http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 12 (5): 447-450, 2009
ISSN 1028-8880

Antioxidant Activities of Methanol Extract of *Sambucus ebulus* L. Flower

M.A. Ebrahimzadeh, S.F. Nabavi and S.M. Nabavi
Pharmaceutical Sciences Research Center, Sari School of Pharmacy,
Mazandaran University of Medical Sciences, Sari, Iran

Abstract: In this study antioxidant activity of methanol extract of *Sambucus ebulus* L. flower was investigated employing various *in vitro* assay systems, i.e., DPPH and nitric oxide radical scavenging, hydrogen peroxide scavenging, reducing power, iron ion chelating power and linoleic acid. IC_{50} for DPPH radical-scavenging activity was 228±12 μg mL^{-1}. The extract showed very high activity in the reducing power assay that was comparable with positive control, vitamin C. The extract showed good nitric oxide-scavenging activity (IC_{50} = 309±14 μg mL^{-1}). It was found that antioxidant activity was dose dependent i.e., activity was increased with the increase of their concentrations. The extract showed very weak activity in iron ion chelating (IC_{50} = 1.3±0.07 mg mL^{-1}). It is showed very good activity in scavenging of hydrogen peroxide. IC_{50} for scavenging of extract was 59.5±3.3 μg mL^{-1}. The extracts exhibited no activity in linoleic acid model. The total phenolic content of flower was 56.3±2.81 mg gallic acid equivalent g^{-1} of extract powder and total flavonoid content was 14.5±0.72 mg quercetin equivalent g^{-1} of extract powder by reference to standard curve.

Key words: Antioxidant activity, nitric oxide, DPPH, *Sambucus ebulus*, reducing power

INTRODUCTION

Free radicals are usually short-lived species but they possess a single unpaired electron, rendering them highly reactive against biologically important macromolecules including DNA, proteins and membrane lipids. To counteract this threat to their integrity, cells have evolved a variety of defense systems based on both water-soluble and lipid-soluble antioxidant species and on antioxidant enzymes. A high proportion of the antioxidant systems of the human body are dependent on dietary constituents (Nehir and Karakaya *et al*., 2004). Consequently, the need to identify alternative natural and safe sources of antioxidant arose and the search for safe and natural antioxidants, especially of plant origin has notably increased in recent years (Nabavi *et al*., 2008b; Pourmorad *et al*., 2007). *Sambucus ebulus* (Caprifoliaceae), a kind of shrub, is widely distributed in Northern forest of Iran (Ebrahimzadeh *et al*., 2006). Iranian traditional medicine uses, in various occasions, the leaves and rhizomes of *S. ebulus* (Caprifoliaceae) in treating some inflammatory cases such as bee and nettle bites, arthritis and sore-throat (Ahmadiani *et al*., 1998). In addition, it has been reported to be an insect repellent, antihemorrhoid, antibacterial toward *Helicobacter pylori*, useful in the treatment of burns and infectious wounds, edema, eczema, urticaria, the cold, inflammation and rheumatism (Tuzlaci and Tolon, 2000; Yesilada *et al*., 1999a, b; Ebrahimzadeh *et al*., 2007). Recently, a significant anti-inflammatory activity was observed by use of the hexane extract of fruits of this plant in the laboratory (Ebrahimzadeh *et al*., 2006). Flavonoids, steroids, tannins, glycosides, cardiac glycosides, caffeic acid derivatives, ebulitins, volatile substances, phenol and flavenoid content of this species was previously reported by Ebrahimzadeh *et al*. (2006, 2008b). To best of our knowledge's there is no scientific report about antioxidant capacity, phenol and flavonoid contents of the *S. ebulus* flowers. The objective of this study is to determine the antioxidant activity of methanol extract of *S. ebulus* flowers, using a set of 6 *in vitro* antioxidant assays including DPPH and nitric oxide radical scavenging, hydrogen peroxide scavenging, reducing power, linoleic acid and iron ion chelating power.

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), ascorbic acid, sulfanilamide, N-(1-naphthyl) ethylenediamine dihydrochloride, EDTA and ferric chloride were purchased from Merck (Germany). All other chemicals were of analytical grade or purer.

Corresponding Author: Muhammad Ali Ebrahimzadeh, Department of Medicinal Chemistry, School of Pharmacy, Mazandaran University of Medical Sciences, Postal Code 48189, Sari, Iran
Tel: +98-151-3543081-3 Fax: +98-151-3543084

Plant material and preparation of freeze-dried extract: *S. ebulus* flower were collected from Sari (Panbe chooleh road) in Mazandaran in July of 2007 and identified by Dr. Bahman Eslami. A voucher (No. 675) has been deposited in the Sari School of Pharmacy Herbarium. Material dried at room temperature and coarsely ground before extraction. Each part was extracted 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 phenolic compounds and flavonoid contents: Total phenolic compound contents were determined by the Folin-Ciocalteau reagent according to the recently published method (Nabavi *et al*., 2008b). Result was expressed as gallic acid equivalents. $AlCl_3$ method was used for determination of total flavonoid content of sample extract (Ebrahimzadeh *et al*., 2008c).

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 extract (Ebrahimzadeh *et al*., 2008c). Vitamin 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: Fe (III) reduction is often used as an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant action (Yildirim *et al*., 2001). The reducing power of extract was determined according to the recently publish research (Nabavi *et al*., 2008b). Different amounts of extract (25-800 μg mL^{-1}) 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 indicated reducing power. Vitamin 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. Quercetin was used as positive control (Nabavi *et al*., 2008b).

Metal chelating activity: The chelating of ferrous ions by extracts was estimated by the recently published research (Ebrahimzadeh *et al*., 2008b).

Determination of antioxidant activity by the 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 peroxidation (Nabavi *et al*., 2008a). The inhibitory capacity of extract was tested against oxidation of linoleic acid by FTC method. The percent inhibition of linoleic acid peroxidation was calculated as:

$$\text{Inhibition (\%)} = 100 - \frac{\text{Absorbance increase of the sample}}{\text{Absorbance increase of the control}} \times 100$$

Vitamin C and BHA used as positive control (Ebrahimzadeh *et al*., 2008c).

Scavenging of hydrogen peroxide: The ability of the extract to scavenge hydrogen peroxide was determined according to the method of Ruch (Nabavi *et al*., 2008b). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). The percentage of hydrogen peroxide scavenging by the extract and standard compounds was calculated as follows:

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

where, A_0 was the absorbance of the control and A_1 was the absorbance in the presence of the sample of extract and standard (Nabavi *et al*., 2008b).

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 means separated by Duncan's multiple range test. The EC_{50} values were calculated from linear regression analysis.

RESULTS AND DISCUSSION

Total phenol and flavonoid contents: Total phenol compounds are reported as gallic acid equivalents by reference to standard curve ($y = 0.0063x$, $R^2 = 0.987$). The total phenolic content of flower of *S. ebulus* was 56.3±2.81 mg gallic acid equivalent g^{-1} of extract powder, respectively. The total flavonoid content of extract was 14.5±0.72 mg quercetin equivalent g^{-1} of extract

powder, respectively, by reference to standard curve ($y = 0.0067x + 0.0132$, $R^2 = 0.999$). 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*., 2008b).

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 (Nabavi *et al*., 2008a). It was found that the radical-scavenging activity of the extract increased with increasing concentration. IC_{50} for DPPH radical-scavenging activity was 228±12 μg mL^{-1}. The IC_{50} values for ascorbic acid, quercetin and BHA were 5.05±0.12, 5.28±0.43 and 53.96±2.13 μg mL^{-1}, respectively.

Reducing power: 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. Figure 1 shows the dose-response curves for the reducing powers of the extract. It was found that the reducing powers of the extract increased with the increase of its concentration. The extract showed very high activity (Fig. 1). The reducing power of extract was comparable with vit. C ($p > 0.01$).

Assay of nitric oxide-scavenging activity: The extract showed moderately good nitric oxide-scavenging activity between 0.2 and 1.6 mg mL^{-1}. IC_{50} of *S. ebulus* extract was 309±14 μg mL^{-1}. However, activity of quercetin was very more pronounced than that of our extract (IC_{50} = 17 μg mL^{-1}), its carcinogenic activity has been reported by Dunnik and Hailey *et al*. (1992). In addition to reactive oxygen species, nitric oxide is also implicated in inflammation, cancer and other pathological conditions (Nabavi *et al*., 2008b).

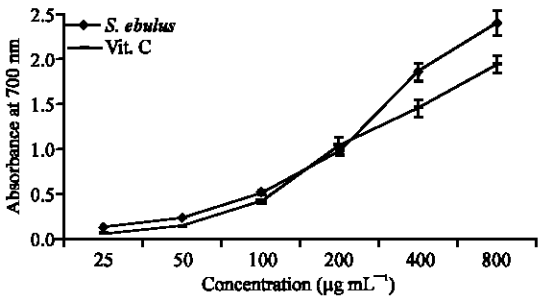

Fig. 1: Reducing power of *Sambucus ebulus* L. flower methanol extract Vit. C used as control

Fe^{2+} chelating ability: Iron chelators mobilize tissue iron by forming soluble, stable complexes that are then excreted in the feces and/or urine. Chelation therapy reduces iron-related complications in human and thereby improves quality of life and overall survival in some diseases such as Thalassemia major (Shinar and Rachmilewitz, 1990; Hebbel *et al*., 1990). In addition, brain iron dysregulation and its association with amyloid precursor protein plaque formation are implicated in Alzheimer's Disease (AD) pathology and so, iron chelation could be considered a rational therapeutic strategy for AD (Reznichenko *et al*., 2006). The transition metal, iron, is capable of generating free radicals from peroxides by Fenton reactions 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. 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 0.2 to 0.8 mg mL^{-1}. 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 *et al*., 1990). Methanol extract of *S. ebulus* flowers had shown very weak activity in iron ion chelating (IC_{50} = 1.3±0.07 mg mL^{-1}). EDTA showed very strong activity (IC_{50} = 18 μg mL^{-1}).

FTC method: The tested extract exhibited very weak activity using the FTC method. The peroxidation inhibition (antioxidant activity) of extract exhibited 86% (at 48th h) only. At the other incubation times (24th and 72nd h), extract showed below 50% inhibition. There were significant differences ($p < 0.001$) among extract and Vit. C or BHA at different incubation times. It suggests that peroxidation inhibition have not any role in antioxidant activity of our extracts and other mechanism may involve.

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*., 2008a). The ability of the extract to effectively scavenge hydrogen peroxide, determined according to the method of Ruch (Nabavi *et al*., 2008a), where it is compared with that of quercetin and ascorbic acid as standards. The extract was capable of scavenging hydrogen peroxide in a concentration-dependent manner. It showed very good activity. IC_{50} for scavenging of extract was 59.5±3.3 μg mL^{-1}. The IC_{50} values for ascorbic

acid and quercetin were 21.4±0.12 and 52.0±3.11 g mL^{-1}, 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*., 2008b).

CONCLUSION

Methanol extract of *S. ebulus* flower exhibited very good but different levels of antioxidant activity in almost all the models studied. The extract had good reducing power activity, DPPH radical-scavenging activity and hydrogen peroxide scavenging. Further investigation of individual compounds, their *in vivo* antioxidant activities and in different antioxidant mechanisms is needed.

ACKNOWLEDGMENT

This research was supported by a grant from the research council of Mazandaran University of Medical Sciences, Iran. This study is dedicated to Mrs. Maryam Nabavi and Seyed Morteza Nabavi.

REFERENCES

Ahmadiani, A., M. Fereidoni, S. Semnanian, M. Kamalinejad and S. Saremi, 1998. Antinociceptive and anti-inflammatory effects of *Sambucus ebulus* rhizome extract in rats. J. Ethnopharmacol., 61: 229-235.

Dunnik, J.K. and J.R. Hailey, 1992. Toxicity and carcinogenicity studies of quercetin, a natural component of foods. Fundam. Applied Toxicol., 19: 423-431.

Ebrahimzadeh, M.A., M. Mahmoudi and E. Salimi, 2006. Antiinflammatory activity of *Sambucus ebulus* hexane extracts. Fitoterapia, 77: 146-148.

Ebrahimzadeh, M.A., M. Mahmoudi, M. Karami, S. Saeedi, A.H. Ahmadi and E. Salimi, 2007. Separation of active and toxic portions in *Sambucus ebulus*. Pak. J. Biol. Sci., 10: 4171-4173.

Ebrahimzadeh, M.A., S.J. Hosseinimehr, A. Hamidinia and M. Jafari, 2008a. Antioxidant and free radical scavenging activity of *Feijoa sallowiana* fruits peel and leaves. Pharmacologyonline, 1: 7-14.

Ebrahimzadeh, M.A., F. Pourmorad and A.R. Bekhradnia, 2008b. Iron chelating activity screening, phenol and flavonoid content of some medicinal plants from Iran. Afr. J. Biotechnol., 7: 3188-3192.

Ebrahimzadeh, M.A., F. Pourmorad and S. Hafezi, 2008c. Antioxidant activities of Iranian corn silk. Turk. J. Biol., 32: 43-49.

Gordon, M.H., 1990. The Mechanism of Antioxidant Action *in vitro*. In: Food Antioxidants. Hudson, B.J.F. (Ed.). Elsevier Applied Science, London, UK., ISBN: 1851664408, pp: 1-18.

Halliwell, B. and J.M.C. Gutteridge, 1990. Role of free radicals and catalytic metal ions in human disease: An overview. Method Enzymol., 186: 1-85.

Hebbel, R.P., A. Leung and N. Mohandas, 1990. Oxidation-induced changes in microheological properties of the red cell membrane. Blood, 76: 1015-1020.

Nabavi, S.M., M.A. Ebrahimzadeh, S.F. Nabavi and M. Jafari, 2008a. Free radical scavenging activity and antioxidant capacity of *Eryngium caucasicum* Trautv and *Froripia subpinata*. Pharmacologyonline, 3: 19-25.

Nabavi, S.M., M.A. Ebrahimzadeh, S.F. Nabavi, A. Hamidinia and A.R. Bekhradnia, 2008b. Determination of antioxidant activity, phenol and flavonoid content of *Parrotia persica* MEY. Pharmacologyonline, 2: 560-567.

Nehir, E.I.S. and S. Karakaya, 2004. Radical scavenging and iron-chelating activities of some greens used as traditional dishes in Mediterranean diet. Int. J. Food Sci. Nutr., 55: 67-74.

Pourmorad, F., M.A. Ebrahimzadeh, M. Mahmoudi and S. Yasini, 2007. Antinociceptive activity of methanolic extract of *Epilobium hirsutum*. Pak. J. Biol. Sci., 10: 2764-2767.

Reznichenko, L., T. Amit, H. Zheng, Y. Avramovich-Tirosh, M.B.H. Youdim, O. Weinreb and S. Mandel, 2006. Reduction of iron-regulated amyloid precursor protein and [beta]-amyloid peptide by (-)-epigallocatechin-3-gallate in cell cultures: Implications for iron chelation in Alzheimer's disease. J. Neurochem., 97: 527-536.

Shinar, E. and E.A. Rachmilewitz, 1990. Oxidation denaturation of red blood cells in thalassemia. Semin. Hematol., 27: 70-82.

Tuzlaci, E. and E. Tolon, 2000. Turkish folk medicinal plants, part III: Sile (Istanbul). Fitoterapia., 71: 673-685.

Yesilada, E., I. Gurbuz and H. Shibata, 1999a. Screening of Turkish anti ulcerogenic folk remedies for anti Helicobacter pylori activity. J. Ethnopharmacol., 66: 289-293.

Yesilada, E., E. Sezik, G. Honda, Y. Takaishi, Y. Takeda and T. Tanaka, 1999b. Traditional medicine in Turkey IX: Folk medicine in Northwest Anatolia. J. Ethnopharmacol., 64: 195-210.

Yildirim, A., A. Mavi and A. Kara, 2001. Determination of antioxidant and antimicrobial activities of *Rumex crispus* L. extracts. J. Agric. Food Chem., 49: 4083-4089.