



Antioxidant and antiradical activities of *Rosa canina* and *Rosa pimpinellifolia* fruits from West Azerbaijan

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Abstract

Rosa canina L. is a member of *Rosaceae* family, which is well-known for its high phenolic contents. These compounds are known to possess antioxidant and antiradical effects. In order to compare the antioxidant and antiradical activity of wild *Rosa* phenolic extracts, two wild *Rosa* species (*Rosa canina* L. and *Rosa pimpinellifolia* L.) were selected from Takab, Oshnavieh and Qasemloo Valley of Urmia, West Azerbaijan province, Iran, in 2011. The fruits of *R. canina* and *R. pimpinellifolia* were collected and then methanolic extracts were prepared from these fruits. The extracts' total phenolic and flavonoid contents and scavenging capacity for radicals nitric oxide, hydrogen peroxide, and DPPH were analyzed. Fruit extracts, respectively, had a range of 176.48 ± 2.71 and 225.65 ± 2.50 mg gallic acid equivalents /100 g methanolic extract in total phenolic content, 0.41 ± 0.02 and 2.02 ± 0.03 mg quercetin/100 g methanolic extract in total flavonoid content, $22.41 \pm 0.64\%$ and $58.10 \pm 0.72\%$ in hydrogen peroxide, $79.16 \pm 0.61\%$ and $87.78 \pm 0.10\%$ in DPPH, and $76.93 \pm 2.31\%$ and $236.76 \pm 16.04\%$ in nitric oxide radical scavenging percentage. The findings suggested that radical scavenging capacities of *R. canina* and *R. pimpinellifolia* extracts in different populations positively correlated with phenolic content.

Keywords: *Rosa* species; antioxidant; antiradical; phenol; flavonoid

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Introduction

The genus *Rosa* with over 100 species, is one of the most widespread members of the *Rosaceae* family. This genus is widely distributed in Europe, Asia, the Middle East and North America (Nilsson, 1997). Antioxidant activity of 50% aqueous methanol extracts of *R. canina* has also been reported, mainly as free radical scavenging and hydrogen peroxide scavenging activities (Serteser et al.,

2008). Mavi et al. (2004) also reported high antioxidant properties of hips of *R. pimpinellifolia*. Such activities can be attributed to the different components present in this species: phenols, carotenoids, vitamin C, tocopherol, pectin, sugar, organic acids, amino acids and essential oils (Ercisli, 2007).

Rosehips are used in many European countries in food products such as tea, Jam, marmalade, and for medical purposes (Demir et al, 2001). In fact, *Rosa* spp has long been used as a herbal tea, vitamin supplement or food product in many European countries, as it is rich in vitamin C. Also, rosehips are a rich source of minerals (K and P), folate, and phenolic

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compounds (Demir et al., 2001). Recent studies revealed that *R. canina* extracts are effective on the inhibition of growth and biofilm formation in methicillin-resistant *Staphylococcus aureus* (MRSA) (Serteser et al., 2008; Quave et al., 2008). The reaction of *Rosa* with biological molecules leads to cell and tissue injury by lipoperoxidation, proteolysis or DNA degradation (Weiss, 1989).

It has been reported that rosehip extract is able to scavenge reactive oxygen species (ROS) (Daels et al., 2002). Among the various natural scavengers of ROS, polyphenolic compounds have received attention. These compounds are widely distributed in plants, constituting an important class of known antioxidative natural substances (Jovanovic et al, 1994).

The aim of this study was to determine and compare phenolic content of *Rosa canina* and *Rosa pimpinellifolia* fruits from West Azarbaijan and evaluating their potential antioxidant and antiradical activity.

Materials and Methods

Plant material

Rosa canina L. and *Rosa pimpinellifolia* L. samples were collected from three regions of West Azerbaijan (Qasemloo Valley of Urmia, Oshnavieh and Takab) during September-October 2011.

Determination of total phenolics content (TPC)

The TPC in extracts was determined using the Folin- ciocalteu reagent (FCR) and gallic acid as standard based on the method described by Slinkard and Singleton (1997). The sample (25 μ L) and 1.25 ml of sodium carbonate (75g/L) were added to 2.5 ml of 10% (v/v) FCR. After 1.5 h of reaction at room temperature, the absorbance was measured at 765 nm in a spectrophotometer (Biowave, S 2100, UK). The results were given as gallic acid equivalent /100 g extract (GAE/100 g extract).

Total flavonoid content (TFC) assay

TFC was determined using aluminium chloride (AlCl₃), and quercetin (standard) as

described by Ordon Ez et al. (2006). 0.1 ml plant extract was added to 0.3 ml distilled water followed by 0.03 ml NaNO₂ (5%). Then, AlCl₃ (0.03 ml, 10%) was added after 5 min and at 25 °C. After further 5 min, the reaction mixture was treated with 0.2 ml of 1 mM NaOH. Finally, the reaction mixture was diluted to 1 ml with water and the absorbance was measured at 510 nm. The results were expressed as mg quercetin / 100 g extract.

DPPH radical scavenging activity

Antioxidant activity of plants extracts was determined based on Blois (1958), where 50 μ l of different concentrations of plant extracts was taken in different test tubes, then 2.5 ml of 0.1 mM DPPH methanolic solution was added to the tubes and shaken vigorously. The tubes were allowed to stand at 25 °C for 20 min. The control was prepared as above using methanol solution instead of extract. The decrease in the absorbance of the formed blue to violet reagent was determined after 20 min at 517 nm and the percentage inhibition activity was calculated using the following formula:

$$\% \text{ inhibition activity} = [A_0 - A_1] / A_0 \times 100$$

A₀ = Absorbance of the control

A₁ = Absorbance of the extract / standard

Hydrogen peroxide radical scavenging assay

A modified version of the method described by Ruch et al. (1989) was used. Extracts (10 μ l) were dissolved in 3.4 ml of a 0.1 M phosphate buffer (pH 7.4) solution and mixed with 600 μ l of a 43 mM solution of hydrogen peroxide (prepared in the same buffer). The concentration of hydrogen peroxide was measured by reading absorbance values at 230 nm of the reaction mixtures. For extracts, a blank sample devoid of hydrogen peroxide was used for background subtraction. Reduction of absorbance in a hydrogen peroxide solution alone due to its degradation was recorded and values were corrected accordingly. The concentration of hydrogen peroxide in the assay medium was determined using a standard curve, and the hydrogen peroxide - scavenging percentage of

the extracts were calculated using the following equation:

$$\text{H}_2\text{O}_2\text{- scavenging (\%)} = (A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}} \times 100$$

Nitric oxide radical scavenging assay

Nitric oxide radical inhibition was estimated using Griess I 11osvoy reaction. (Garrat, 1964). Griess I 11osvoy reagent was modified by using naphthyl ethylene diaminedihydrochloride (0.1% w/v) instead of 1-naphthylamine (5%). The reaction mixture (3 ml) containing sodium nitroprusside (10 M, 2 ml), phosphate buffer saline (0.5 ml), and phenolic extract (1mg/ml) was incubated at 25 °C for 150 min. After incubation, 0.5 mL of the reaction mixture was mixed with 1 mL of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for completing diazotization. Then 1ml of naphthyl ethylene diaminedihydrochloride was added, mixed, and allowed to stand for 30 min at 25 °C. A pink colored chromophore was formed in diffused light. The absorbance of the solution was measured at 540 nm against the corresponding blank solutions. The nitric oxide scavenging capacities of the extracts were calculated using the following equation:

$$\text{Nitric oxide radical scavenging (\%)} = \frac{A_{\text{Blank}} - A_{\text{Sample}}}{A_{\text{Sample}}} \times 100$$

Statistical analysis

All the assays were carried out in triplicate. The results were expressed as mean values and standard error (SE) of the mean or standard deviation (SD) of the mean. The differences between the populations were analyzed using one-way analysis of variance (ANOVA). The statistical analysis was carried out using SPSS v. 11.5 program.

Results

In this study, the average of TPC in 3 populations from *R. canina* and *R. pimpinellifolia* of methanolic extracts was 199.43 ± 4.7 mg gallic acid equivalents/100 g extract.

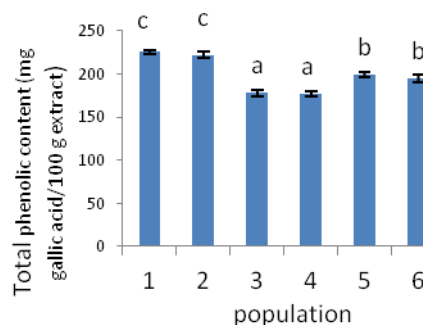


Fig. I. Total phenolic content of 2 species of *R. canina* and *R. pimpinellifolia* in three populations' extracts (mg Gallic acid equivalents/100 g methanolic extract): 1- *R. canina* from Takab, 2- *R. pimpinellifolia* from Takab, 3- *R. canina* from Urmia, 4- *R. pimpinellifolia* from Urmia, 5- *R. canina* of Oshnavieh, 6- *R. pimpinellifolia* from Oshnavieh. Means of 3 replicates with standard errors ($P < 0.05$)

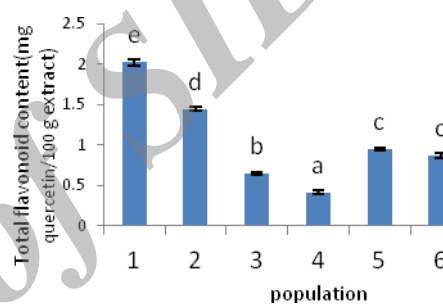


Fig. II. Flavonoid content of 2 species of *R. canina* and *R. pimpinellifolia* in three populations' extracts (mg Quercetin equivalents/100 g methanolic extract): 1- *R. canina* from Takab, 2- *R. pimpinellifolia* from Takab, 3- *R. canina* from Urmia, 4- *R. pimpinellifolia* from Urmia, 5- *R. canina* from Oshnavieh, 6- *R. pimpinellifolia* from Oshnavieh. Means of 3 replicates with standard errors ($P < 0.05$)

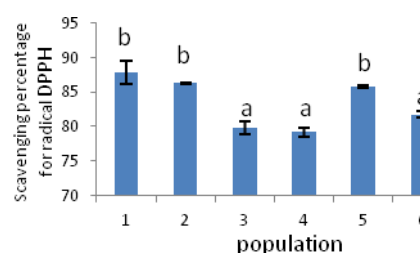


Fig. III. Scavenging percentage for radical DPPH in 2 species of *R. canina* and *R. pimpinellifolia* in three populations' extracts: 1- *R. canina* from Takab, 2- *R. pimpinellifolia* from Takab, 3- *R. canina* from Urmia, 4- *R. pimpinellifolia* from Urmia, 5- *R. canina* from Oshnavieh, 6- *R. pimpinellifolia* from Oshnavieh (mean \pm S.E., $n = 3$, $P < 0.05$).

The maximum TPC in extracts was 225.65 ± 2.50 mg/g for *R. canina* from Takab and minimum total phenolics content was $176.48 \pm$

2.71 mg/g for *R. pimpinellifolia* from Urmia. Results of statistical comparison showed that TPC of *R. canina* and *R. pimpinellifolia* from Takab were not significantly different. Also total phenolic compounds of *R. canina* and *R. pimpinellifolia* from Urmia were not significantly different. Similarly, TPC of *R. canina* and *R. pimpinellifolia* from Oshnavieh were not significantly different (Figure I).

The average TFC in 3 populations methanolic extracts was 1.05 ± 0.13 mg quercetin/100 g extract. Maximum TFC in extracts was 2.02 ± 0.03 mg quercetin /100 g for population of *R. canina* from Takab and minimum TFC was 0.41 ± 0.02 mg quercetin /100 g for population of *R. pimpinellifolia* from Urmia. The findings also showed that TFC in populations of *R. canina* and *R. pimpinellifolia* from Oshnavieh was not significantly different (Figure II). In this investigation, the average DPPH radical scavenging percentage in 3 population methanolic extract was $83.41 \pm 0.86\%$. Maximum DPPH radical scavenging percentage was 87.78 ± 0.10 % for population of *R. canina* from Takab and the minimum DPPH radical scavenging percentage was $79.16 \pm 0.61\%$ for population of *R. pimpinellifolia* of Urmia (Figure III).

DPPH radical scavenging activity between *R. canina* and *R. pimpinellifolia* from Takab with *R. canina* from Oshnavieh was not significantly different. *R. canina* and *R. pimpinellifolia* from Urmia were also not significantly different from *R. pimpinellifolia* from Oshnavieh. The mean hydrogen peroxide radical scavenging percentage in 3 populations' methanolic extract was 40.54 ± 2.8 %. Maximum hydrogen peroxide radical scavenging percentage was 58.10 ± 0.72 % for population of *R. canina* from Takab and the minimum hydrogen peroxide radical scavenging percentage was 22.41 ± 0.64 % for population of *R. pimpinellifolia* from Urmia (Figure IV). Moreover, hydrogen peroxide radical scavenging assay of *R. canina* and *R. pimpinellifolia* from Oshnavieh did not show a significant difference. In this investigation the average nitric oxide radical scavenging percentage in 3 populations' methanolic extract was 186.10 ± 13.97 %. The maximum nitric oxide radical scavenging percentage was 236.76 ± 16.04 % for population of *R. canina* from Takab and the minimum nitric

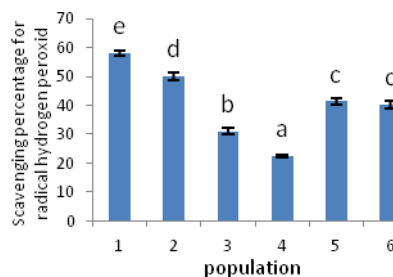


Fig. IV. Scavenging percentage for radical hydrogen peroxide in 2 species of *R. canina* and *R. pimpinellifolia* in three populations' extracts: 1- *R. canina* from Takab, 2- *R. pimpinellifolia* from Takab, 3- *R. canina* from Urmia, 4- *R. pimpinellifolia* from Urmia, 5- *R. canina* from Oshnavieh, 6- *R. pimpinellifolia* from Oshnavieh (mean \pm S.E., n=3, P 250. < 0.05).

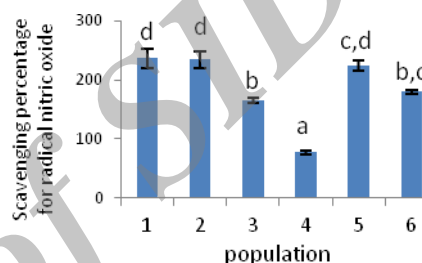


Fig. V. Scavenging percentage of radical nitric oxide in 2 species of *R. canina* and *R. pimpinellifolia* in three populations' extracts: 1- *R. canina* from Takab, 2- *R. pimpinellifolia* from Takab, 3- *R. canina* from Urmia, 4- *R. pimpinellifolia* from Urmia, 5- *R. canina* from Oshnavieh, 6- *R. pimpinellifolia* from Oshnavieh (mean \pm S.E., n=3, P < 0.05).

oxide radical scavenging percentage was 76.93 ± 2.3 % for population of *R. pimpinellifolia* from Urmia (Figure V). Nitric oxide radical scavenging assay of *R. canina* and *R. pimpinellifolia* from Takab were not significantly different. Finally, radical scavenging capacities of *R. canina* and *R. pimpinellifolia* extracts in different populations positively correlated with phenolic content (Table I).

Discussion

Phenolic compounds are considered as a major group of compounds that contribute to the antioxidant activities of botanical materials because of their scavenging ability on free radicals due to their hydroxyl groups (Djeridane et al., 2006; Balasundram et al., 2005). The antioxidant capacity of phenolic compounds is mainly due to their redox properties, which allow

Table 1

Correlations among the total phenol content, DPPH radical scavenging, peroxide radical scavenging, and nitric oxide radical scavenging of 2 species of *R. canina* and *R. pimpinellifolia* in three populations.

	Phenol content (mg GAE/g extract)	DPPH radical scavenging	peroxide radical scavenging	nitric oxide radical scavenging
Phenol content	1	0.86	0.93	0.93

them to act as reducing agents, hydrogen donors, singlet oxygen quenchers or metal chelators (Balasundram et al., 2006). Phenolics are believed to be the major phytochemicals responsible for antioxidant activity of plant materials (Javanmardi et al., 2003; Pizzale et al., 2002). The average TPC reported by Montazeri et al. (2011) was 424.6 ± 1.8 mg GAE /g extract. TPC for *R. canina* extract reported by Ercisli (2007) was 96 mg GAE/g extract. In previous studies, the TPC of rose species were found to range from 55 to 122 mg GAE/g DW (Gao et al., 2000; Olsson et al., 2005) which is in accordance with our findings.

Flavonoids are regarded as one of the most widespread groups of natural constituents found in plants (Patel et al., 2010). TFC for *R. canina* extract reported by Daels et al. (2002) was 14.71 mg/100 g extract. The average TFC for *R. canina* by Ghazghazi et al. (2010) was 0.33 ± 0.01 mg RE/ml. The average TFC reported by Montazeri et al. (2011) was 23.6 ± 4.2 mg quercetin/g extract. Our findings are also in agreement with these reports.

DPPH is a compound that possesses a nitrogen free radical and is readily destroyed by a free radical scavenger. This assay was used to test the ability of the antioxidative compounds functioning as proton radical scavengers or hydrogen donors (Singh and Rajini, 2004). DPPH radical scavenging percentage for *R. canina* L. reported by Wenzig et al. (2008) was 25 µg/ml.

It was demonstrated that the *in vivo* and *ex vivo* inhibitory effects of *R. canina* against H₂O₂ were in a dose-dependent manner (Daels et al., 2002). Significant correlation was observed between H₂O₂ scavenging activity and the total phenol content of *R. canina* (Kilicgun and Altiner, 2010). H₂O₂ inhibition activity for *R. canina* extract reported by Serteser et al. (2008) was 65.21 ± 4.8 % which is in accordance with our findings.

Nitric oxide (NO) is a highly reactive molecule that participates in signal transduction

in the cardiovascular and immune systems. It is often characterized by contrasting actions as it can exhibit antioxidant and pro-oxidant functions as well as anti-apoptotic and proapoptotic effects. Also, the role of NO in carcinogenesis has not been entirely clarified and observations have been reported demonstrating its ability to both stimulate and inhibit tumor growth (Sokolowska et al., 2003). Thus, if some substances can scavenge nitrosamine or its precursor substance such as nitrite, it probably has protective functions.

Finally, the findings suggest that radical scavenging capacities of *R. canina* and *R. pimpinellifolia* extracts in different populations positively correlated with phenolic content. Currently, there has been an increased interest globally to identify antioxidant compounds from plant sources which are pharmacologically potent and have low or no side effects for use in protective medicine and the food industry.

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