peroxide itself is not very reactive, it can sometimes cause cytotoxicity by giving rise to hydroxyl radicals in the cell. Thus, removing H_2O_2 is very important throughout food systems (Nabavi *et al.,* 2008a).

In conclusion, all extracts exhibited different levels of antioxidant activities in all the models studied. Further investigation of individual compounds, their in vivo antioxidant activities and in different antioxidant mechanisms is needed.

ACKNOWLEDGMENTS

This research was partially supported by a grant from the research council of Medical Sciences University of Mazandaran, Iran.

REFERENCES

Alali FQ, Tawaha K and El-Elimat T (2007). Determination of (-)-demecolcine and (-)colchicine content in selected Jordanian Colchicum species. *Pharmazie*, **62**(10): 739-742.

Amin G, Salehi Surmaghi MH, Yasa N, Aynehchi Y, Dehmoobed-Sharifabadi A, Emami M, Shidfar MR, Amin M, Moghadami M, Kordbacheh P and Zeini F (2002). Screening of Iranian plants for antifungal activitypart 2. *DARU*, **10**(2): 78-89.

Atta-ur-Rahman, Parveen S, Khalid A, Farooq A, Ayatollahi SAM and Choudhary MI (1998). Acetylcholinesterase inhibiting triterpenoidal alkaloids from *Buxus hyrcana*. *Heterocycles*, **49**: 481-488.

Atta-ur-Rahman and Choudhary MI (1999). Diterpenoid and steroidal alkaloids. *Nat. Prod. Rep.,* **71**: 619-635.

Babar ZU, Ata A and Meshkatalsadat MH (2006). New bioactive steroidal alkaloids from *Buxus hyrcana*. *Steroids,* **71**(13-14): 1045-51.

Basova NE, Rozengart EV and Suvorov AA (2006). Cholinergic activity of isoquinoline alkaloids from the showy autumn crocus (*Colchicum speciosum Stev*.). Dokl Biochem Biophys, pp.40627-40631.

Choudhary MI, Shahnaz S, Parveen S, Khalid A, Mesaik MA, Ayatollahi SA and Atta-ur-Rahman (2006). New cholinesterase-inhibiting triterpenoid alkaloids from *Buxus hyrcana. Chem. Biodivers,* **3**(9):1039-1052.

Churadze LI, Iavich PA, Beridze PZ and Chikhladze ChA (1976). Quantitative determination of colchamine and colchicine. *Farmatsiia*, **25**(5): 41-44.

Cu JQ, Perineau F and Gaset A (1992). Volatile components of violet leaves. *Phytochemistry*, **31**(2): 571-573.

Dehpour AA, Ebrahimzadeh MA, Nabavi SF and Nabavi SM (2009). Antioxidant activity of methanol extract of *Ferula assafoetida* and its essential oil composition. *Grasas Aceites*, **60**(4): 405-412.

Durant J, Chantre P, Gonzalez G, Vandermander J, Halfon P, Rousse B, Guedon D, Rahelinirina V, Chamaret S, Montagnier L and dellamonica P (1998). Efficacy and safety of Buxus sempervirens L. preparations (SPV30) in HIV asymptomatic patients: a multicentre, randomized, double-blind, placebo-controlled trail. *Phytomedicine*, **5**: 1-10.

Duthie GG (1993). Lipid peroxidation. *Eur. J. Clin. Nutr.,* **47**(11): 759-764.

Ebrahimzadeh MA, Pourmorad F and Bekhradnia AR (2008a). Iron chelating activity screening, phenol and flavonoid content of some medicinal plants from Iran. *Afr. J. Biotechnol*., **32**: 43-49.

Ebrahimzadeh MA, Pourmorad F and Hafezi S (2008b). Antioxidant activities of Iranian corn silk. *Turk. J. Biol.,* **32**: 43-49.

Ebrahimzadeh MA and Bahramian F (2009a). Antioxidant activity of *Crataegus pentagina subsp. elbursis* fruits extracts used in traditional medicine in Iran. *Pak. J. Biol. Sci.,* **12**(5): 413-419.

Ebrahimzadeh MA, Ehsanifar S and Eslami B (2009b). Sambucus ebulus elburensis fruits: A good source for antioxidants. *Pharmacognosy Magazine,* **4**(19): 213-218.

Ebrahimzadeh MA, Nabavi SM, Nabavi SF and Eslami B (2009c). Antioxidant activity of aqueous extract of *Pyrus boissieriana* fruit. *Pharmacologyonline*, **1**: 1318-1323.

Ebrahimzadeh MA, Nabavi SF and Nabavi SM (2009d). Antioxidant activities of methanol extract of *sambucus ebulus L.* flower. *Pak. J. Biol. Sci.*, **12**(5): 447-450.

Ghasemi K, Ghasemi Y and Ebrahimzadeh MA (2009). Antioxidant activity, phenol and flavonoid contents of 13 Citrus specis peels and tissues. *Pak. J. Pharm. Sci*., **22**(3): 277-281.

Gordon MH (1990). The mechanism of antioxidant action *in vitro*. *In*: Hudson BJF (editor). Food antioxidants. Elsevier Applied Science. London, pp.1-18.

Halliwell B (1997). Antioxidants: the basics - what they are and how to evaluate them. *Adv. Pharmacol.,* **38**: 3-20.

Halliwell B and Gutteridge JMC (1990). Role of free radicals and catalytic metal ions in human disease: an overview. *Methods Enzymol.,* **186**: 1-85. http://www.altnature.com/Accessed July 26, 2009.

Ireland DC, Colgrave ML, Nguyencong P. Daly NL and Craik DJ (2006). Discovery and Characterization of a Linear Cyclotide from *Viola odorata*: Implications for the Processing of Circular Proteins. *J. Mol. Biol.,* **357**: 1522-1535.

Kazazi H, Rezaei K, Ghotb-Sharif SJ, Emam-Djomeh Z and Yamini Y (2007). Supercriticial fluid extraction of flavors and fragrances from *Hyssopus officinalis L*. cultivated in Iran. *Food Chem.,* **105**: 805-811.

Kerrola K, Galambosi B and Kallio H (1994). Volatile components and odor intensity of four phenotypes of hyssop (*Hyssopus officinalis L.)*. *J. Agri. Food Chem*., **42**: 776-781.

Khattak SG, Naeemuddin Gilani S and Ikram M (1985). Antipyretic studies on some indigenous Pakistani medicinal plants. *J. Ethnopharmacol.,* **14**(1): 45-51.

Khazaie HR, Nadjafi F and Bannayan M (2008). Effect of irrigation frequency and planting density on herbage biomass and oil production of thyme (*Thymus vulgaris*) and hyssop (*Hyssopus officinalis*). *Industrial Crops and Products*, **27**: 315-321.

NoyanAlpan N and Ziegler MF (1973). Tumor inhibitors. 88. The antileukemia principles of *Colchicum speciosum*. *Lloydia*, **36**(3): 338-340.

Matsuura H, Miyazaki H, Asakawa C, Amano M, Yoshihara T and Mizutani J (2004). Isolation of α-glusosidase inhibitors from hyssop (*Hyssopus officinalis*). *Phytochemistry*, **65**: 91-97.

Meshkatalsadat MH, Mollataghi A and Ata A (2006). New triterpenoidal alkaloids from *Buxus hyrcana*. *Z. Naturforsch B*. **61**: 201-206.

Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Hamidinia A and Bekhradnia AR (2008a). Determination of antioxidant activity, phenol and flavonoids content of *Parrotia persica Mey.* Pharmacologyonline, 2: 560-567

Nabavi SM, Ebrahimzadeh MA, Nabavi SF and Jafari M (2008b). Free radical scavenging activity and antioxidant capacity of *Eryngium caucasicum Trautv* and *Froripia subpinnata*. *Pharmacologyonline,* **3**: 19-25.

Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Fazelian M and Eslami B (2009a). *In vitro* antioxidant and free radical scavenging activity of *Diospyros lotus* and *Pyrus boissieriana* growing in Iran. *Pharmacognosy Magazine,* **4**(18): 123-127.

Nabavi SM, Ebrahimzadeh MA, Nabavi SF and Bahramian F (2009b). *In vitro* antioxidant activity of *Phytolacca americana* berries. *Pharmacologyonline*, **1**: 81-88.

Omidbaigi R (2000). Production and processing of medicinal plants, Vol. 3, Astan Quds Razavi Publications, Behnashr Co. Mashad, Iran, pp.27-31.

Pham-Huy LA, He H and Pham-Huyc C (2008). Free Radicals, Antioxidants in Disease and Health. *International Journal of Biomedical Science,* **4**(2): 89-96.

Souri E, Amin G, Dehmobed-Sharifabadi A, Nazifi A and Farsam H (2004). Antioxidative activity of sixty plants from Iran. *Iran J. Pharm. Res.,* **3**: 55-59.

Terao J and Piskula MK (1997). Flavonoids as inhibitors of lipid peroxidation in membranes. In Rice-Evans CA and Packer L. (editor), Flavonoids in health and disease. Marcel Dekker. New York, pp.277-295.

Tognolini M, Barocelli E, Ballabeni V, Bruni R, Bianchi A, Chiavarini M and Impicciatore M (2006). Comparative screening of plant essential oils Phenylpropanoid moiety as basic core for antiplatelet activity. *Life Sci.,* **78**: 1419-1432.

Willcox JK, Ash SL and Catignani GL (2004). Antioxidants and prevention of chronic disease. *Critical Reviews in Food Science and Nutrition,* **44**: 275-295.

Yen GC and Chen HY (1995). Antioxidant activity of various tea extracts in relation to their antimutagenicity. *J. Agri. Food Chem.,* **43**(1): 27-32.