DIETARY SUPPLEMENTS AND TRADITIONAL MEDICINE

Phytochemical Composition and Antioxidant Activity of Petals of Six *Rosa* Species from Iran

SHAHLA SHAMEH, BAHMAN HOSSEINI[1], and ABOLFAZL ALIREZALU
Faculty of Agriculture, Department of Horticultural Sciences, Urmia University, Urmia, Iran
RAMIN MALEKI
Iranian Academic Center for Education, Culture and Research (ACECR), Research Department of Chromatography, Urmia, Iran

Background: **The petals of *Rosa* species are used in the food industry and various traditional medicinal products, but few studies exist on the phytochemical composition and antioxidant activity of petals of *Rosa* L. species grown in Iran.** ***Objective:*** **Phytochemical characteristics and antioxidant activity and some phenolic compounds of petals of six *Rosa* L. species were studied.** ***Methods:*** **Total phenolic content, total flavonoid content, and antioxidant activity were determined using Folin–Ciocalteu reagent, aluminum chloride method, ferric-reducing antioxidant power (FRAP), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays, respectively. An HPLC system was used for quantitative analysis of phytochemical compounds. Hierarchical cluster analysis (HCA) and principal component analysis (PCA) were performed among the variables analyzed using Minitab software. Also, heat maps were used to visualize phytochemical characteristics and antioxidant activity in each species using GraphPad Prism software.** ***Results:*** **The amount of total phenol content, total flavonoid content, and antioxidant activity were in the range of 25.13–52.01 mg gallic acid equivalents/g dry weight (DW), 0.61–0.82 mg quercetin equivalents/g DW, 11.47–20.93 μmol Fe^{++}/g DW (FRAP), and 31.66–74.44% (DPPH), respectively. The *p*-coumaric acid (647.28 μg/g DW) and chlorogenic acid (24.37–135.23 μg/g DW) were found to be the most abundant phenolic compounds in the extracts of rose petals. The HCA and PCA revealed three distinct categories of species based on phytochemical composition and antioxidant activity.** ***Conclusions:*** **These results showed that phytochemical characteristics of different rose species widely correlated with species type and are promising sources of natural antioxidants beneficial for use in the food or pharmaceutical industries.** ***Highlights:*** **Iran is one of the main centers for genetic diversity of *Rosa* L. The petals of *Rosa* species are used in the food industry and various traditional medicinal products, but few studies exist on the phytochemical composition and antioxidant activity of petals of *Rosa* L. species grown in Iran. Antioxidant activity and phytochemical compound of Six *Rosa* L. species petals grown in Iran were studied. Phenolic compounds in petals of *Rosa* were analyzed by HPLC. The color parameters, amount of total phenolic, total flavonoids, antioxidant activity and some individual phenolic compounds were significantly variable amongst *Rosa* species.**

The genus *Rosa*, with over 100 species, is one of the most widespread members of the Rosaceae family (1). Different species of the rose genus (*Rosa* spp.) make it the most valuable genus of the Rosaceae family. Wild rose is geographically spread in Europe, Turkey, Iran, Russia, Afghanistan, Pakistan, and Iraq (2), and there are 10 native species of this medicinal shrub in Iran (3). In traditional folk medicine, petals, fruit, and leaves of this genus are applied in the treatment of various diseases such as nephritis, common cold, flu, coughing, bronchitis, eczema, itching, and biliary diseases (4).

Wild rose (such as *R. dumalis* Bechst, *R. canina* L., *R. pulverulenta* M.Bieb) hips contain malic acid, citric acid, ascorbic acid (0.5–1.7%), flavonoids (rutin, quercetin, kaempferol, myristin, catechin), carotenoids (beta-carotene, lycopene), vitamins (B, C, E, K), sugars and mucilage, 8–10% seed oils (palmitic acid, stearic acid, linoleic acid, etc.), pectin, and volatile phenolic acids (caffeic acid, coumaric acid, vanillic acid, ferulic acid, etc.; 5). Various organs of rose could be a rich source of antioxidants because of the high phenolic compositions and some well-known antioxidant compounds, namely catechin, rutin, quercetin, kaempferol, and myristin, and phenolic compounds such as gallic acid, caffeic acid, coumaric acid, and vitamin C (5).

Preharvest and postharvest conditions and processing methods are important factors that may impact phenolic compounds in plants. The amount of total phenolic compounds, such as flavonoids and phenolic acids, in plant organs is affected by genetic variations among different species or even within the same species. Many studies have been conducted on the antioxidant activity and phytochemical compounds of *Rosa* species fruits (6–10), but few studies exist on petals of rose species. The petals of *Rosa* species are used in the food industry and various traditional medicinal products (11). With reference to rose species, several studies have been performed on the petals of *R. damascena* because of the high phenolic compound and essential oil content (12–15).

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[1]Corresponding author's e-mails: b.hosseini@urmia.ac.ir, pbhosseini@yahoo.com
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To the best of our knowledge, no study has been performed to date in Iran on the phenolic compound screening and antioxidant activities of *Rosa* species petals. The main aim of this study was to evaluate the phytochemical and natural antioxidant composition of six species of rose petals in the northwest of Iran.

Experimental

Plant Material

Fresh flowers of wild growing species of roses (*R. foetida* Herrm, *R. hemisphaerica* Herrm, *R. webbiana* Wall. ex Royle, *R.* × *damascena* Herrm, *R. canina* L., *R. moschata* Herrm; Figure 1) were collected from three provinces of Iran (Table 1) in 2016. The flowers were dried at room temperature (25°C) after sampling and stored in dry and cool conditions until analysis.

Preparation of Plant Extracts

Flowers of each species were ground to homogenize particle size before extraction. 1 g each of powder samples were extracted by ultrasound for 30 min at 25°C (Elmasonic) using methanol–water (80:20, 25 mL), and the extracted samples were filtered after extraction.

HPLC Analysis

Methanolic extract of samples was centrifuged for 7 min at 8000 rpm. Supernatant was filtered through a polyamide filter and transferred to a micro tube before injection into the HPLC system. The separation, identification, and determination amount of quantitative phenolic acids were performed with an HPLC 1100 series model manufactured by Agilent America. The HPLC system with a diode array detector was monitored at 250 nm (quercetin and chlorogenic acid), 272 nm (gallic acid, cinnamic acid, and apigenin), and 310 nm (caffeic acid, rutin, and *p*-coumaric acid), respectively. An HPLC column (250 mm × 4.6 mm, C18, ZORBAX Eclipse XDB) at 25°C was used. The injection volume was 20 µL and the flow rate was maintained at 1 mL/min. HPLC analyses were performed with four solvent delivery system quaternary pump, degasser system, column oven set at 25°C. The mobile phases were 1% aqueous acetic acid solution (solvent A) and acetonitrile (solvent B). A gradient elution was performed by varying the proportion of solvent B to solvent A. Solvent A was water containing 1% acetic acid, and solvent B was acetonitrile. The gradient conditions were as follows: 0–5 min, 10% B; 5–10 min, 25% B; and 10–15 min, 65% B. The stock solution of 1 mg/mL concentration was prepared by dissolving 1 mg standard (gallic acid, caffeic acid, *p*-coumaric acid, chlorogenic acid, cinnamic acid, rutin, quercetin, apigenin) in 0.5 mL HPLC methanol followed by sonication for 10 min, and the resulting volume was diluted to 1 mL with the solvent for the mobile phase (acetonitrile and 1% aqueous acetic acid, 1:9). To draw the curve, the standard dilution of the standard (1 mg/mL) was diluted to six concentrations (5, 10, 20, 30, 40, and 60), each of which was separately injected into the HPLC system, and the calibration curve was obtained by plotting the peak region against the concentration of each sample with a square correlation coefficient $R^2 < 0.99$ representing the linearity measurements.

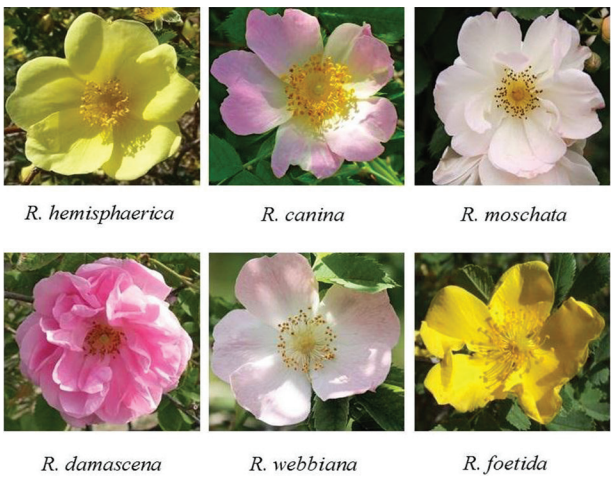

Figure 1. Picture of six *Rosa* species.

Table 1. Sampling locations of *Rosa* species

Species	Locality	Longitude	Latitude	Height, m
R. canina	Urmia	37°27'04.17″	44°55'15.53″	1700
R. moschata	Urmia	37°27'04.17″	44°55'15.53″	1700
R. damascena	Urmia	37°43'15.14″	45°10'47.22″	1284
R. webbiana	Boukan	36°29'07.81″	46°14'11.54″	1442
R. hemisphaerica	Oshnavieh	37°18'24.06″	45°07'00.42″	1441
R. foetida	Baneh	36°11'46.33″	46°10'18.80″	1585

Total Phenolic Content

Total phenolic content (TPC) was measured using the Folin–Ciocalteu method. Extract (100 µL) was removed from the main solution and brought to a volume of 1 mL (10-fold dilution). Then 1.6 mL of deionized water was added to 200 µL of the diluted sample. Next, 200 µL Folin was added to the mixture; after 5 min, 2 mL of 7% sodium carbonate was added; finally, the mixture was diluted to a volume of 5 mL with deionized water. After that, the samples were placed at room temperature for 30–45 min. Finally, the absorbance at 760 nm was measured by a spectrophotometer. Deionized water was used as a control and gallic acid as the standard. Gallic acid standard curve was plotted based on gallic acid and results were reported as mg gallic acid equivalents (GAE)/g dry weight (DW; 16).

Total Flavonoid Content

The total flavonoid content (TFC) of the petal extracts was determined using the $AlCl_3$ reagent. Briefly, the extract solution (0.5 mL) was mixed with 1.5 mL of 80% methanol, 0.1 mL of 10% aluminum chloride hexahydrate ($AlCl_3$), 0.1 mL of 1 M potassium acetate (CH_3COOK), and 2.8 mL of deionized water. After 40 min, the mixture absorption was read to the control at a wavelength of 415 nm. For plotting a standard curve, quercetin was used. The TFC was reported based on mg of quercetin equivalents (QUE)/g DW (17).

Antioxidant Activity by DPPH Assay

To measure the antioxidant activity by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method with 0.004 percentage of inhibitory concentration, 5 µL of methanol extract of samples was placed in a test tube and a 2000 µL solution of DPPH (pre-prepared) was added. The resulting solution was shaken and was kept at laboratory temperature for 30 min, then the absorbance of the solution at wavelength 517 nm was read using a spectrophotometer. To provide a control, the procedure described above was followed using 50 µL of 80% ethanol instead of the extract (18).

$$\mathrm{RSA} = \frac{(\mathrm{Abs\,control})\,t=30\,\mathrm{min} - (\mathrm{Abs\,sample})\,t=30\,\mathrm{min}}{(\mathrm{Abs\,control})\,t=30\,\mathrm{min}} \times 100$$

where RSA = radical scavenging activity; Abscontrol = absorbance control; t = time; and Abssample = absorbance sample.

Antioxidant Activity by FRAP Assay

50 µL of sample extracts were mixed together with 3 mL of fresh reagent ferric-reducing antioxidant power (FRAP), containing 300 mM sodium acetate buffer (pH 3.6), more 2,4,6-tri-2-pyridyl-s-triazine, and iron-ferric chloride. The resulting mixture was positioned in a warm water bath (37°C) for 30 min at a wavelength of 593 nm, and its absorbance as the uptake compared with control was read using a spectrophotometer. $FeSO_4 \cdot 7H_2O$ was used in order to plot a standard curve, and resulting data are based on µmol Fe^{++}/g DW stated (19).

Statistical Analysis

All of the analyses were done in triplicate with an experiment in completely randomized design. SAS 9.1.3 software was used for statistical data analysis. The Duncan's multiple-range test was used for means comparison and determination of statistical significance at the $P < 0.05$ probability level. Hierarchical cluster analysis (HCA) and principal component analysis (PCA) were performed among the variables analyzed using Minitab software. Also, heat maps were used to visualize phytochemical characteristics and antioxidant activity in each species using GraphPad Prism software.

Results and Discussion

TPC and TFC

Figures 2 and 3 show the TPC and TFC in six species of *Rosa* petals. The results showed that TPC was significantly affected by the type of *Rosa* species, which is similar to other studies on medicinal plants (20). The TPC in different species of rose petals was varied from 25.13 to 52.01 mg GAE/g DW. The highest values of TPC were observed in *R. canina* species and the lowest values in *R. webbiana* species. Kumar et al. determined that the amounts of TPC of roses were varied from 14.5 g to 25.4 g GAE/100 g of fresh weight (FW; 13). Rop et al. determined that the TPC in 12 cultivars of edible flowers was from 253 to 528 mg GAE/100 g FW (21). In another study, the TPC of *Rosa* hips ranged from 55 to 122 mg GAE/g DW (22).

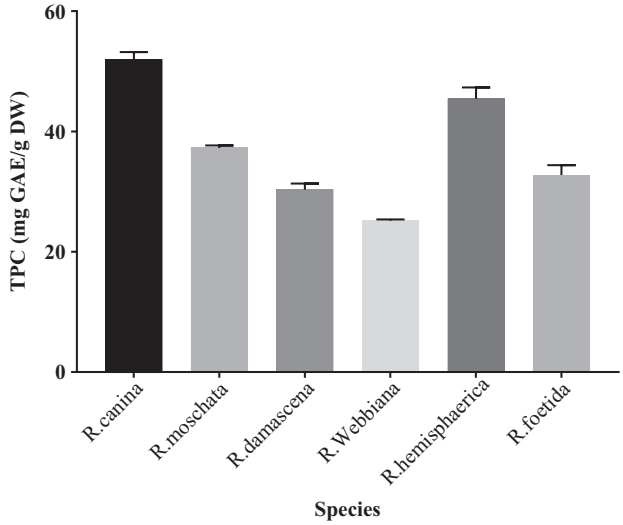

Figure 2. Total phenolic content of different species of *Rosa*.

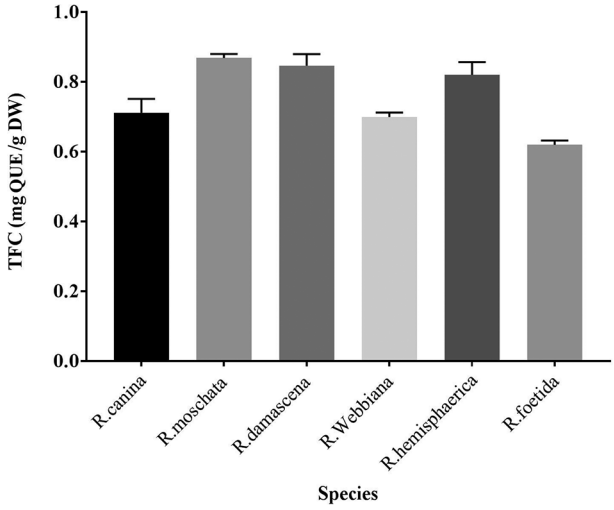

Figure 3. Total flavonoid content of different species of *Rosa*.

The results showed that the TFC in different species of roses varies from 0.61 to 0.86 mg QUE/g DW. The highest TFC was observed in petals of *R. moschata* and the lowest was in *R. foetida*. The TFC is influenced by the interaction between varieties and parts of plants. Environmental factors also have a significant contribution to the TFC in plants (23). Adamczak et al. reported the amount flavonoid content in *R. canina* hips (41 mg/100 g DW) and *R. rubiginosa* hips (72 mg/100 g DW; 24). Ghazghazi et al. reported the average total flavonoids for *R. canina* as 0.33 ± 0.01 mg rutin equivalent/mL (12).

Antioxidant Activity

In this study, the antioxidant activity of different species of roses was evaluated with FRAP and DPPH assay. Antioxidant activity by DPPH assay was different, from 31.66 to 74.44% (Figure 4). The highest antioxidant activity was observed in petals of *R. webbiana* species and the lowest in petals of *R. foetida* species. Results showed that the antioxidant activity of petals by FRAP assay was different, from 11.47 to 20.93 µmol Fe^{++}/g DW (Figure 5). The highest antioxidant activity was observed

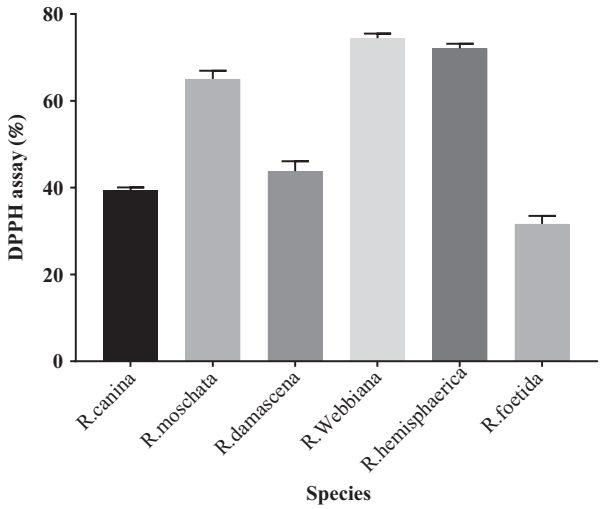

Figure 4. Antioxidant activity of different species of *Rosa* by DPPH assay.

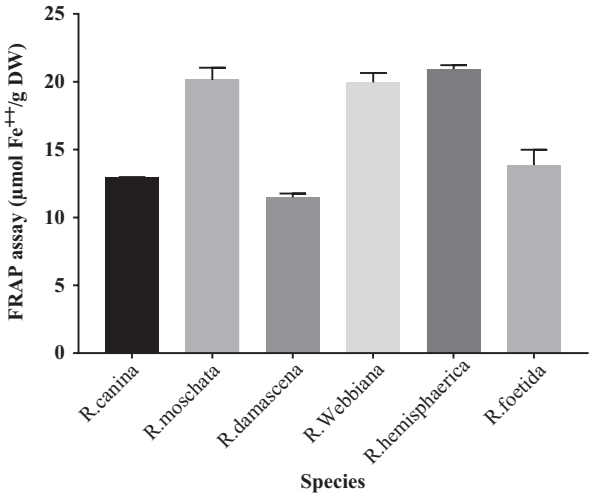

Figure 5. Antioxidant activity of different species of *Rosa* by FRAP assay.

in *R. hemisphaerica* petals and the lowest antioxidant activity in *R. damascena* petals.

The phenolics and flavonoids from herbs, including quercetin, rutin, isoquercetin, chlorogenic acid, and apigenin, were suggested to be the compounds with strong radical-scavenging activity (25). Most of the reports regarding antioxidant activity of *Rosa* species deal with hips and leaves. Nowak and Gawlik-Dziki reported the antioxidant activity range (by DPPH assay) in rose leaves from 83.4 to 95.7% (26). Also, Fattahi et al. reported the antioxidant activity range in rose hips from 79.16 (*R. pimpinellifolia*) to 87.78% (*R. canina*; 27). This shows that antioxidant activity and phenolic compounds can be affected by secondary metabolites such as vitamin C and carotenoids (28). Furthermore, environmental stresses such as cold and drought may increase phenolic compounds and antioxidant activity (29).

Phytochemical Compounds Analyses

In this study, eight individual phenolic compounds (i.e., gallic acid, caffeic acid, chlorogenic acid, *p*-coumaric acid, rutin, apigenin, cinnamic acid, and quercetin) were measured by HPLC in six *Rosa* L. species petals. Figure 6 represents the chromatograms of the above-mentioned standards. Table 2 summarizes the values of phenolic compounds in all six samples analyzed in this study. The results of the study showed that amounts of phytochemical compounds were significantly varied among species ($P \leq 0.05$). Results showed that the amounts of phenolic acids varied in the studied species in this order: gallic acid from 22.71 to 38.54 µg/g DW, caffeic acid from 1.50 to 64.18 µ/g DW, chlorogenic acid from 24.37 to 135.22 µg/g DW, *p*-coumaric acid from 20.68 to 647.28 µg/g DW, and cinnamic acid from 0.136 to 0.360 µg/g DW. The greatest amount of gallic acid was present in species of *R. hemisphaerica* petals and the least amount was in species of *R. damascena*

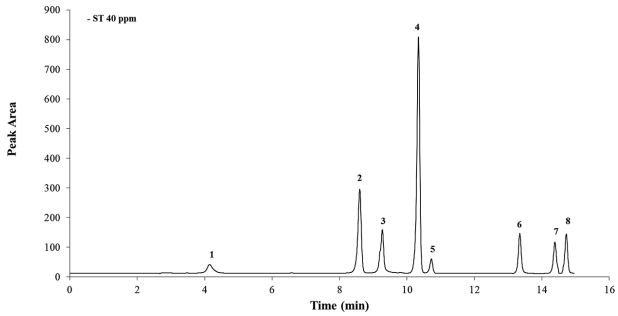

Figure 6. HPLC chromatograms of eight phytochemical standards: (1) gallic acid, (2) caffeic acid, (3) chlorogenic acid, (4) rutin, (5) *p*-coumaric acid, (6) quercetin, (7) cinnamic acid, and (8) apigenin.

Table 2. Phytochemical composition in petals of different *Rosa* species[a]

Species	Gallic acid, µg/g DW	Caffeic acid, µg/g DW	Chlorogenic acid, µg/g DW	Rutin, µg/g DW	*p*-Coumaric acid, µg/g DW	Quercetin, µg/g DW	Cinnamic acid, µg/g DW	Apigenin, µg/g DW
R. canina	ND[b]	3.74 ± 0.24[c]	34.45 ± 0.31[c]	5.48 ± 0.16[d]	143.55 ± 4.52[c]	3.65 ± 0.34[e]	0.36 ± 0.03[f]	1.03 ± 0.05[e]
R. moschata	ND	29.03 ± 0.02[d]	57.35 ± 0.39[e]	6.13 ± 0.14[d]	205.71 ± 1.29[d]	5.44 ± 0.16[d]	0.36 ± 0.01[f]	1.04 ± 0.05[e]
R. damascena	22.71 ± 0.0[d]	1.50 ± 0.25[g]	126.21 ± 0.53[d]	7.40 ± 0.55[f]	647.28 ± 0.43[f]	7.26 ± 0.10[f]	0.24 ± 0.02[d]	1.25 ± 0.05[d]
R. webbiana	ND	5.53 ± 0.30[e]	24.37 ± 0.50[g]	4.25 ± 0.16[e]	ND	5.27 ± 0.15[d]	0.34 ± 0.02[f]	1.04 ± 0.07[e]
R. hemisphaerica	38.54 ± 0.26[f]	4.19 ± 0.02[c]	135.23 ± 0.35[f]	1.34 ± 0.14[c]	155.18 ± 1.48[e]	7.65 ± 0.23[f]	0.22 ± 0.00[d]	2.31 ± 0.07[f]
R. foetida	ND	64.18 ± 0.32[f]	58.34 ± 0.16[e]	1.68 ± 0.23[c]	20.68 ± 0.27[g]	1.42 ± 0.10[c]	0.13 ± 0.01[e]	0.44 ± 0.00[c]

[a] All results were significant at the 1% level.

[b] ND = Not detected.

[c-g] All results labeled with the same footnote symbol are not significantly different using a LSD test.

petals. The greatest amount of caffeic acid was present in the *R. foetida* flowers and the least amount was in *R. damascena* flowers. The greatest amount of chlorogenic acid was present in the petals of *R. hemisphaerica* and the least amount was in the petals of *R. webbiana*. The greatest amount of *p*-coumaric acid was present in the petals of *R. damascena* species and the least amount was in the petals of the *R. foetida* species. The greatest amount of cinnamic acid was present in *R. canina* and *R. moschata* flowers and the least amount was observed in flowers of *R. foetida* species. For flavonoid compounds, the rutin amounts ranged from 1.34 to 7.40 µg/g DW, quercetin from 1.42 to 7.63 µg/g DW, and apigenin from 0.44 to 2.31 µg/g DW. The greatest amount of rutin was in the petals of *R. damascena* and the least was in the petals of *R. hemisphaerica*. The greatest amount of quercetin was in *R. hemisphaerica* and the least amount was in the petals of the *R. foetida*. The greatest amount of apigenin was present in petals of *R. hemisphaerica* and the least was observed in petals of *R. foetida* species.

Studies have shown that significant differences were obtained among the phenolic compound contents in different *Rosa* species. Nowak and Gawlik-Dziki reported that the highest amount of quercetin was observed in leaves of *R. gallica* (15.81 mg/g DW) and the lowest was observed in leaves of *R. agrestis* (3.68 mg/g DW) and determined that the amounts of kaempferol compounds of roses varied between 0.73 and 9.41 mg/g DW (26). Abdel-Hameed et al. reported four compounds in the methanol extract fraction: rutin (9.55 ± 0.09 mg/g), kaempferol-3-glucose (8.19 ± 0.22 mg/g), quercetin (7.75 ± 0.44 mg/g), and quercetin-3-glucose (6.73 ± 0.27 mg/g), whereas apigenin was not detected (30).

HCA, PCA, and Heat Map Visualization

The cluster analysis was carried out by the Ward linkage method (Figure 7a). The resulting dendrogram has three major groups. Phenolic compounds of petals (i.e., gallic acid, caffeic acid, chlorogenic acid, *p*-coumaric acid, rutin, apigenin, and quercetin) were the principal classification factor. In the first cluster, *R. damascena* was the species with the highest content of phenolic compounds. In the second cluster, *R. foetida* and *R. webbiana* were included because of the similarity of total flavonoid compounds and antioxidant activity. In the third cluster, *R. canina*, *R. moschata,* and *R. hemisphaerica* were included because of the similarity of total flavonoid compounds, phenolic compounds, and antioxidant activity. PCA classification confirmed the results of cluster analysis (Figure 7b). A PCA was performed, reducing the multidimensional structure of the data and providing a two-dimensional map to explain the variance observed. The first two components of the PCA explained 65% of the total variance (43% for component 1 and 22% for component 2). The first component is highly positively correlated with TPC, apigenin, quercetin, gallic acid, caffeic acid, and chlorogenic acid. The second principal component separates the samples according to *p*-coumaric acid, rutin, and antioxidant activity (DPPH assay). The heat map in Figure 8 summarizes quantitative data on phytochemical characteristics and antioxidant activity distribution in petals of *Rosa* species. A color was associated with the amounts of compounds, from white for low concentrations to blue for high concentrations.

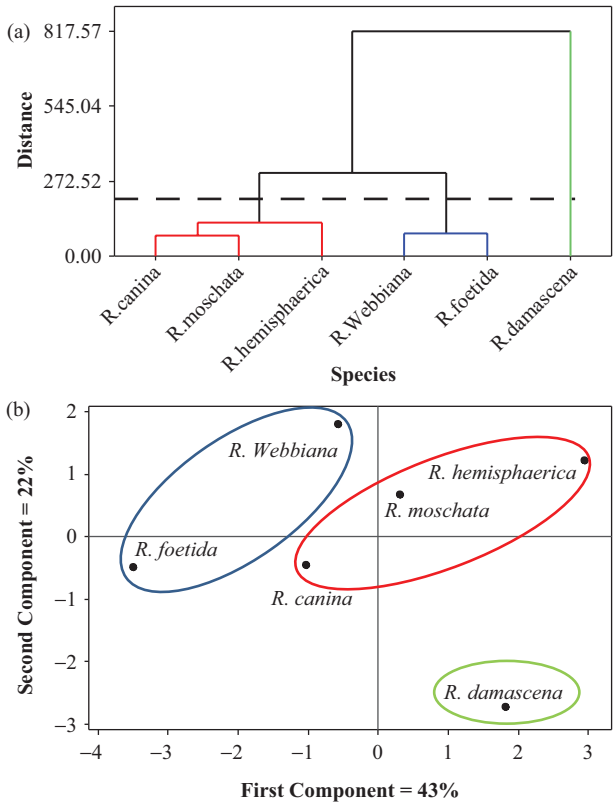

Figure 7. (a) Hierarchical cluster analysis (HCA) and (b) principal component analysis (PCA) of rose species based on phytochemical composition and antioxidant activity data.

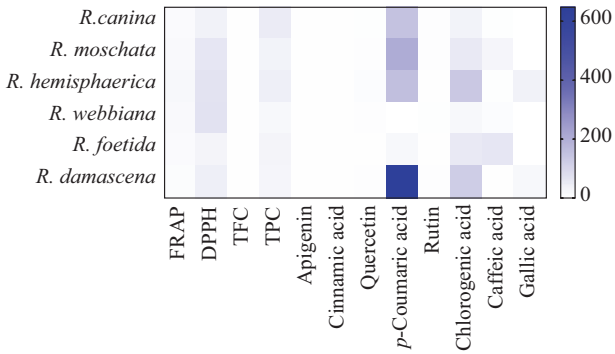

Figure 8. Phytochemical composition and antioxidant activity distribution in petals of rose species with heat map visualization, from white for low concentrations to blue for high concentrations. DPPH = 2,2-diphenyl-1-picrylhydrazyl; FRAP = ferric-reducing antioxidant power; TFC = total flavonoid content; TPC = total phenolic content.

Conclusions

Findings of this study showed that the petals of *Rosa* species could be considered as rich sources of phytochemicals and natural antioxidants. To the best of our knowledge, this is the first report regarding antioxidant activity and determination of phytochemical compounds (gallic acid, chlorogenic acid, caffeic acid, *p*-coumaric acid, cinnamic acid, rutin, quercetin, and apigenin) in petals of *Rosa* species grown in Iran. Different species of the genus *Rosa* showed high levels of phytochemical

compounds as well as antioxidant activity. Thus, this could provide valuable data for planning breeding strategies, as well as for selecting species with high phenolic contents for producing natural antioxidants beneficial as industrial crops for the food and pharmaceutical industries.

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