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Original Article

Determination of Total Flavonoid and Phenolic Contents and *In Vitro* Evaluation of Antioxidant Effects of *Rosa persica*

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Abstract

Background: *Rosa persica* (RP), a member of the *Rosaceae* family, is known for its high phenolic content. These compounds reportedly possess antiradical and antioxidant activities. This study aimed to investigate the total flavonoid and phenolic contents of three parts of RP and their potential antioxidant and antiradical activities using two methods (ferric reducing ability of plasma [FRAP] and 1,1-diphenyl-2-picrylhydrazyl [DPPH] assays).

Methods: Flowers, fruits, and seeds of RP were collected from Hamedan Province, Iran, and the hydroalcoholic extracts were prepared from each part separately. Then, the total flavonoid and phenolic contents of the extracts were determined, and the antioxidant activities were evaluated using the FRAP and DPPH assays.

Results: The total phenolic content of the fruit, seed, and flower extracts was 137.56 ± 9.98, 191.79 ± 20.51, and 203.15 ± 4.58 mg of gallic acid equivalent/1 g of hydroalcoholic extract, respectively. The total flavonoid content of the fruit, seed, and flower extracts was 74.72 ± 15.60, 70.00 ± 16.12, and 120.53 ± 0.00 mg of rutin/1 g of hydroalcoholic extract, respectively. The FRAP assay results of the fruit, seed, and flower extracts were 3.95 ± 0.49, 13.92 ± 1.78, and 11.32 ± 173 µmol of ferrous sulfate.$7H_2O$/1 g of hydroalcoholic extract, respectively. The IC_{50} values of the extracts in the DPPH assay were 899.8, 45.83, and 70.75 µg/mL.

Conclusion: The results suggest that extracts from different parts of RP are rich sources of flavonoids and phenolic compounds as natural antioxidants and can be used to prevent deleterious effects caused by free radicals.

Keywords: Antioxidant activity, Flavonoid content, *In vitro,* Oxidative stress, Phenolic content, *Rosaceae*

Introduction

Oxidative stress is defined as an imbalance between the production of reactive oxygen/nitrogen species (ROS/RNS) and the organism's ability to neutralize them using antioxidant defense systems [1, 3]. Oxidative stress can result from increased ROS/RNS generation or a degeneration of the antioxidant protective ability [4, 6]. Antioxidants are compounds that inhibit the initiation or propagation of oxidative chain reactions, thereby delaying or terminating the oxidation of other molecules [7, 8]. They can also be categorized as synthetic or natural [9, 10]. For

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example, nutrients such as vitamin E (alpha-tocopherol), vitamin C (ascorbic acid), beta-carotene, polyphenols, carotenoids, flavonoids, and antioxidant enzymes are natural antioxidants that play an essential protective role against the damaging effects of ROS/RNS [11, 13]. In contrast, phenolic compounds are the main source of synthetic antioxidants, including butylated hydroxyanisole, butylated hydroxytoluene, propyl gallate, and tertiary butylhydroquinone, which have been extensively applied in the food industry to minimize lipid oxidation in food products [14, 18]. Nevertheless, some studies indicate that synthetic antioxidants have toxicological and carcinogenic effects on cells of experimental animals [14, 19, 23]. The assessment of the antioxidant effects of plant extracts has recently received considerable attention. Herbs and spices are widely considered innocuous sources of natural antioxidants [24].

The genus *Rosa* L. (*Rosaceae*) consists of over 200 species and 180000 cultivars, spread throughout the subtropical and temperate areas of the Northern Hemisphere [25, 26]. The fruit and flowers of these deciduous shrubs make these plants suitable for planting in gardens. The plants also show strong resistance to harsh environmental conditions [27, 28]. The medical, perfumery, and food industries have long used plants from the *Rosa* L. genus [29]. In addition to high ascorbic acid (vitamin C) content, rose hips contain other vitamins, minerals, fruit acids, sugars, tannins, tocopherol, bioflavonoids, pectin, carotenoids, organic acids, essential oils, and amino acids. Rose hips have also been recognized as effective plant parts that boost the immune system against infections, especially the common cold. [30, 31]. Other parts of *Rosa persica* (RP), such as roots, leaves, and flowers, have various applications in Chinese medicine. For example, the roots have been used to treat injuries, rheumatic arthritis, uterine prolapse, and anus prolapse; and the leaves have been applied to treat furuncles, carbuncles, and burns [32, 33].

RP Michx. ex Juss., syn. *Hulthemia persica* (Michx.), Bornm., R. berberifolia Pall. is a dwarf suckering and procumbent shrub that grows up to 50 cm in height and produces single flowers with yellow petals of up to 15 mm long with a red base, purple anthers, and rounded, bristly, blackish hips; and has many curved prickles and straight spines (Fig. 1). This plant is native to the desert and steppe areas of Iran, Afghanistan, Central Asia, and northwestern Siberia. It is common in Iran and grows as a weed, which is utilized for fuel [34, 36]. To the best of our knowledge, the antioxidant activity of RP has rarely been directly studied. A new approach is therefore needed to evaluate the antioxidant activity of various parts of this plant. This in vitro study aimed to determine the total phenolic and flavonoid contents of different parts of RP from Hamedan to assess their potential antiradical and antioxidant activities using ferric reducing ability of plasma (FRAP) and 1,1-diphenyl-2-picrylhydrazyl [DPPH] assays.



Figure 1. Rosa persica

Materials and Methods

Chemicals

All solvents and chemical reagents used in this investigation, including DPPH, rutin, gallic acid, Folin-Ciocalteu reagent (FCR), sodium bicarbonate, $AlCl_3$, ascorbic acid, 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ), hydrochloric acid, $FeCl_3$, acetate buffer solution, ferrous sulfate.$7H_2O$, and ethanol, were purchased from Merck (Darmstadt, Germany)

Plant Materials

The flowers, seeds, and fruit of RP were harvested from Hamedan Province, Iran, from May to October 2015. An expert taxonomist from the School of

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Pharmacy, Hamadan University of Medical Sciences, Hamadan, Iran, identified the plant materials.

Extract Preparation

The dried and powdered fruit (216 g), flowers (103 g), and seeds (70 g) of RP were macerated in hydro-ethanol solution (water/ethanol ratio: 2:8 v/v) at room temperature for 72 h. The maceration was repeated three times. Then, each extract (macerate) was filtered and concentrated under reduced pressure.

Measurement of Antioxidant Activity

Measurement of Free Radical-Scavenging Activity (1,1-Diphenyl-2-Picrylhydrazyl [DPPH] Assay)

The radical-scavenging activity of the extracts was evaluated to assess antioxidant activity, following methods previously described by Kerdar et al., with some modifications. Briefly, 200 µL of 0.15 mM DPPH• solution in ethanol was added to 20 µL of each sample at various concentrations of 1000, 500, 250, 125, 100, 50, 25, 10, 5, 2.5, and 1.25 µg/mL. Test tubes were vortexed and incubated at room temperature in the dark for 30 min. The absorbance values were then measured at 518 nm using a Synergy HDX ELISA reader. The following equation was used to calculate the inhibition percentage of DPPH radical:

$$I\ DPPH^{\cdot}\ \% = \frac{\mathrm{Ac} - \mathrm{As}}{\mathrm{Ac}} \times 100$$

A total of 200 µL of DPPH• solution and 20 µL of ethanol were used as a negative control. Furthermore, rutin and vitamin C solutions at concentrations of 1000, 500, 250, 125, 100, 50, 25, 10, 5, 2.5, and 1.25 µg/mL were utilized as a positive control. A logarithmic regression curve was obtained by plotting the inhibition percentage of DPPH radical against sample concentration to evaluate the IC_{50} value (inhibitory concentration), defined as the sample concentration (µg/mL) needed to decrease DPPH absorbance by 50% [37].

The Ferric Reducing Ability of Plasma (FRAP Assay)

The amounts of 3 mL of a FeCl3 solution (20 mmol/L) mixed in 30 mL of an acetate buffer (0.3 mol/L, pH 3.6) and 3 mL of a TPTZ solution (10 mmol/L) mixed with hydrochloric acid (40 mmol/L) were utilized to prepare the FRAP reagent. Then, 10 µL of FeSO4 ferrous sulfate.7H_2O (as standard) and sample solutions in ethanol (1000, 500, 250, 125 µg/mL) were mixed with 300 µL of FRAP solution. The absorbance was measured at 593 nm using a Synergy HDX ELISA reader. Next, a linear calibration curve (absorbance versus concentration) was obtained. The plot appeared to be linear across the assay range (1000-125 µg/mL, r2 > 0.99). The total antioxidant capacity of each extract (as µmol of ferrous sulfate.7H_2O equivalent [RE]/g of extract) was assessed according to the standard curve [38].

Measurement of Total Phenolic Content (TPC)

FCR was used to evaluate the TPC of the extracts using gallic acid as the reference compound. A total of 150 µL of FCR (diluted 1/10) was mixed with 30 µL of gallic acid and the sample solution in ethanol at different concentrations of 1000, 750, 500, 300, 150, 100, 50, 25, 10, and 5 µg/mL, then incubated at room temperature. Then, 120 µL of sodium carbonate solution (75 mg/mL) was added after 10 minutes. The ultimate solution was completely mixed and stored in the dark for 30 minutes. Absorbance was determined at 765 nm using a Synergy HDX ELISA reader. A linear calibration curve (absorbance versus concentration) was then obtained. The plot was linear across the ranged assay (300-25 µg/mL, r2>0.99). The TPC of each extract (mg of gallic acid rutin equivalent [RE]/g of extract) was determined using a standard curve [39].

Measurement of Total Flavonoid Content (TFC)

$AlCl_3$ and rutin (standard) were applied to assess the total flavonoid content (TFC) as described by Ordonez et al. The amounts of 100 µL of $AlCl_3$ solution (20mg/mL) and 100 µL of CH_3COOK 1 M were mixed with 100 µL of rutin and sample solution in ethanol at the concentrations of 1000, 750, 500, 300, 150, 100, 50, 25, 10, and 5 µg/mL. After 40 min of incubation at room temperature, absorbance was read at 415 nm using a Synergy HDX ELISA reader. A linear calibration curve of absorbance and concentration was then achieved. Across the assay range (150-10 µg/mL, r**2**>0.99), the plot appeared to be linear. The TFC for the extracts (as mg of RE/g of extract) was calculated according to the standard curve [39].

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Statistical Analysis

All experiments were performed in triplicate. IC_{50} values were reported with their corresponding 95% confidence limits. The TFC, TPC, and FRAP (mg/g) were presented as mean ± SEM. The statistical analysis of the DPPH test was performed using GraphPad Prism 6.07 for Windows (GraphPad Software, San Diego, California, USA). All statistical analysis of TFC, TPC, and FRAP was performed using Excel software (Microsoft).

Results

To evaluate the antioxidant capacity of plant extracts, researchers have used various methods, among which the FRAP and DPPH assays are widely used and effective to measure the free-radical-scavenging activity and antioxidant capacity. Moreover, they are widely used to evaluate the antioxidant activity of hydrogen-donating and chain-breaking antioxidants [40].

Polyphenolic compounds are an essential part of the human diet due to their existence in all plant structures. Food phenolics have attracted considerable interest in recent decades owing to their free radical-scavenging and antioxidant activities. For example, polyphenols protect endogenous antioxidant enzymes in the human body, which enables these phenolic compounds to show indirect antioxidant activities [41]. Thus, in this investigation, the total phenolic and flavonoid contents of the hydroalcoholic extracts obtained from three parts of RP were determined, and their antioxidant capacities were evaluated using two methods.

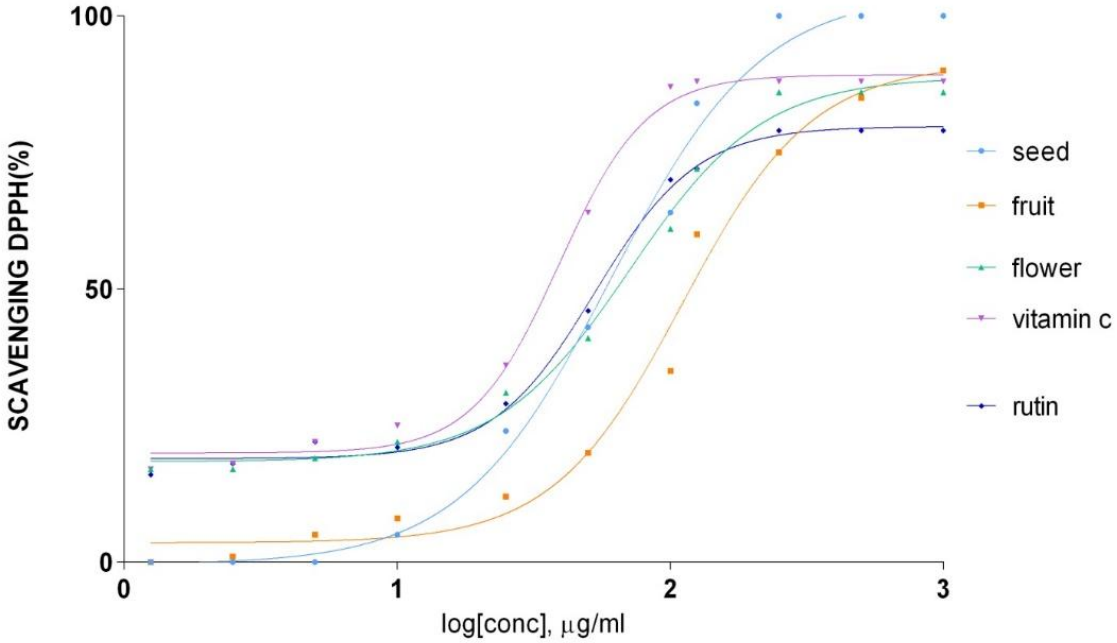


Figure 2. Determination of Free Radical-Scavenging and Antioxidant Activities of the Fruit, Seeds, and Flowers Extracts of *Rosa persica* Using DPPH Assay. Each point demonstrates the mean of three experiments, and the vertical bars indicate the SEM.

The DPPH assay results (Figure 2 and Table 1) showed that all extracts exhibited a considerable concentration-dependent antiradical effect; however, they showed different inhibitory activities. The seed extract possessed the highest free radical-scavenging activity (IC_{50} = 45.83 [35.79-58.68] µg/mL), whereas the fruit extract had the lowest activity (IC_{50}= 899.8 [650.97-1227.3] µg/mL). The IC_{50} (DPPH•) of the extracts rose in value in the following order: fruit < flowers < seeds.

Table 1. The IC50 Values of Different Parts of *Rosa persica* in DPPH Assay

Parts of *Rosa persica*/Standard	Amount of IC_{50} (µg/mL)	Range of IC_{50} (µg/mL)
Flowers	70.75	58.89 to 85.01
Fruit	899.8	650.97 to 1227.3
Seeds	45.83	35.79 to 58.68
Rutin	52.26	46.17 to 59.14
Vitamin C	39.49	34.96 to 44.62

Abbreviations: DPPH, 1,1-diphenyl-2-picrylhydrazyl; IC_{50}, inhibitory concentration.

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Discussion

Based on the results in Table 2, the extracts showed significant differences in total phenolic content ranging from 137.56 to 203.15 mg/g. Among the tested RP extracts, the flower extract had the highest TPC (203.15 ± 4.58 mg/g), while the fruit extract had the lowest (137.56 ± 9.98 mg/g). The TPC of the extracts decreased in the following order: flowers > seeds > fruit (Table 2). Table 2 also revealed that the TFC of the extracts varied considerably, ranging from 70.00 to 120.53 mg/g. Among the tested extracts, the flower extract exhibited the highest TFC value (120.53 ± 0.00 mg of rutin/1g of extract). By contrast, the seed extract demonstrated the lowest value (70.00 ± 16.12 mg of rutin/1g of extract). The TFC values decreased in the following order: flowers > fruit > seeds (Table 2).

Thus, the tested plant parts in this study possess high antioxidant properties and can also be considered rich sources of phenolic compounds. The total antioxidant activity (FRAP assay) of the extracts was similar to the DPPH• scavenging capacity. Since total extracts are composed of various compounds with different activities, the antioxidant activity of RP can be a result of the other compounds in the extracts that may exert antioxidant activity. The results confirm that various plant parts of the genus *Rosa* are considerable natural antioxidant sources. Moreover, the results of this study are consistent with previous findings that rose hips have strong antioxidant capacity [42, 44]. Hence, the free radical-scavenging and antioxidant activities may be due to phenolic compounds in *Rosa* species. On the other hand, the antioxidant activities in these plants can be explained by other components that do not contain phenolic structures [45, 46].

Conclusion

This study investigated the total flavonoid and phenolic contents of various parts of RP for their potential antiradical and antioxidant activities using the FRAP and DPPH methods. The results revealed that among the hydroalcoholic extracts of the flowers, seeds, and fruit of RP, the hydroalcoholic extract of the seeds exhibited the highest free radical-scavenging activity (IC50 = 45.83 µg/mL) and the highest total antioxidant activity (FRAP assay). Furthermore, the hydroalcoholic extract of flowers possessed the highest TPC value (203.155 ± 4.589 mg/g) and TFC (120.535 ± 0.007 mg of rutin/1g of extract). The findings also suggest that hydroalcoholic extracts from different parts of RP are rich sources of natural antioxidants, such as phenolic and flavonoid compounds, and can be applied to inhibit detrimental effects induced by free radicals. Besides, this conclusion follows from the fact that the antioxidant activities of the hydroalcoholic extract of various parts (seeds, fruit, and flowers) of RP are not correlated with the phenolic and flavonoid contents. This may constitute the object of future studies.

Conflict of Interest

The authors declared no conflicts of interest.

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