Antioxidant Activities of Methanol Extract of Sambucus ebulus L. Flower

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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. IC₅₀ 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 (IC₅₀ = 306±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 (IC₅₀ = 1.3±0.07 mg mL⁻¹). It is showed very good activity in scavenging of hydrogen peroxide. IC₅₀ 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 Kamkaya 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; Pournorad 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 (Ahmadnami et al., 1998). In addition, it has been reported to be an insect repellent, antihelmintic, antibacterial toward Helicobacter pylori, useful in the treatment of burns and infectious wounds, edema, eczema, urticaria, the cold, inflammation and rheumatism (Tuzlacı and Tolen, 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, ebultins, volatile substances, phenol and flavonoid 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 activity, 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-picrylhydrazyl (DPPH), potassium ferricyanide were purchased from Sigma Chemicals Co., (USA). Gallic acid, quercetin, Butylated hydroxyanisole (BHA), ascorbic acid, sulfanilamide, N-(1-raphthyl) ethylenediamine dihydrochloride, EDTA and ferric chloride were purchased from Merck (Germany). All other chemicals were of analytical grade or purer.

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Plant material and preparation of freeze-dried extract: *S. ebulis* flower were collected from Sari (Panbe chooleh road) in Mazandaran in July of 2007 and identified by Dr. Behman Esfandi. 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. 

**DPPH radical-scavenging activity:** The stable 1,1-diphenyl-2-picrylhydrazyl 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 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 (Yıldırım et al., 2001). The reducing power of extract was determined according to the recently published 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 [K₃Fe(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 FeCl₃ (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:

\[
\text{Scavenged} \left[ \text{H}_2\text{O}_2 \right] (\%) = \frac{A_0 - A_1}{A_0} \times 100
\]

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 Mean±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 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 \(y = 0.0063x, \text{R}^2 = 0.987\). The total phenolic content of flower of *S. ebulis* 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).

**DPHH 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 \( \mu \text{g mL}^{-1} \).
The IC\(_{50}\) values for ascorbic acid, quercetin and BHA were
5.05±0.12, 5.28±0.43 and 53.96±2.13 \( \mu \text{g mL}^{-1} \),
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\(^{3+}\) 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 \( \text{mg mL}^{-1} \). IC\(_{50}\) of \( S. \text{ebulus} \) extract was
309±14 \( \mu \text{g mL}^{-1} \). However, activity of quercetin was
very more pronounced than that of our extract
(IC\(_{50}\) = 17 \( \mu \text{g mL}^{-1} \)), 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).

**Fe\(^{3+}\) 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\(^{3+}\) causes the production of oxynitrides
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\(^{3+}\)-ferrozine complex was decreased dose-dependently,
i.e., the activity was increased on increasing
concentration from 0.2 to 0.8 \( \text{mg mL}^{-1} \). 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 \( S. \text{ebulus} \)
flowers had shown very weak activity in iron ion chelating
(IC\(_{50}\) = 1.3±0.07 \( \mu \text{g mL}^{-1} \)). EDTA showed very strong
activity (IC\(_{50}\) = 18 \( \mu \text{g mL}^{-1} \)).

**FTC method:** The tested extract exhibited very weak
activity using the FTC method. The peroxidation
inhibition (antioxidant activity) of extract exhibited 80% (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 \( \text{H}_2\text{O}_2 \) by
extracts may be attributed to their phenolics, which can
donate electrons to \( \text{H}_2\text{O}_2 \), 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 \( \mu \text{g mL}^{-1} \). The IC\(_{50}\) values for ascorbic

![Graph](image-url)
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 H₂O₂ 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.

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REFERENCES


