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Antioxidant Activity and Flavonoid Estimation in Rosa multiflora and Rosa wichuraiana Fruits and Flowers

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The fruits of Rosa multiflora Thunberg and Rosa wichuraiana Crépin are oriental medicine resources used complementary in management dropsy, edema and nocturnal enuresis in Korea. The objective of the present study was to evaluate the antioxidant activity and the content of kaempferol and quercetin of Rosa multiflora and Rosa wichuraiana fruits and flowers. Crude ethanol extracts of the species' fruits and flowers from the two Rosa species were fractionized with hexane, ether, ethyl acetate and water, and antioxidant activities of the resulting fractions were evaluated in vitro using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity and superoxide anion radical scavenging activity. The content of kaempferol and quercetin was quantified by high-performance liquid chromatography (HPLC) analyses. The water fraction of R. multiflora and ethyl acetate fraction of R. wichuraiana exhibited the highest DPPH free radical scavenging activity, which are generally proportionally to concentration, and the ethyl acetate fraction of fruit and ether fraction of the flower from the two Rosa species exhibited the highest superoxide anion radical scavenging activity. Meanwhile, the ethyl acetate and ether fraction of flower and fruit from the two Rosa species contained high level content of kaempferol and quercetin. These findings indicate that the antioxidant activity and the content of kaempferol and quercetin of Rosa multiflora and Rosa wichuraiana is dependent on solvent fraction. Moreover, both Rosa species fruits and flowers are promising sources of antioxidant phytochemicals, which further supports their use in complementary oriental medicine resource in Korea.

Keywords: Antioxidant; Kaempferol; Quercetin; Rosa multiflora; Rosa wichuraiana.

Species belonging to the genus *Rosa* (Rosaceae) are widely distributed in temperate and subtropical regions of the northern hemisphere. Numerous *Rosa* species are used for medical purposes or ornamentals. In Korea, the fruits of *Rosa multiflora* called Yeongsil are used to treat dropsy, edema, constipation, nocturnal enuresis and the species' flowers are used to treat malaria and bleeding[1-4]. *Rosa multiflora* is a perennial shrub with thorny stems and has alternate compound leaves, generally with five to eleven sharply toothed leaflets, and tolerance for a broad range of soil, moisture and light conditions. The principal components of the species' fruit include quercetin

glycosides, kaempferol glycosides, methyl gallate, and lycopene which is red pigment of fruits[5-7] and several studies have reported that *Rosa multiflora* exhibits antimicrobial, antioxidant, melanogenesis-inhibiting, and anti-inflammatory activities[8,9]. Meanwhile, the closely related species *Rosa wichuraiana* is native to Japan, Korea, east China and Taiwan, where it grows best in lowland thickets, near ocean. The species can be differentiated from *R. multiflora* by its thicker and more lustrous leaves. Interestingly, the somatic hybridization and propagation of *R. wichuraiana* has been studied to use the species as a novel source of disease resistance in ornamental rose breeding[10-12]. However, the bioactivity of the species has yet to be investigated.

The production of free radicals in the human body can induce oxidative stress, which can damage DNA, proteins, and lipids, ultimately causing various chronic illnesses (e.g., cancer and cardiovascular disease)[13-15]. However, even though antioxidants can be used to reduce the incidence of reactive oxygen species (ROS; e.g., superoxide anion radicals, hydroxyl radicals, non-freeradical species, and single oxygen)[16,17], the use of synthetic antioxidants has been associated with negative side effects[18]. Thus, the bioactive constituents and antioxidant activities of natural sources have received increasing interest.

Flavonoids, like kaempferol and quercetin, are plant secondary metabolites with polyphenolic structure and various biological activities[19]. Furthermore, kaempferol and quercetin have been identified in multiple plant species used in traditional medicine[20]. The most important characteristic of flavonoid compounds is their antioxidative activity, and many studies have reported linear relationships between flavonoid content and antioxidant activity[21-23]. This is the first study to compare the antioxidant activities and flavonoid contents of *R. multiflora* and *R. wichuraiana*.

MATERIALS AND METHODS

Chemicals and solvents used for this study were purchased from Sigma Chemicals (St Louis, MO, USA) and Difco (Detroit, MI, USA).

Rosa multiflora fruits and flowers were collected from Suncheon, Korea (34°54'27''N, 127°34'52''E), in September 2015 and May 2016, and *R. wichuraiana* fruits and flowers were collected from Goheung, Korea (34°43'65''N, 127°49'47''E), in September 2015 and June 2016. The plant was authenticated by one of the authors (K. W. Yun), and voucher specimens (SCNU 2015 201 and SCNU 2016 53, respectively) were also collected and were deposited in the herbarium of Sunchon National University. The collected flowers and fruits were air-dried for 14 d.

The air-dried fruit and flower of the two Rosa species was ground into powder. The samples (100 g) were soaked in 1,000 mL of ethanol and kept at room temperature for 24 hr and then filtered through filter paper (Whatman No.2). The crude ethanol extract was fractionized with 500 mL of hexane and then the top layer was concentrated (comprising the hexane fraction). The remaining layer was successively fractionized with 500 mL of diethyl ether and then ethyl acetate (forming the ether and ethyl acetate fraction). The remaining residue was the water fraction. Finally, each fraction was concentrated (*in vacuo*, 30 °C) to 30 mL for the subsequent measurement of antioxidant activity and flavonoid contents.

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity of each fraction was measured using a modified version of the method described by Blois[24]. Briefly, 140 µL DPPH (0.075 mM, in methanol) was added to fraction aliquots to reach final concentrations of 3.13–100.00 µg/mL, gently mixed, and incubated in the dark at 25 °C for 30 min. Butylhydroxy toluene (BHT; 100 µg/mL) and ethanol (210 µL) were used as positive and negative controls, respectively. After incubation, the optical density at 517 nm (OD_{517}) of each reaction mixture was measured using an ELISA Reader (Color techno system Co., Tokyo, Japan), and DPPH free radical scavenging activity was calculated as follows:

Scavenging activity (%) = (1 – [absorbance of sample] / [absorbance of control]) × 100%.

The superoxide anion radical scavenging activity of each fraction was measured according to Fridovich[25]. Superoxide radicals were generated in 0.4 mL potassium phosphate buffer (0.1M, pH 7.5) that contained 1 mL xanthine (0.4 mM), 1 mL nitro blue tetrazolium chloride (NBT, 0.24 mM) solution, 1 mL xanthine oxidase (0.2 unit/mL) and 0.1 mL *Rosa* extract fraction. The reaction mixtures were

incubated at 37 °C for 20 min. After incubation, the optical density at 560 nm (OD_{560}) of each reaction mixture was measured using an ELISA Reader. Lower the absorbance value, higher the superoxide radical scavenging activity was observed. The IC_{50} value was inversely correlated with antioxidant activity of the tested fractions; lower IC_{50} value indicated higher antioxidant activity [26]

For quantitative estimation of kaempferol and quercetin, each of the four extract fractions of two *Rosa* species' fruits and flowers was lyophilized using lyophilizer (Ilsin Co, Korea), and the lyophilized powders were stored in airtight bottles at "5°C until used. Kaempferol and quercetin standards (30 µg/mL) were prepared using methanol. High- performance liquid chromatographic (HPLC) was performed using Aglient 1200 series system (Agilent Technologies Inc., Santa Clara, CA, USA) that was equipped with a ZORBAX SB-C_{18} column (4.6 mm × 150 mm, 3.5 µm; Agilent). Ultra-distilled water and acetonitrile were used as mobile phases A and B, respectively. The flow rate and injection volume were 1.0 mL/min and the 5 µL, respectively, and the elution profile was as follows: 15% B for 0–1.5 min; 17% B for 3–4 min; 20-35% B for 7–14 min. The elution was monitored in the UV range, and the data for quantitative analysis were acquired at 360 nm. The retention time of quercetin and kaempferol was 7.63 min and 10.98 min, respectively. The kaempferol and quercetin contents were quantified using the external standard calibration method[27].

Data were expressed as mean ± standard deviation values (n = 3). Statistical analysis was performed using SPSS software (version 24.0; SPSS Inc., Chicago, IL, USA). The significance of differences between means was evaluated using Duncan's test.

RESULTS AND DISCUSSION

Table 1 shows the yield of the four fractions of ethanol extract from *R. multiflora* and *R. wichuraiana*. Solvents of increasing polarity were used to fractionate the crude ethanol extracts. The determination of stable DPPH radicals scavenging is a widely used and common method for the relatively rapid evaluation of antioxidant activity[28,29]. The DPPH free radical scavenging activity of the two *Rosa* species is shown in Table 2. The DPPH radical scavenging activities of hexane and water fraction of *R. multiflora* fruit extract are 82.93% and 79.10% at 50 µg/mL DPPH, whereas that of ether fraction is 69.57% at 12.5 µg/mL DPPH. The DPPH radical scavenging activity of the ethyl acetate fraction of *R. multiflora* fruit was greater than that of BHT. The DPPH radical scavenging activities of the hexane, ether, ethyl acetate, and water fraction of the *R. multiflora* flower were 70.07%, 79.27%, 82.54%, and 95.28%, respectively, at DPPH concentration of 100 µg/mL. The activities of hexane, ether, ethyl acetate, and water fractions of *R. wichuraiana* flower were 37.15%, 47.42%, 83.04%, and 77.58%, respectively, at DPPH concentration of 100 µg/mL. The DPPH radical scavenging activity of the ether fraction of the *R. wichuraiana* flower was 69.70% at a DPPH concentration of 50 µg/mL, and the DPPH radical scavenging activities of ethyl acetate and water fractions of the *R. wichuraiana* flower were greater than that of BHT, regardless of DPPH concentration. Park et al.[4] reported that the DPPH radical scavenging activities of ethanol, methanol, and acetone extracts of *R. multiflora* roots were 80% at a DPPH concentration of 100 µg/mL and that the scavenging activity of an aqueous extract was 40% at a DPPH concentration of 50 µg/mL. The DPPH radical scavenging activities of aqueous and ethanol extracts of *Potentilla supina* (Rosaceae) were 25.2% and 35.97%, respectively, at 25 µg/mL DPPH, whereas those of methanol extracts of three *Rosa* species were 64.5%, 51.8%, and 43.6%, respectively, at 100 µg/mL DPPH[30-32]. In the present study, the ethyl acetate and water fractions exhibited greater DPPH free radical scavenging activities than the other two fractions, and the *R. multiflora* extract fractions exhibited greater DPPH free radical scavenging activity than the *R. wichuraiana* extract fractions.

Superoxides are radicals that contain an oxygen atom with unpaired electrons. Despite having low chemical reactivity, superoxides can generate highly reactive species, such as hydroxyl radicals and the protonated form of superoxide. The superoxide anion radical scavenging activity of the two *Rosa* species are shown in Table3. In the present study, IC_{50} was calculated as the concentration that caused a 50% reduction in the superoxide anion radical concentration. The superoxide anion radical scavenging activities

of ether fraction from *R. multiflora* and *R. wichuraiana* flower were 0.06 and 0.09 mg/mL, respectively, whereas those of the ethyl acetate fractions of the *R. multiflora* and *R. wichuraiana* fruit extracts were 0.14 and 0.08 mg/mL.. The superoxide anion radical scavenging activities of the ethyl acetate fractions of the fruit extracts and the ether fractions of flower extracts were greater than those of the other fractions, regardless of species. The superoxide anion radical scavenging activity of ethyl acetate fraction of *Sanguisorba officinalis* (Rosaceae) extract was 40% at a concentration of 1,000 μg/mL, and the scavenging activity of water extract of *Prunus sargentii* was 40 % at a concentration of 500 ppm[33,34]. Superoxide radicals are powerful oxidizing agents that can react with biological membranes and induce tissue damage. Moreover, these radicals decompose to singlet oxygen, hydroxyl radical, or hydrogen peroxide molecules and may be associated with the onset of a various pathological conditions, including rheumatoid arthritis and cancer[35,36].

Kaempferol and quercetin are present in many plant species that are commonly used in traditional medicine[20]. The compounds have been associated with reduced risk of pancreatic cancer, and quercetin, in particular, is effective against prostate cancer[37]. Kaempferol is a markedly active inhibitor of COX-2 transcriptional activation and exhibits antimicrobial activity against *Propionibacterium acnes*[38,39]. One of the goals of the present study was to measure the kaempferol and quercetin contents of fractions of two *Rosa* species fruit and flower extracts (Table 4; Fig. 3; Fig.4). Unlike the observed for extraction yield, the flavonoids content did not show dependence on the yield. The content of kaemferol was 10.35 mg% and 6.21 mg% in ethyl acetate fraction of *R. multiflora* and *R. wichuraiana* flower, these are the highest contents of kaempferol. Among the fruit fractions of the two *Rosa* species, quercetin was detected only in the ether fraction of *R. multiflora*. The water and ethyl acetate fractions of the *R. multiflora* and *R. wichuraiana* flower extracts contained more quercetin than the other two fractions, and no quercetin was detected in fractions of the *R. wichuraiana* fruit extract. The ether fractions of both the *R. multiflora* and *R. wichuraiana* fruit extracts contained more quercetin than the other fractions, and more quercetin was detected in the ethyl acetate fractions of the two *Rosa* species flower extracts. In contrast to the previous studies[21-23,40], the findings of the present study did not show strong correlation between flavonoid contents and antioxidant activity. Previous studies[41,42] have reported that the

Table 1. Yield of each fraction from *Rosa multiflora* and *R. wichuraiana*

Plant	Plant organ	Extract fraction	Yield (%)
Rosa multiflora	Fruit	Hexane	0.63±0.12 [d]
		Ether	1.24±0.22 [c]
		Ethyl acetate	2.35±0.26 [b]
		Water	6.43±0.31 [a]
	Flower	Hexane	0.94±0.26 [d]
		Ether	1.87±0.32 [c]
		Ethyl acetate	3.91±0.34 [b]
		Water	10.18±0.69 [a]
Rosa wichuraiana	Fruit	Hexane	0.4±0.04 [cd]
		Ether	0.7±0.07 [c]
		Ethyl acetate	1.46±0.18 [b]
		Water	4.78±0.52 [a]
	Flower	Hexane	0.78±0.13 [d]
		Ether	1.71±0.17 [c]
		Ethyl acetate	4.01±0.39 [b]
		Water	9.08±0.72 [a]

[a] Values with different letters in the same column were significantly ($p < 0.05$) different. Duncan's test should be compared within each part of a plant.

Table 2. DPPH free radical scavenging activity of solvent fractions from two *Rosa* species

Plant	Plant organ	Extract fraction	DPPH free radical scavenging activity (±SD, %)[a] Concentration (μg/mL) 100	50	25	12.5	6.25	3.13	BHT (100 μg/ml)
Rosa multiflora	Fruit	Hexane	72.06±0.81^{b}	82.93±0.22^{a}	61.94±1.83^{c}	35.44±1.87^{d}	18.26±0.75^{e}	9.70±1.30^{f}	69.00±3.47
		Ether	82.40±0.64^{a}	89.87±0.11^{a}	87.14±2.71^{a}	69.57±6.06^{b}	48.12±4.74^{c}	29.93±4.70^{d}	
		Ethyl acetate	91.03±0.14^{a}	92.07±0.11^{a}	93.19±0.16^{a}	94.81±0.30^{a}	95.56±0.03^{a}	91.66±3.01^{a}	
		Water	95.22±0.52^{a}	79.10±8.06^{a}	53.89±8.60^{b}	35.47±6.56b^{c}	22.19±9.64^{cd}	14.01±9.19^{d}	
	Flower	Hexane	70.07±0.89^{a}	42.66±1.54^{b}	22.28±1.10^{c}	12.61±2.12^{d}	5.67±2.49^{e}	2.43±2.30^{e}	
		Ether	79.27±0.41^{ab}	86.36±0.53^{a}	70.82±7.23^{b}	44.53±7.68^{c}	22.60±1.39^{d}	11.10±0.43^{e}	
		Ethyl acetate	82.54±0.27^{a}	89.15±0.65^{a}	92.03±0.19^{a}	93.60±0.10^{a}	86.19±4.95^{a}	57.83±7.86^{b}	
		Water	95.28±0.22^{a}	95.66±0.76^{a}	95.74±0.76^{a}	94.38±1.61^{a}	80.29±14.42^{a}	50.08±14.08^{b}	
Rosa wichuraiana	Fruit	Hexane	72.38±0.36^{a}	57.72±0.76^{b}	31.86±2.54^{c}	18.13±1.39^{d}	8.56±2.79^{e}	3.98±3.54^{e}	
		Ether	78.52±4.38^{b}	88.34±2.98^{a}	91.80±2.01^{a}	87.13±0.40^{a}	66.85±0.94^{c}	44.21±1.13^{d}	
		Ethyl acetate	92.33±0.94^{a}	94.03±0.80^{a}	94.90±1.00^{a}	91.88±2.13^{a}	77.01±5.83^{b}	53.94±7.84^{c}	
		Water	86.95±0.89^{a}	77.60±19.08^{a}	65.13±38.00^{a}	58.35±49.55^{a}	49.49±50.87^{a}	36.20±40.00^{a}	
	Flower	Hexane	37.15±0.59^{a}	26.31±0.54^{b}	13.55±4.24^{c}	7.71±2.55^{d}	3.69±2.47^{de}	1.03±1.82^{e}	
		Ether	47.42±1.38^{b}	69.70±2.60^{a}	66.28±12.57^{a}	48.44±7.23^{b}	29.01±4.84^{c}	16.08±3.18^{cd}	
		Ethyl acetate	83.04±2.01^{c}	88.61±1.30^{b}	91.90±1.43^{ab}	92.48±0.46^{ab}	93.27±1.83^{a}	94.01±1.92^{a}	
		Water	77.58±8.42^{b}	85.88±4.45^{ab}	89.91±2.92^{a}	92.10±1.38^{a}	93.21±1.48^{a}	93.86±1.16^{a}	

[a] Values with different letters in the same line were significantly ($p < 0.05$) different.

kaempferol and quercetin contents of *Rubus idaeus* (Rosaceae) and *Prunus cerasus* leaves are 2.38 and 5.05 mg/kg, respectively. Liaudanskas et al.[43] reported a strong correlation between total phenolic contents and radical scavenging and reducing activities of the *Rosa* fruits grown in Lithuania, and another study[44] reported that the ethyl acetate fraction of *Rosa multiflora* flower extract had greater phenolic content and antioxidant activity than other tested fractions.

Table 3. The superoxide anion radical scavenging activity of solvent fractions from two *Rosa* species

Plant	Plant organ	Extract fraction	IC_{50} (±SD, mg/ml)[a]
Rosa multiflora	Fruit	Hexane	0.31±0.05[a]
		Ether	0.24±0.02[b]
		Ethyl acetate	0.14±0.04[b]
		Water	0.34±0.02[a]
	Flower	Hexane	0.36±0.01[a]
		Ether	0.06±0.00[c]
		Ethyl acetate	0.20±0.00[b]
		Water	-
Rosa wichuraiana	Fruit	Hexane	0.24±0.01[b]
		Ether	0.18±0.00[c]
		Ethyl acetate	0.08±0.00[d]
		Water	0.70±0.00[a]
	Flower	Hexane	0.16±0.01[b]
		Ether	0.09±0.01[b]
		Ethyl acetate	0.69±0.04[a]
		Water	0.14±0.02[b]

[a] Values with different letters in the same column were significantly ($p < 0.05$) different. Duncan's test should be compared within each part of a plant.

Table 4. The contents of kaempferol and quercetin in solvent fractions from two *Rosa* species

Plant	Plant organ	Extract fraction	Kaempferol (±SD, mg%)[a]	Quercetin (±SD, mg%)[a]
Rosa multiflora	Fruit	Hexane	-	-
		Ether	0.47±0.03[a]	5.78±0.79[a]
		Ethyl acetate	-	0.93±0.14[b]
		Water	-	0.20±0.14[b]
	Flower	Hexane	-	-
		Ether	1.50±0.41[b]	1.53±0.61[b]
		Ethyl acetate	10.35±0.77[ab]	4.53±0.54[bc]
		Water	12.13±0.89[a]	9.16±0.82[a]
Rosa wichuraiana	Fruit	Hexane	-	-
		Ether	-	2.73±0.00[a]
		Ethyl acetate	-	0.72±0.35[b]
		Water	-	0.82±0.44[b]
	Flower	Hexane	-	-
		Ether	1.73±0.52[b]	2.01±0.69[b]
		Ethyl acetate	6.21±0.61[a]	11.69±1.01[a]
		Water	1.18±0.46[b]	2.26±0.99[b]

[a] Values with different letters in the same column were significantly ($p < 0.05$) different

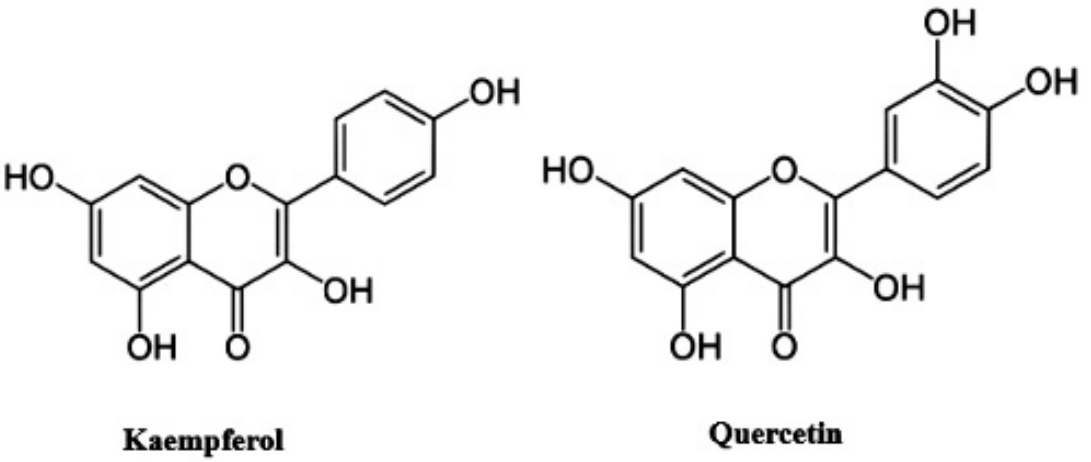

Fig. 1. The structure of kaempferol and quercetin

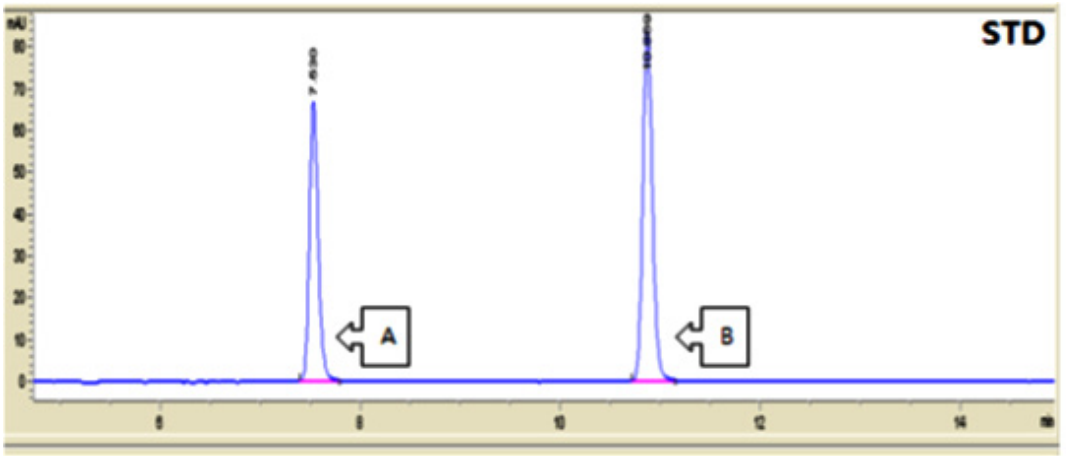

Fig. 2. The chromatogram of kaempferol and quercetin standard by HPLC (A, Quercetin; B, Kaempferol)

CONCLUSION

The antioxidant activity and flavonoid contents of various fractions (hexane, ether, ethyl acetate, and water) of ethanol extract from *R. multiflora* and *R. wichuraiana* used complementary in Korea were evaluated. The greater antioxidant activity by DPPH assay exhibited in the ethyl acetate or water fraction of two *Rosa* species extracts, whereas greater superoxide anion radical scavenging activity was shown in the ether or ethyl acetate fraction. The content of flavonoid, such as kaempferol and quercetin in the four fractions is not reflected clearly in the DPPH and superoxide anion radical scavenging activity. However, the findings of the study still suggest that the two *Rosa* species could be useful as natural antioxidants or food additives.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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