Free Radicals Scavenging and Antioxidant Activity of Vicia sojakii

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Some species of Vicia genus (Papilionaceae) are used in traditional medicine. In the present study, free radical scavenging and antioxidant activity of aerial parts of V. sojakii were investigated in six different models. IC50 for DPPH radical-scavenging activity was 440.4 ± 13.2 mg mL-1. It showed good nitric oxide-scavenging activity between 0.2 and 1.6 mg mL-1. Extract showed very potent Fe2+ chelating ability. IC50 was 72 ± 2.8 mg mL-1. Extract exhibited low activity in linoleic acid peroxidation test. The total amount of phenolic compounds in extract was determined as gallic acid equivalents (47.8 ± 1.6 mg . g-1) and total flavonoid contents were calculated as quercetin equivalents (18.8 ± 0.47mg . g-1) from a calibration curve. Antioxidant activity may be attributed, at least in part, to the presence of phenols and flavonoids in the extract.

Keywords: Antioxidant activity, Chelating ability, Vicia sojakii

Reactive oxygen species have been found to play an important role in the initiation and/or progression of various diseases such as atherosclerosis, inflammatory injury, cancer, and cardiovascular disease [1]. Recent studies have investigated the potential of plant products as antioxidants against various diseases induced by free radicals [2]. In addition, it has been determined that the antioxidant effect of plant products is mainly attributed to phenolic compounds, such as flavonoids and phenolic acids [3]. Among the various medicinal plants, some endemic and edible species are of particular interest because they may be used for producing raw materials or preparations containing phytochemicals with significant antioxidant capacities and health benefits [4-6]. Vicia genus member of Papilionaceae family has 45 species in Iran [7]. The extract from V. sativum showed insecticidal activity. Also it has exhibited hepatoprotective effect against CCI4 induced hepatotoxicity [8]. Antioxidant activities of V. faba [9], V. charca and V. sativa [10] and V. sativum [8] have been reported previously. Also anti-inflammatory and antinociceptive activity of V. sativa [11] and antimicrobial and cytotoxic activity of V. faba [9] have been reported. V. sojakii is native to Iran [7] and there is no report on its biological activity. The aim of this study was to determine the antioxidant activity of V. sojakii aerial parts in order to understand the usefulness of this plant.

Experimental part
Plant material and preparation of extract
V. sojakii aerial parts were collected from Golestanak, Iran, in summer 2008. Aerial parts were dried at room temperature. Dried materials were coarsely ground before extraction. 100g of aerial parts were extracted by percolation method using methanol for 24 h at room temperature. The extract was then separated from the sample residue by filtration through Whatman No.1 filter paper. This procedure was repeated three times. The resultant extracts were concentrated in a rotary evaporator until crude semi-solid extracts were obtained (16.5 %).

Determination of total phenolic compounds and flavonoid content
Total phenolic compound contents were determined by the Folin-Ciocalteau method [12,13]. The extract sample (0.5 mL) was mixed with 2.5 mL of 0.2 N Folin-Ciocalteau reagent for 5 min and 2.0 mL of 75 g L-1 sodium carbonate were then added. The absorbance of reaction was measured at 760 nm after 2 h of incubation at room temperature. Results were expressed as gallic acid equivalents. Total flavonoids content was determined by colorimetric method [12,13]. Briefly, 0.5 mL solution of plant extract 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 min. The absorbance of the reaction mixture was measured at 415 nm with a double beam spectrophotometer (Perkin Elmer). Total flavonoid contents were calculated as quercetin from a calibration curve.

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 [14,15]. Different concentrations of extract were added, at an equal volume, to methanolic solution of DPPH (100 mM). After 15 min at room temperature, the absorbance was recorded at 517 nm. The experiment was repeated for three times. Vitamin C, BHA and quercetin were used as standard controls. IC50 values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals.

Reducing power determination
The reducing power of V. sojakii was determined according to the method [16]. 2.5 mL 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 mixture indicated increased reducing power. Vitamin C was used as positive control.

Assay of nitric oxide-scavenging activity
For this experiment, sodium nitroprusside (10 mM), in phosphate-buffered saline, was mixed with different concentrations of each extracts dissolved in water and
but with an equivalent amount of water, served as control. 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 [17].

Metal chelating activity
The chelating of ferrous ions by V. sojakii was estimated according to our recently published paper [18]. Briefly, the extract (25 to 400 mg mL⁻¹) was added to a solution of 2 mM FeCl₂ (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–Fe²⁺ complex formation was calculated as \( \frac{(A_0 - A_s)}{A_0} \times 100 \), where \( A_0 \) was the absorbance of the control, and \( A_s \) was the absorbance of the extract/standard. \( \text{Na}_2\text{EDTA} \) was used as positive control.

Determination of antioxidant activity by the FTC Method
This method was adopted from Osaka and Namiki [19]. Twenty mg mL⁻¹ of samples dissolved in 4 mL of 95% (v/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 screwcap 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) 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 screwcap 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 the sample/absorbance increase of the control) / 100]). All tests were run in duplicate, and analyses of all samples were run in triplicate and averaged. Vitamin C and BHA were used as positive control.

Data analysis
Experimental results were expressed as means ± SD of 3 determinations. The data were analyzed by analysis of variance (p < 0.05) and the means separated by Duncan’s multiple range test.

Results and Discussions
Total phenol and flavonoid contents
The total phenolic content was 47.8 ± 1.6 mg gallic acid equivalent g⁻¹ of extract powder by reference to standard curve (\( y = 0.0054x + 0.0628, \ r^2 = 0.987 \)). The total flavonoid content was 18.8 ± 0.47 mg quercetin equivalent g⁻¹ of extract powder, by reference to standard curve (\( y = 0.0063x, \ r^2 = 0.999 \)). This plant shown high total phenol and flavonoid contents. 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 [20]. Studies have shown that increasing levels of flavonoids in the diet could decrease certain human diseases [21].

DPPH radical-scaavenging activity
The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples [22]. 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 donation [23].

\[ \text{DPPH}^+ + \text{antioxidant} \rightarrow \text{DPPH-H} + \text{antioxidant}^- \] (Purple color) (Yellow color)

The IC₅₀ for DPPH radical-scavenging activity was 440.4 ± 13.2 μg mL⁻¹. The IC₅₀ values for vitamin C, quercetin and BHA were 1.26 ± 0.11, 1.32 ± 0.07 and 13.49 ± 1.04 μg mL⁻¹, respectively. Phenol and flavonoid contents of this plant may lead to its good DPPH-scavenging activity.

Reducing power of extracts
Fe (III) reduction is often used as an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant action [15]. In this assay, the presence of reductants (antioxidants) in the samples would result in the reducing of Fe³⁺ to Fe²⁺ by donating an electron. Amount of Fe²⁺ complex can be then 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 V. sojakii extract. It was found that the reducing powers of extract also increased with the increase of its concentrations. The extract exhibited a moderate reducing power at 100 and 800 μg mL⁻¹.

Fig.1. Reducing power of V. sojakii aerial parts. Vitamin C used as control.

Assay of nitric oxide-scavenging activity
The extract also showed weak nitric oxide (NO) scavenging activity between 0.1 and 1.6 mg mL⁻¹. The procedure is based on the principle that, sodium nitroprusside in aqueous solution at physiological \( p\text{H} \) spontaneously generates NO which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Scavengers of NO compete with oxygen, leading to reduced production of nitrite ions. The % inhibition was increased with increasing concentration of the extract. IC₅₀ for V. sojakii was 949.2 ± 37.9 μg mL⁻¹ for V. sojakii and 17.01 ± 0.03 mg mL⁻¹ for quercetin. In addition to reactive oxygen species, NO is also implicated in inflammation, cancer and other pathological conditions [24]. The plant/plant products may have the property to counteract the effect of NO formation and in turn may be of considerable interest in preventing the ill effects of excessive NO generation in the human body. Further, the scavenging activity may also help to arrest the chain of reactions initiated by excess generation of NO that are detrimental to human health.

Fe²⁺ chelating activity of extract
Abundant evidence exists from in vitro experiments that iron can catalyze the production of oxyradicals when iron
is available in a redox-active form. Ferrous iron chelates can react with lipid hydroperoxides (LOOH) to form alkoxyl radicals (LO•): LOOH + Fe2+ → Fe3+ + OH• + LO•. Lipid peroxyl radicals can perpetuate the chain reaction of lipid peroxidation by extracting hydrogen atoms from nearby lipids or can react with other cell constituents. Iron also can catalyze the production of hydroxyl radicals (OH•) through Fenton chemistry. In this reaction, the OH• is produced from hydrogen peroxide: Fe2+ + H2O2 → Fe3+ + OH• + OH•. The hydroxyl radical is extremely reactive and can attack many cell constituents, including lipids, nucleic acids, and proteins [25]. 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, cancer or Wilson’s disease [26]. These processes can be delayed by iron chelation and deactivation. Minimizing Fe2+ concentration in Fenton reactions affords protection against oxidative damage. Ferrozine can quantitatively form complexes with Fe2+. In the presence of other chelating agents, the complex formation is disrupted with the result that the red color of the complexes decreases. In this assay, both extract and EDTA showed very strong activity (IC50 = 18 mg mL−1). It was reported that ferrous ion before ferrozine. The absorbance of Fe2+-ferrozine complex was decreased dose-dependently, capturing ferrous ion before ferrozine. The absorbance of complex, suggesting that it has chelating activity and the complexes decreases. In this assay, both extract and the formation is disrupted with the result that the red color of the extract had very potent Fe2+ chelatory activity. Further investigation of individual compounds and the mechanisms of activities are needed.

Conclusions

V. sojakii aerial parts exhibited good but different levels of antioxidant activity in some of models studied. The extract had very potent Fe2+ chelatory activity. Further investigation of individual compounds and the mechanisms of activities are needed.

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References