

# Compound Identification of Selected Rose Species and Cultivars: an Insight to Petal and Leaf Phenolic Profiles

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**ABSTRACT.** Using high-performance liquid chromatography/mass spectrometry, leaf and petal phenolic profiles of four rose (*Rosa*) species (*R. canina*, *R. glauca*, *R. rubiginosa*, *R. sempervirens*) traditionally used for medicinal purposes and three modern rose cultivars (Rosarium Uetersen, Ulrich Brunner Fils, Schwanensee) were determined. An abundance of phenolic constituents was identified: seven different anthocyanins and 31 flavonols in petals; 30 flavonols, 14 phenolic acids, and their derivatives; 15 flavanols; and 20 hydrolysable tannins in leaves. Additionally, petal color was measured with a colorimeter and regression analysis indicated a strong correlation between color parameter  $a^*$  and total anthocyanin content. The content and composition of phenolic compounds varied significantly among species and cultivars and plant organs investigated. Distinct differences in the distribution of leaf phenolic compounds were observed, especially between *Rosa* species and modern rose cultivars. In general, leaves of analyzed species were richer in content of most phenolic groups and individual components compared with cultivars. Multivariate statistical analysis clustered the investigated species and cultivars into three distinct groups. Among species, leaves of *R. canina* stood out with their high and varied phenolic content. Conversely, leaves of the susceptible cultivar Schwanensee appeared most dissimilar as a result of their low levels of phenolic constituents.

The genus *Rosa* contains more than 200 species distributed in Europe, Asia, the Middle East, and North America (Li et al., 2013). The genus is represented by 22 species in the Slovenian flora, among which *R. canina*, *R. glauca*, *R. rubiginosa*, and *R. sempervirens* are most frequent (Martincic et al., 1999). Numerous rose cultivars are widely planted in gardens and parks for their aesthetic value and native plants are harvested for their fruit and flowers.

Medicinal benefits of the genus *Rosa* have been reported in many studies and specific plant tissue has been used for different purposes (Nowak and Gawlik-Dziki, 2007). Rose hips comprise several biologically active compounds and are famous for their high content of vitamins, particularly vitamin C (Hvattum, 2002; Roman et al., 2013). Fragrance compounds in rose petals are praised in perfumery and food industries (Farooq et al., 2012). Similarly, rose leaves have been used in Chinese and European medicine for centuries as ingredients in common cold remedies (Coruh and Ercisli, 2010; Fenglin et al., 2004). Health-beneficial properties of *Rosa* leaves may be attributed to their content of phenolics, which are known to possess a wide spectrum of bioactive functions such as antioxidant and anti-inflammatory effects (Sroka, 2005). Interspecific variation in the levels of bioactive compounds and their healing potential led to selective harvest of specific rose species for traditional uses. In Slovenia, hips, petals, and leaves of *R. canina* have

been favored for their healing powers, but other indigenous rose species have not been used to the same extent.

A number of studies have been published on the phenolic and mineral composition of rose hips in connection to their antioxidant activity (Ghazghazi et al., 2012; Hvattum, 2002; Roman et al., 2013). Petal phenolic antioxidants have also been identified in several rose species and cultivars (Biolley et al., 1994; Cai et al., 2005; Mikanagi et al., 1995; Schmitzer et al., 2010, 2012). However, research on rose leaf polyphenols is still partial and scarce. Different authors report total phenolic content, total flavonoid, or total flavonol aglycone levels in leaf extracts (Ghazghazi et al., 2012; Nowak and Gawlik-Dziki, 2007) of *Rosa* species, but studies targeted at identification of individual phenolic compounds are limited.

The aim of the present study was to identify and quantify phenolic compounds in petals of several indigenous rose species in Slovenia and compare them with the phenolic profiles of certain modern rose cultivars. Moreover, rose leaf phenolic profiles were established and individual phenolic compounds have been identified and quantified for the first time in selected rose species. The qualitative and quantitative differences in leaf phenolic compounds among analyzed rose species and cultivars are discussed.

## Materials and Methods

**PLANT MATERIAL.** Four rose species: *R. glauca* (pink flowers), *R. canina* ssp. *canina* (white to pale pink flowers), *R. sempervirens* (white flowers), and *R. rubiginosa* (pink flowers), and three cultivars: Rosarium Uetersen, Ulrich Brunner Fils (both with pink flowers), and Schwanensee (white flowers with light pink center), were selected for the study. Plants were located at the Botanical Garden and Arboretum Ljubljana (lat. 46.18° N,

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long. 14.61° E, altitude 250 m) and leaves and petals for the analysis were sampled in June 2013. Flowers were collected and analyzed in developmental stage fully open flower (Schmitzer et al., 2010) and petal color measurements were recorded. Leaves were collected at their mature stage and only the first three fully expanded leaves on the branch were analyzed. Plant material was frozen in liquid nitrogen and stored at -20 °C until further analysis.

**PETAL COLOR MEASUREMENTS.** Flower color was measured by a portable colorimeter (CR-10 Chroma; Minolta, Osaka, Japan) with C illuminant. The colorimeter was calibrated with a white standard calibration plate before use. In the CIE  $L^* a^* b^*$  system of color representation, the  $L^*$  value corresponds to a dark-bright scale and represents the relative lightness with a range from 0 to 100 (0 = black, 100 = white). Color parameters  $a^*$  and  $b^*$  extend from -60 to 60;  $a^*$  negative is for green and  $a^*$  positive is for red and  $b^*$  negative is for blue and positive for yellow. The hue angle ( $h^\circ$ ) is expressed in degrees from 0 to 360, where 0° = red, 90° = yellow, 180° = green, and 270° = blue. Color was measured in the middle of each petal (three replicates per flower; 10 flowers per repetition) to ensure equal measurement conditions.

**EXTRACTION AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY DETERMINATION OF PHENOLIC COMPOUNDS.** Flower petals (combined samples consisting of five to 15 flowers) and leaves (combined samples consisting of 10 leaves) were ground to a fine powder with liquid nitrogen and 1 g of powder was extracted with 6 mL methanol containing 3% (v/v) formic acid and 1% (w/v) 2,6-Di-*tert*-butyl-4-methylphenol (BHT) in an ultrasonic bath for 1 h. Samples were centrifuged for 7 min at 12,000  $g_n$ . Supernatant was filtered through a polyamide filter (Chromafil AO-20/25; Macherey-Nagel, Düren, Germany) and transferred to a vial before injection into the high-performance liquid chromatography (HPLC) system. Samples were analyzed using a Thermo Finnigan Surveyor HPLC system (Thermo Scientific, San Jose, CA) with a diode array detector at 280 nm (phenolic acids and their derivatives, hydrolysable tannins, flavanols), 350 nm (glycosides of quercetin, kaempferol, myricetin, and isorhamnetin), and 530 nm (anthocyanins). A HPLC column (150 × 4.6 mm, Gemini 3  $\mu$ m C18; Phenomenex, Torrance, CA) protected with a Phenomenex security guard column operated at 25 °C was used. The injection volume was 20  $\mu$ L and the flow rate maintained at 0.6 mL·min<sup>-1</sup>. The elution solvents were aqueous 0.1% formic acid in double-distilled water (A) and 0.1% formic acid in acetonitrile (B). Samples were eluted according to the linear gradient from 5% to 20% B in the first 15 min followed by a linear gradient from 20% to 30% B for 5 min, then an isocratic mixture for 5 min, followed by a linear gradient from 30% to 90% B for 5 min, and then an isocratic mixture for 15 min before returning to the initial conditions (Wang et al., 2002). Phenolics were further identified using a mass spectrometer (LCQ Deca XP MAX; Thermo Scientific) with an electrospray ionization interface operating in negative/positive ion mode using multiple-stage mass spectrometry (MS<sup>n</sup>) scanning mode from  $m/z$  115 to 1500. The injection volume was 10  $\mu$ L and the flow rate maintained at 0.6 mL·min<sup>-1</sup>. Capillary temperature was 250 °C, the sheath gas and auxiliary gas were 20 and 8 units, respectively, and the source voltage was 4 kV for negative ionization and 0.1 kV for positive ionization. Spectral data were elaborated using Excalibur software (Thermo Scientific). The identification of compounds was confirmed by comparing retention times and their spectra as

well as by adding the standard solution to the sample and by fragmentation. The content of phenolic compounds was assessed from peak areas and quantified with the use of corresponding external standards. For compounds lacking standards, quantification was carried out using similar compounds as standards. Thus, galloylquinic acid, methyl gallate hexoside, digalloyl quinic acid, trigalloyl hexose, digalloyl pentose, ellagic acid hexoside, digalloyl glucose isomer, vescalagin, and compounds with a hexahydroxydiphenic (HHDP) moiety were quantified with the calibration curve of ellagic acid, *p*-coumaric acid hexoside, *cis*- and *trans*-5-*O*-*p*-coumaroylquinic acid by *p*-coumaric acid, caffeoyl hexose by caffeic acid, sinapic acid hexoside by sinapic acid, all procyanidins by the standard curve of procyanidin B2, all kaempferol compounds by kaempferol-3-glucoside, and quercetin (Q) compounds (except Q-rutinoside, Q-galactoside, Q-glucoside, Q-rhamnoside, Q-arabinofuranoside, and Q-xyloside) by quercetin-3-galactoside. Anthocyanins were quantified with the standard curves of cyanidin-3-glucoside, pelargonidin-3-glucoside, and peonidin-3-glucoside, respectively. Total anthocyanins, total quercetin and kaempferol glycosides, total flavonols, total phenolic acids, hydrolysable tannins, and flavanols were calculated as the sum of all identified phenolics of the group. All compounds were expressed on a fresh weight basis in micrograms per gram.

**CHEMICALS.** The standards used to determine the phenolic compounds in samples were caffeic, ellagic, sinapic and chlorogenic acid (5-caffeoylquinic acid), quercetin-3-*O*-rutinoside, cyanidin-3-*O*-glucoside, pelargonidin-3-*O*-glucoside, and peonidin-3-*O*-glucoside from Sigma-Aldrich (Steinheim, Germany); and (+)-epicatechin, *p*-coumaric acid, procyanidin B2, kaempferol-3-*O*-glucoside, quercetin-3-*O*-glucoside, quercetin-3-*O*-xyloside, quercetin-3-*O*-arabinofuranoside, quercetin-3-*O*-galactoside, and quercetin-3-*O*-rhamnoside from Fluka (Buchs, Switzerland). The chemicals for the sample preparation and mobile phases were methanol, BHT, and acetonitrile from Sigma-Aldrich and formic acid from Fluka. The water used in mobile phase was bidistilled and purified with a Milli-Q water purification system by Millipore (Bedford, MA).

**STATISTICAL ANALYSIS.** The results were analyzed using Statgraphics Plus 4.0 (Manugistics, Rockville, MD) program using one-way analysis of variance. Differences in phenolic content between species/cultivars were estimated with Duncan's multiple range test between means ( $P < 0.05$ ). Multiple-variable analysis with Pearson's correlation coefficient ( $r$ ) was calculated between color variables  $a^*$ ,  $L^*$ , and  $C$  and total and individual anthocyanin content at  $P < 0.05$ . Multivariate statistical analysis (hierarchical cluster analysis, discriminate analysis, and classification) was conducted to interpret the differences in secondary metabolites among species and cultivars.

## Results and Discussion

**PETAL COLOR MEASUREMENTS.** Color parameter  $a^*$  represents the amount of red coloration of plant tissue (Lancaster et al., 1997) and thus increases from white to reddish colored petals. Highest values were measured on the surface of the 'Ulrich Brunner Fils' and 'Rosarium Uetersen' and *R. glauca* petals and lowest on *R. sempervirens* petals (Table 1). Values of color parameter  $b^*$ , which indicates yellow (positive values) and blue hues (negative values) (Lancaster et al., 1997), were higher in species and cultivars with (predominantly) white petals (*R. sempervirens*, *R. canina*, and 'Schwanensee') and lower

Table 1. Petal color parameters and anthocyanin content of analyzed rose species and cultivars.

Rosa species/cultivar	Flower color	Color parameter (mean $\pm$ SE)				Anthocyanin [mean $\pm$ SE ( $\mu\text{g}\cdot\text{g}^{-1}$ FW)]			
		$a^*$	$b^*$	$L^*$	$h^\circ$	Cy-di-glu <sup>2</sup>	Cy-glu	Pg-di-glu	total AC
<i>R. canina</i>	Pale pink	8.6 $\pm$ 0.3 c <sup>3</sup>	4.2 $\pm$ 0.1 e	72.3 $\pm$ 0.2 e	24.5 $\pm$ 0.9 a	19.4 $\pm$ 0.9 a	—	5.2 $\pm$ 0.5 a	28.6 $\pm$ 1.5 a
<i>R. glauca</i>	Pink	49.3 $\pm$ 0.7 e	-11.2 $\pm$ 0.6 a	36.6 $\pm$ 0.5 a	347.0 $\pm$ 0.7 c	569.8 $\pm$ 8.3 c	26.2 $\pm$ 1.8 b	15.4 $\pm$ 0.4 b	726.9 $\pm$ 11.6 c
<i>R. sempervirens</i>	White	2.9 $\pm$ 0.0 a	8.5 $\pm$ 0.1 f	76.2 $\pm$ 0.2 g	71.3 $\pm$ 0.6 b	13.9 $\pm$ 0.7 a	—	—	13.9 $\pm$ 1.7 a
<i>R. rubiginosa</i>	Pink	35.8 $\pm$ 0.9 d	-6.6 $\pm$ 0.3 b	55.7 $\pm$ 0.6 d	349.7 $\pm$ 0.4 d	353.2 $\pm$ 8.3 b	31.8 $\pm$ 1.4 b	4.8 $\pm$ 0.1 a	393.9 $\pm$ 8.6 b
Rosarium Uetersen	Pink	50.8 $\pm$ 0.4 e	-2.1 $\pm$ 0.1 d	49.5 $\pm$ 0.3 c	357.3 $\pm$ 0.2 f	580.2 $\pm$ 20.9 c	48.6 $\pm$ 3.4 c	59.0 $\pm$ 2.4 c	699.1 $\pm$ 24.7 c
Ulrich Brunner Fils	Pink	50.9 $\pm$ 0.7 e	-4.6 $\pm$ 0.2 c	46.7 $\pm$ 0.5 b	354.5 $\pm$ 0.5 e	717.2 $\pm$ 41.3 d	25.5 $\pm$ 1.2 b	—	855.8 $\pm$ 45.8 d
Schwanensee	White	6.9 $\pm$ 0.2 b	3.9 $\pm$ 0.1 e	73.8 $\pm$ 0.2 f	23.3 $\pm$ 0.6 a	39.7 $\pm$ 1.4 a	11.4 $\pm$ 1.1 a	—	51.1 $\pm$ 2.7 a

<sup>2</sup>Cy-di-glu = cyanidin-3,5-diglucoside; Cy-glu = cyanidin-3-glucoside; Pg-di-glu = pelargonidin-3,5-diglucoside; total AC = sum of all anthocyanins analyzed; — = compound not present in samples.

<sup>3</sup>Different letters (a–g) in rows denote statistically significant differences in color parameters, individual and total anthocyanins by Duncan's multiple range test at  $P < 0.05$  among species/cultivars.

in pink-flowered species and cultivars (*R. glauca*, *R. rubiginosa*, and 'Ulrich Brunner Fils'). An inverse relationship between the parameters  $a^*$  and  $b^*$  was also observed in previous research when comparing flowers of different cultivars of groundcover rose (Schmitzer et al., 2010). Analysis of lightness coefficient ( $L^*$ ) and hue angle ( $h^\circ$ ) similarly revealed statistically significant differences among studied species and cultivars. The highest values of parameter  $L^*$  were observed in flowers of species and cultivars associated with highest  $a^*$  value and lowest  $b^*$  value (*R. sempervirens*, *R. canina*, and 'Schwanensee'). Flower petals of the latter were also characterized by lowest values of the  $h^\circ$  parameter.

**ANTHOCYANINS AND FLAVONOLS IN ROSE PETALS.** Anthocyanins are the principal pigments in rose petals attributing intense red to mauve color hues. Flower color investigation of roses so far has shown that four major anthocyanins, 3-glucosides and 3,5-diglucosides of cyanidin (Cy) and peonidin (Pn) can be detected in flowers of wild *Rosa* species and also pelargonidin (Pg) 3-glucoside and Pg-3,5-diglucoside in *Rosa* cultivars (Biolley et al., 1994; Kumar et al., 2008; Mikanagi et al., 1995, 2000). Rarely, Pg-based anthocyanins can be accumulated in *Rosa* plant parts (Cai et al., 2005; Eugster and Märki-Fischer, 1991). In this study two major anthocyanins have been identified in analyzed rose petals, namely Cy-3,5-diglucoside and Cy-3-glucoside [Table 1 (only major anthocyanins presented)], which is in accordance with our earlier studies (Schmitzer et al., 2010). Additionally, Cy-3-rutinoside, Pg-3,5-diglucoside, Pg-3-glucoside, Pn-3,5-diglucoside, and Pn-3-glucoside have been confirmed in petals of specific *Rosa* species or cultivars (Table 2), but their content only accounted several percent of total anthocyanin content level (data not shown). Occurrence of cyanidin, pelargonidin, and peonidin-based anthocyanins has been reported in different red rose cultivars (Biolley et al., 1994; Mikanagi et al., 2000) and higher levels of Cy-3,5-diglucoside quantified in analyzed rose species and cultivars are in agreement with the reported color expression of cyanidins, generally contributing to more intense red hues of plant tissue (Tatsuzawa et al., 2012). Significant differences in anthocyanin composition and content were determined among roses analyzed (Table 1). Highest levels of Cy-3,5-diglucoside, Cy-3-glucoside, and consequently total anthocyanins were quantified in pink-flowering species and cultivars (*R. glauca*, 'Ulrich Brunner Fils', and 'Rosarium Uetersen'). Although the petals of 'Ulrich Brunner Fils' were characterized by highest levels of total anthocyanins, they only accumulated three anthocyanin glycosides compared with the petals of 'Rosarium Uetersen' in which five anthocyanins have been identified. Anthocyanin composition is in tight connection with color expression (Lancaster et al., 1997; Schmitzer et al., 2009) and regression analysis indicated a strong correlation between color parameter  $a^*$  and total anthocyanin content (Pearson's correlation coefficient = 0.97,  $r^2 = 94.9\%$ ), similar to the reports of Schmitzer et al. (2010).

Thirty-one flavonols have been detected in rose petals; their content varied significantly among species and cultivars analyzed. As a result of a similar ultraviolet spectrum of individual components of each phenolic group and limited availability of external standards, HPLC–mass spectroscopy was used for reliable peak identification (Table 2). The presence of seven major Q glycosides (Q-rutinoside, Q-glucoside, Q-glucuronide, Q-arabinofuranoside, Q-galactoside, Q-xyloside, and Q-rhamnoside) and five major kaempferol (K) glycosides (K-diglucoside,

Table 2. Phenolic compounds in leaves and petals of *Rosa* species/cultivars, their mass-to-charge ratio ( $m/z$ ) values of the molecular masses and main fragments [second- ( $MS^2$ ) and third-generation ( $MS^3$ ) product ion] in positive ( $[M-H]^+$ ), and negative ion mode ( $[M-H]^-$ ) identified with electrospray ionization mass spectrometry (ESI-MS).

Phenolic group	$m/z$ $[M-H]^-$ or $[M-H]^+$	$(m/z)$ of the main fragments by ESI-MS		Tentative identification	Plant part <sup>y</sup>
		$MS^2$ $[M-H]^-$ or $[M-H]^+$	$MS^3$		
Anthocyanins <sup>z</sup>	611	449	287	Cyanidin-3,5-diglucoside	P
	449	287		Cyanidin-3-glucoside	P
	595	449	287	Cyanidin-3-rutinoside	P
	595	433	271	Pelargonidin-3,5-diglucoside	P
	433	271		Pelargonidin-3-glucoside	P
	625	463	301	Peonidin-3,5-diglucoside	P
	463	301		Peonidin-3-glucoside	P
Flavonols	609	301		Quercetin-3-rutinoside	PL
	463	301		Quercetin-3-galactoside	PL
	463	301		Quercetin-3-glucoside	PL
	477	301		Quercetin-3-glucuronide	PL
	433	301		Quercetin-3-arabinopyranoside	L
	433	301		Quercetin-3-arabinofuranoside	PL
	433	301		Quercetin-3-xyloside	PL
	447	301		Quercetin-3-rhamnoside	PL
	609	463, 447	301	Quercetin-hexoside-rhamnoside 1-2	PL
	615	463	301	Quercetin-galloylhexoside 1-5	PL
	585	433	301	Quercetin-galloylpentoside 1-3	L
	599	447	301	Quercetin-galloylrhamnoside	L
	625	463	301	Quercetin-dihexoside	P
	651	609, 447	301	Quercetin-acetyl-hexoside-rhamnoside	P
	595	433	301	Quercetin-hexosyl-pentoside	P
	609	447	285	Kaempferol-di-hexoside	P
	431	285		Kaempferol-3-rhamnoside	PL
	593	285		Kaempferol-3-rutinoside	L
	447	285		Kaempferol-3-galactoside	P
	447	285		Kaempferol-3-glucoside	PL
	461	285		Kaempferol-3-glucuronide	PL
	489	285		Kaempferol-acetylglucoside	PL
	599	447, 285		Kaempferol-galloylhexoside 1-2	PL
	417	285		Kaempferol-pentoside 1-3	PL
	579	447	285	Kaempferol-pentoside-hexoside	P
	593	447	285	Kaempferol-rhamnoside-hexoside 1-4	P
	635	593, 431	285	Kaempferol-acetyl-hexoside-rhamnoside	P
	447	315		Isorhamnetin-3-arabinoside	L
	461	315		Isorhamnetin-3-rhamnoside	L
	493	317		Myricetin-3-glucuronide	L
	463	317		Myricetin-3-rhamnoside	L
Flavanols	289	245		Catechin	L
	289	245		Epicatechin	L
	577	451, 425, 407, 289,		Procyanidin dimer 1-5	L
	865	577, 695, 451, 425, 407, 289		Procyanidin trimer 1-6	L
	1153	865, 695, 575, 577, 407, 289		Procyanidin tetramer 1-2	L
Phenolic acids and their derivatives	353	191, 179		3-caffeoylquinic acid	L
	353	173, 179		<i>cis</i> -5-caffeoylquinic acid	L
	353	191, 179		<i>trans</i> -5-caffeoylquinic acid	L
	325	163		<i>p</i> -coumaric acid hexoside	L
	341	179		Caffeoyl hexose	L
	353	173, 179		4-caffeoylquinic acid	L
	385	223, 205, 152		Sinapic acid hexoside	L
	337	191, 163		<i>cis</i> -5- <i>p</i> -coumaroylquinic acid	L

Continued next page

Table 2. Continued.

Phenolic group	$m/z$ [M-H] <sup>-</sup> or [M-H] <sup>+</sup>	(m/z) of the main fragments by ESI-MS		Tentative identification	Plant part <sup>y</sup>
		MS <sup>2</sup> [M-H] <sup>-</sup> or [M-H] <sup>+</sup>	MS <sup>3</sup>		
	337	191, 163		<i>trans</i> -5- <i>p</i> -coumaroylquinic acid	L
	301	257, 229, 185		Ellagic acid	L
	345	183		Methyl gallate hexoside	L
	463	301		Ellagic acid hexoside	L
	433	301		Ellagic acid pentoside 1-2	L
Hydrolysable tannins	343	191, 169		Galloyl quinic acid	L
	495	343	191, 169	Digalloyl quinic acid	L
	635	483, 465, 423, 271, 193		Trigalloyl hexose 1-2	L
	453	313, 327, 285, 169		Digalloyl pentose	L
	483	439, 331, 313, 271, 169	313, 287	Digalloyl glucose isomer	L
	783	481, 301, 275	257, 229	Di-HHDP glucose 1-2	L
	785	633, 615, 419, 301, 275	257	HHDP digalloyl glucose isomer 1-3	L
	933	631, 451, 301		Vescalagin isomer 1-4	L
	935	633, 301		Galloyl bis HHDP glucose	L
	937	767, 785, 465, 741, 635, 301		Trigalloyl HHDP glucose 1-2	L

<sup>z</sup>Anthocyanins were obtained in the positive ion mode ([M-H]<sup>+</sup>), other phenolic groups in negative ion mode ([M-H]<sup>-</sup>).

<sup>y</sup>Plant part (in which individual compound was determined): P = petals; L = leaves; PL = petals and leaves.

HHDP = hexahydroxydiphenyl.

K-rhamnoside, K-glucoside, K-glucuronide, and K-galactoside) in addition to an abundance of species/cultivar specific flavonols was confirmed (Table 3; content levels of major flavonols are represented) in rose petals comparable to previous research (Cai et al., 2005; Kumar et al., 2009; Mikanagi et al., 2000).

The highest content of total quercetin glycosides was determined in flowers of 'Ulrich Brunner Fils' and the two major

quercetin glycosides identified in the petals were Q-arabinofuranoside and Q-galactoside. In other *Rosa* species and cultivars, the abundance of specific quercetin glycosides varied greatly; Q-glucoside was the major quercetin in petals of *R. canina*, *R. sempervirens*, and *R. rubiginosa*, and Q-rhamnoside in petals of *R. glauca*. Other authors similarly report high levels of quercetin glucoside, arabinoside, rhamnoside, and other glycosides in

Table 3. Flavonols in petals of *Rosa* species and cultivars.

Flavonol <sup>z</sup>	<i>Rosa</i> species/cultivar						
	<i>R. canina</i>	<i>R. glauca</i>	<i>R. sempervirens</i>	<i>R. rubiginosa</i>	Rosarium Uetersen	Ulrich Brunner Fils	Schwanensee
	[mean ± SE (μg·g <sup>-1</sup> FW)]						
1	—	37.6 ± 1.3 a <sup>y</sup>	—	502.7 ± 10.8 d	—	164.2 ± 12.1 b	344.9 ± 6.2 c
2	629.0 ± 9.7 d	259.4 ± 3.6 b	372.8 ± 7.1 c	775.0 ± 12.9 e	12.6 ± 0.4 a	846.8 ± 44.2 f	353.9 ± 1.5 c
3	309.4 ± 7.2 c	92.5 ± 1.8 b	—	—	5.5 ± 0.2 a	93.1 ± 1.9 b	—
4	115.5 ± 0.8 a	178.0 ± 2.2 a	112.9 ± 1.2 a	—	—	1279.7 ± 47.4 c	906.3 ± 22.7 b
5	62.4 ± 1.9 a	—	—	17.4 ± 0.3 a	—	1480.2 ± 41.0 c	207.1 ± 1.8 b
6	17.0 ± 0.9 a	—	16.1 ± 0.6 a	112.4 ± 5.3 c	—	139.9 ± 5.8 d	69.5 ± 1.5 b
7	438.7 ± 5.0 e	573.8 ± 16.3 f	131.7 ± 9.7 b	302.8 ± 3.7 c	37.9 ± 1.7 a	455.4 ± 7.8 e	410.7 ± 5.2 d
8	333.8 ± 3.8 d	126.9 ± 0.6 b	—	51.4 ± 1.7 a	217.3 ± 7.7 c	—	—
9	273.6 ± 4.9 c	249.6 ± 1.3 b	407.6 ± 1.7 d	521.9 ± 10.4 f	123.9 ± 6.1 a	113.9 ± 3.3 a	439.0 ± 5.8 e
10	273.5 ± 1.8 c	40.8 ± 0.5 a	2607.6 ± 27.7 f	2463.0 ± 14.3 e	42.3 ± 1.7 a	130.1 ± 4.8 b	973.7 ± 24.4 d
11	182.0 ± 4.2 c	—	—	—	13.7 ± 0.7 a	22.9 ± 1.4 b	—
12	—	—	35.3 ± 1.4 a	44.5 ± 0.6 b	—	99.4 ± 2.1 c	260.2 ± 5.3 d
Total Q	2125.7 ± 19.4 d	1646.6 ± 16.0 c	684.1 ± 17.6 b	1834.3 ± 21.0 c	90.9 ± 3.0 a	5086.8 ± 170.8 f	2361.9 ± 32.2 e
Total K	1211.9 ± 9.9 c	576.4 ± 9.3 a	3875.8 ± 40.6 e	6512.9 ± 43.3 f	574.6 ± 22.8 a	1040.8 ± 60.1 b	3033.1 ± 52.3 d
% Q	63.7 ± 0.2 e	74.1 ± 0.4 f	15.0 ± 0.3 b	22.0 ± 0.1 c	13.7 ± 0.2 a	83.0 ± 0.8 g	43.8 ± 0.2 d
% K	36.3 ± 0.2 c	25.9 ± 0.4 b	85.0 ± 0.3 f	78.0 ± 0.1 e	86.3 ± 0.2 g	17.0 ± 0.8 a	56.2 ± 0.2 d

<sup>z</sup>Flavonol, quercetin (Q) compounds (1–7): 1 = Q-3-rutinoside, 2 = Q-3-glucoside, 3 = Q-3-glucuronide, 4 = Q-3-arabinofuranoside, 5 = Q-3-galactoside, 6 = Q-3-xyloside, 7 = Q-3-rhamnoside; kaempferol (K) compounds (8–12): 8 = K-di-hexoside, 9 = K-3-rhamnoside, 10 = K-3-glucoside, 11 = K-3-glucuronide, 12 = K-3-galactoside; total Q = sum of quercetin compounds; total K = sum of kaempferol compounds; % Q = percent of total quercetin compounds among flavonols; % K = percent of total kaempferol compounds among flavonols, — = compound not present in samples.

<sup>y</sup>Different letters (a–g) within individual phenolic compound denote statistically significant differences among *Rosa* species and cultivars by Duncan's multiple range test at  $P < 0.05$ .

FW = fresh weight.

petals of different rose species (Barros et al., 2013; Kumar et al., 2009; Schieber et al., 2005).

Based on their fragmentation pattern and ultraviolet spectral data, 17 compounds have been tentatively identified as kaempferol glycosides in rose petals (Table 2). Most species contained high levels of K-glucoside, which is in accordance with the results of Schieber et al. (2005) who investigated phenolic compounds in *R. damascena* flowers and Barros et al. (2013) who identified phenolics in *R. micrantha* flower petals. Several species and cultivars in this study also contained high levels of K-rhamnoside similar to *R. damascena*, *R. bourboniana*, and *R. brunonii* petal profiling reported by Kumar et al. (2009). In quantitative terms, the species with the highest total kaempferol content was *R. rubiginosa*; its petals contained predominantly K-glucoside and K-rhamnoside previously identified in petals of *R. damascena* (Schieber et al., 2005) and *R. micrantha* (Barros et al., 2013). Contrary, the petals of *R. glauca* and ‘Rosarium Uetersen’ contained less than one-tenth the level of total kaempferol glycosides compared with *R. rubiginosa*.

**LEAF PHENOLIC COMPOSITION.** Thirty different flavonols have been tentatively identified in leaves of different rose species and cultivars (Table 2). MS<sup>n</sup> confirmed the presence of the prevailing quercetin and kaempferol glycosides and in some species/cultivars also isorhamnetin and myricetin glycosides (*R. canina*, *R. sempervirens*, and ‘Schwanensee’). Porter et al. (2012) correspondingly reported an abundance of kaempferol and quercetin glycosides in leaves of *R. spinosissima* and quercetin glycosides have been detected in leaves of *R. rugosa* (Hashidoko, 1996) and *R. sericea* (Li et al., 2013). Nowak and Gawlik-Dziki (2007) measured total leaf phenolic content, and quercetin and kaempferol levels of different *Rosa* species including *R. canina* and *R. rubiginosa* but did not study their specific chemical composition. The same applies to research on *R. sempervirens*

and *R. canina* leaves by Ghazghazi et al. (2010, 2012) and thus the identification of individual components in leaves of selected *Rosa* species was not comprehensive. In the present research, seven major quercetin glycosides have been identified in rose leaves (Q-arabinofuranoside, Q-galactoside, Q-glucoside, Q-glucuronide, Q-rhamnoside, Q-rutinoside, Q-xyloside). Q-arabinofuranoside, Q-xyloside, and Q-rhamnoside have been determined in all studied species and cultivars. Particularly high levels of the latter were characteristic for *R. sempervirens* and *R. canina* leaves (Table 4). Shetty et al. (2011) similarly detected several quercetin and kaempferol glycosides in leaves of *R. hybrida* ‘Smart’ asserting Q-rutinoside and Q-rhamnoside occurred in highest concentrations.

Three predominant kaempferol glycosides (K-rhamnoside, K-glucuronide, K-glucoside) have been determined in leaves of selected rose species and cultivars (Table 4) in addition to five minor kaempferol compounds (Table 2). Generally, the content of K-glycosides in rose leaves was considerably lower compared with petals and only K-rhamnoside was present in leaves of all studied species and cultivars. Shetty et al. (2011) similarly identified this glycoside as the prevalent kaempferol in *R. hybrida* leaves. The highest content of K-rhamnoside was again determined in *R. canina* leaves, which was correspondingly the species with the highest content of total kaempferol, quercetin, and flavonol glycosides (Fig. 1A). High levels of K-rhamnoside were also detected in ‘Ulrich Brunner Fils’ and *R. rubiginosa*. The leaves of ‘Schwanensee’ contained characteristically low levels of total kaempferol and also quercetin glycosides. The percentage of total quercetin and total kaempferol glycosides varied among the studied species and cultivars (Table 4) but generally, quercetin glycosides were the predominant flavonols in most rose leaves, which is in accordance with the research of Nowak and Gawlik-Dziki (2007) on different *Rosa* species and cultivars.

Table 4. Flavonols in leaves of *Rosa* species and cultivars.

Flavonol <sup>a</sup>	<i>Rosa</i> species/cultivar						
	<i>R. canina</i>	<i>R. glauca</i>	<i>R. sempervirens</i>	<i>R. rubiginosa</i>	Rosarium Uetersen	Ulrich Brunner Fils	Schwanensee
	[mean ± SE (μg·g <sup>-1</sup> FW)]						
1	330.7 ± 18.5 c <sup>y</sup>	62.0 ± 5.5 a	377.3 ± 14.1 d	48.6 ± 1.7 a	26.9 ± 1.8 a	334.2 ± 21.6 c	135.5 ± 11.4 b
2	194.9 ± 13.3 b	—	—	292.3 ± 6.8 c	—	194.4 ± 17.4 b	114.4 ± 11.4 a
3	186.7 ± 9.9 bc	54.8 ± 8.4 a	191.0 ± 16.8 c	325.9 ± 6.4 d	80.9 ± 5.7 a	148.7 ± 8.9 b	88.5 ± 7.3 a
4	411.8 ± 4.2 e	56.8 ± 7.7 b	—	316.0 ± 8.0 d	108.1 ± 9.1 c	121.5 ± 8.5 c	18.1 ± 2.5 a
5	2601.8 ± 267.6 d	1428.2 ± 127.1 c	2909.8 ± 118.7 d	1002.4 ± 51.9 b	1021.6 ± 115.7 bc	545.9 ± 59.9 a	175.4 ± 19.8 a
6	37.1 ± 3.6 b	—	—	73.5 ± 3.6 c	5.6 ± 0.4 a	25.9 ± 2.9 ab	172.8 ± 16.7 d
7	125.9 ± 14.4 c	12.2 ± 1.1 a	51.9 ± 6.0 b	14.8 ± 0.6 a	10.6 ± 1.4 a	53.5 ± 4.6 b	48.2 ± 1.3 b
8	160.3 ± 36.0 e	84.9 ± 3.1 bc	88.2 ± 10.4 cd	133.0 ± 5.6 de	29.8 ± 5.4 a	140.5 ± 13.5 e	39.2 ± 3.2 ab
9	158.9 ± 32.4 b	—	—	71.0 ± 2.2 a	90.4 ± 8.2 a	—	—
10	—	—	—	93.5 ± 3.3 b	16.9 ± 2.4 a	—	—
Total Q	4201.1 ± 80.8 e	1515.4 ± 149.5 b	3651.3 ± 168.5 d	2449.9 ± 96.0 c	1451.1 ± 125.3 b	1699.3 ± 128.6 b	776.8 ± 77.6 a
Total K	473.2 ± 34.7 d	157.5 ± 14.6 b	140.9 ± 11.6 b	294.8 ± 10.6 c	132.8 ± 12.2 b	168.6 ± 16.5 b	50.9 ± 4.2 a
% Q	90.9 ± 1.0 b	91.3 ± 0.7 b	96.3 ± 0.3 d	89.2 ± 0.3 a	91.8 ± 0.5 b	90.4 ± 0.1 b	93.8 ± 0.2 c
% K	9.1 ± 0.1 c	8.7 ± 0.7 c	3.7 ± 0.2 a	10.8 ± 0.3 d	8.2 ± 0.5 c	9.6 ± 0.1 c	6.2 ± 0.2 b

<sup>a</sup>Flavonol, quercetin (Q) compounds (1–7): 1 = Q-3-arabinofuranoside, 2 = Q-3-galactoside, 3 = Q-3-glucoside, 4 = Q-3-glucuronide, 5 = Q-3-rhamnoside, 6 = Q-3-rutinoside, 7 = Q-3-xyloside; kaempferol (K) compounds (8–10): 8 = K-3-rhamnoside, 9 = K-3-glucuronide, 10 = K-3-glucoside; total Q = sum of quercetin compounds; total K = sum of kaempferol compounds; % Q = percent of total quercetin compounds among flavonols; % K = percent of total kaempferol compounds among flavonols; — = compound not present in samples.

<sup>y</sup>Different letters (a–e) within individual phenolic compound denote statistically significant differences among *Rosa* species and cultivars by Duncan’s multiple range test at *P* < 0.05.

FW = fresh weight.

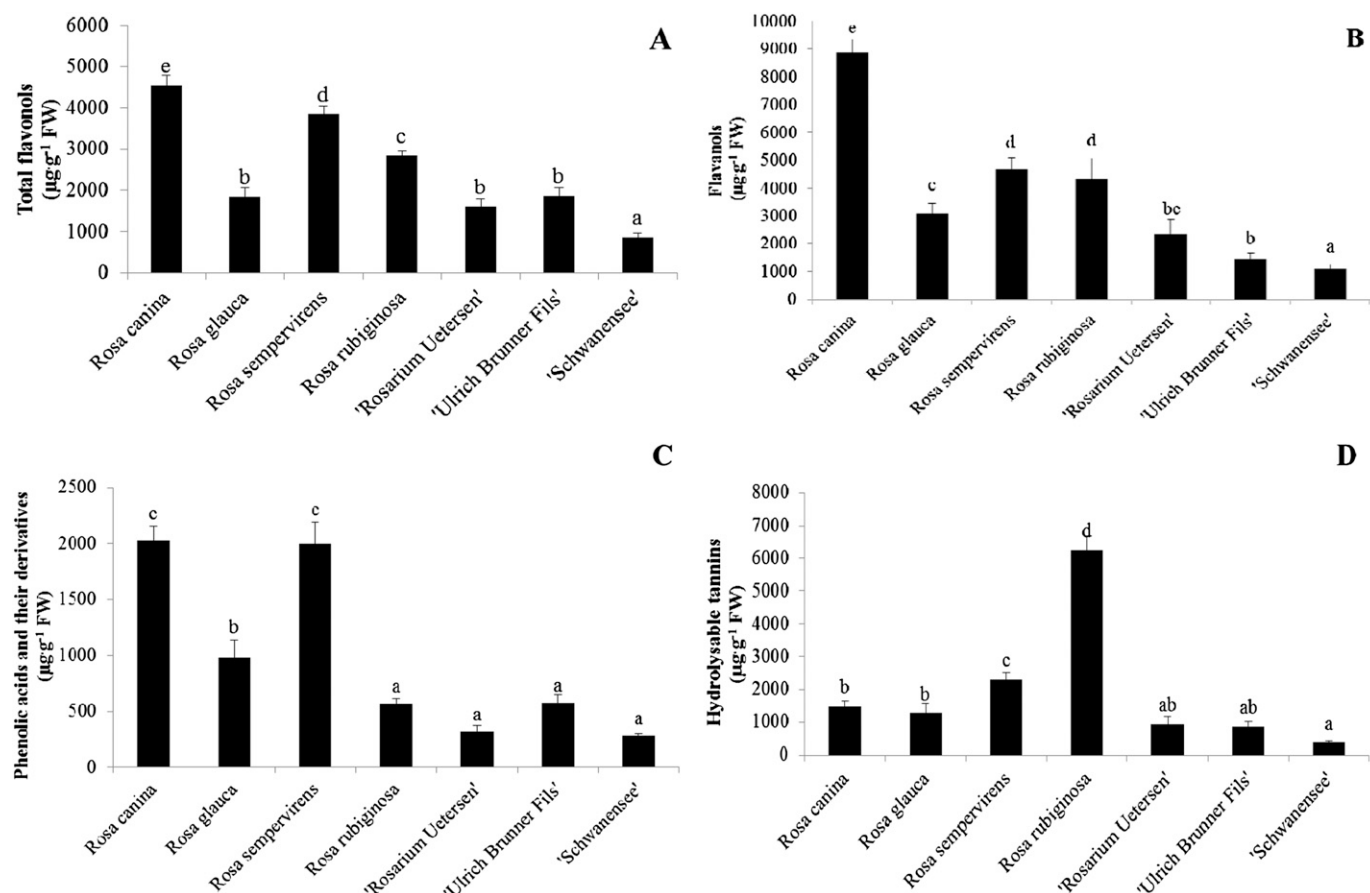


Fig. 1. Content of total flavonols (A), flavonols (B), phenolic acids and their derivatives (C), and hydrolysable tannins (D) in leaves of *Rosa* species and cultivars. Bars represent SE. Different letters (a–e) within individual phenolic groups denote statistically significant differences among *Rosa* species and cultivars by Duncan's multiple range test at  $P < 0.05$ .

In addition to flavonols, an abundance of flavonols, phenolic acids, and their derivatives and hydrolysable tannins were determined in leaves of selected *Rosa* species and cultivars using MS<sup>n</sup> (Table 2). Rosaceae can generally be characterized as a family rich in catechin and proanthocyanidin secondary metabolites (Hoffmann et al., 2012). From the group of flavonols, catechin was detected in all studied species and cultivars (Table 5) with the highest content measured in leaves of *R. canina* and the lowest in leaves of 'Schwanensee'. Oppositely, epicatechin could only be identified in 'Schwanensee'

leaves. Catechin and epicatechin have previously been determined in extracts of *R. damascena* leaves and different organs of *R. rugosa* (Hashidoko, 1996). High levels of catechin were recently also quantified in root tips of *R. ×hybrida* (Hoffmann et al., 2012) and in *R. micrantha* flowers (Barros et al., 2013). According to Baydar and Baydar (2013), catechin and epicatechin represent the most important phenolic constituents of *R. damascena* leaves. *R. canina* leaves contained several procyanidin di- and trimers, and in *R. canina*, *R. glauca*, and *R. sempervirens*, procyanidin tetramers have also been

Table 5. Flavonols in leaves of *Rosa* species and cultivars.

	<i>Rosa</i> species/cultivar						
	<i>R. canina</i>	<i>R. glauca</i>	<i>R. sempervirens</i>	<i>R. rubiginosa</i>	Rosarium Uetersen	Ulrich Brunner Fils	Schwanensee
Flavanol <sup>2</sup>	[mean ± SE (µg·g <sup>-1</sup> FW)]						
1	3655.1 ± 211.6 d <sup>3</sup>	1592.0 ± 210.4 c	1485.4 ± 147.5 c	1215.3 ± 255.0 c	1151.3 ± 272.5 bc	620.0 ± 116.2 ab	517.6 ± 45.2 a
2	1506.2 ± 70.4 c	598.6 ± 61.6 b	664.3 ± 57.9 b	1502.7 ± 246.8 c	176.2 ± 32.8 a	123.8 ± 12.5a	131.5 ± 19.8 a
3	3534.4 ± 180.8 e	750.8 ± 74.6 ab	2336.9 ± 186.8 d	1398.3 ± 224.0 c	1026.5 ± 209.3 bc	697.2 ± 70.5 ab	386.4 ± 58.2 a
4	171.8 ± 14.3 a	151.5 ± 21.0 a	196.0 ± 18.1a	—	—	—	—
5	—	—	—	—	—	—	76.1 ± 10.7

<sup>2</sup>Flavanol: 1 = sum of procyanidin dimers 1, 2, 3, 4, 5; 2 = sum of procyanidin trimers 1, 2, 3, 4, 5, 6; 3 = catechin; 4 = sum of procyanidin tetramers 1, 2; 5 = epicatechin; — = compound not present in samples.

<sup>3</sup>Different letters (a–e) within specific groups of phenolic compounds denote statistically significant differences among *Rosa* species and cultivars by Duncan's multiple range test at  $P < 0.05$ .

FW = fresh weight.

determined. Generally, the rose species in this study contained significantly higher levels of procyanidins and were also richer in total flavanol content compared with the studied cultivars (Table 5; Fig. 1B).

Similar to the flavanol content, diversity and content of phenolic acid (and their derivatives) varied significantly among analyzed species and cultivars (Table 6; Fig. 1C). *Cis*- and *trans*-5-caffeoylquinic acid (chlorogenic acid) have been determined in all studied species and cultivars. Shetty et al. (2011) also detected chlorogenic, neochlorogenic, and an unknown phenolic acid in leaves of *R. hybrida* 'Smart' and reported that chlorogenic acid was the most abundant phenolic acid present. Comparatively, this is in accordance with our results because *cis*-5-caffeoylquinic acid content was highest among all phenolic acids in *R. canina*, *R. glauca*, *R. rubiginosa*, and 'Rosarium Uetersen' leaves; in 'Ulrich Brunner Fils', the predominant phenolic acid was 3-caffeoylquinic acid (neochlorogenic acid). In contrast, in leaves of *R. sempervirens*, ellagic acid was the main phenolic acid constituent and 'Schwanensee' was characterized by high content levels of ellagic acid pentosides.

More than eight minor phenolic acids were tentatively identified in analyzed rose leaves (Table 2). Similarly, Baydar and Baydar (2013) also report low levels of different phenolic acids such as caffeic, chlorogenic, *p*-coumaric, ferulic, and gallic acids in leaves of *R. damascena*.

Twenty hydrolysable tannins have been tentatively identified in rose leaves (Table 2). Despite the research of Miyasaki et al. (2013) who reported ellagic acid as the most important active compound of *R. rugosa* extracts, free ellagic acid was only identified in leaves of *R. sempervirens*, *R. glauca*, and 'Rosarium Uetersen' (Table 6). In leaves of the latter two, it did not represent the major constituent of their leaves. However, numerous conjugated forms and isomers of ellagic acid have been determined in *Rosa* leaves in the present study, many

reported for the first time (Tables 2 and 7). Most importantly, hydrolysable tannins (ellagitannins) have been determined in leaves of selected *Rosa* species and cultivars consisting of one or more HHDP moieties esterified to a polyol, usually glucose (Haslam, 1996; Koponen et al., 2007). They are important in plant physiology because they provide protection against microbial decay. This ability is linked to their characteristic feature to form strong complexes with proteins and polysaccharides and consequently inhibiting microbial growth (Ascacio-Valdés et al., 2011). Ellagitannins are widely distributed in the Hamamelidaceae, Dilleniaceae, and Rosaceae and have frequently been used as chemotaxonomic markers (Haslam, 1996). As a result of their strong antioxidant properties, they are also important in the human diet, especially in the prevention of degenerative diseases (Ascacio-Valdés et al., 2011; Haslam, 1996; Sroka, 2005). The species with the highest total hydrolysable tannin content was *R. rubiginosa* (Fig. 1D), in which the highest levels of HHDP digalloyl glucose isomers, galloyl bis HHDP glucose and trigalloyl HHDP hexose, have been measured. Along with HHDP digalloyl glucose, also di-HHDP glucose isomers and vescalagin isomers have been determined in all studied species and cultivars. The latter was highest in 'Rosarium Uetersen'. The lowest total hydrolysable tannin content was determined in 'Schwanensee'.

Multivariate statistical analysis clustered the analyzed rose species and cultivars into three distinct groups (Fig. 2). Leaf phenolic profile was the principal classification factor and *R. canina* stood out as the species with the highest content and abundance of most phenolic compounds. This is in accordance with the reports of Nowak and Gawlik-Dziki (2007) who also measured highest levels of phenolics in different *R. canina* cultivars. *R. sempervirens* and *R. rubiginosa* represented the second group based on their high content of free and conjugated forms of ellagic acid, flavanols, and phenolic acids and were thus closest to *R. canina*. *R. glauca* was the most

Table 6. Phenolic acids and their derivatives in leaves of *Rosa* species and cultivars.

Phenolic acids and their derivatives <sup>z</sup>	<i>Rosa</i> species/cultivar						
	<i>R. canina</i>	<i>R. glauca</i>	<i>R. sempervirens</i>	<i>R. rubiginosa</i>	Rosarium Uetersen	Ulrich Brunner Fils	Schwanensee
	[mean ± SE (μg·g <sup>-1</sup> FW)]						
1	—	118.2 ± 20.3	—	—	—	—	—
2	—	—	—	—	58.4 ± 15.0 b	—	28.3 ± 2.4 a
3	473.0 ± 46.2 c <sup>y</sup>	182.9 ± 31.4 a	433.6 ± 37.5 c	154.3 ± 11.0 a	—	306.1 ± 53.5 b	—
4	24.5 ± 2.4	—	—	—	—	—	—
5	1120.9 ± 57.4 c	283.6 ± 28.2 b	130.8 ± 10.5 a	298.2 ± 26.2 b	91.0 ± 18.5 a	104.0 ± 10.5 a	57.7 ± 8.7 a
6	50.0 ± 4.1 d	14.2 ± 2.8 b	29.6 ± 4.5 c	—	—	—	1.1 ± 0.1 a
7	—	—	82.2 ± 12.5 b	—	—	25.3 ± 2.5 a	20.5 ± 2.5 a
8	97.3 ± 8.1 b	108.8 ± 18.2 b	105.0 ± 8.4 b	97.7 ± 5.7 b	26.3 ± 5.0 a	38.8 ± 5.5 a	13.9 ± 1.8 a
9	6.0 ± 0.3 a	9.0 ± 1.5 b	17.1 ± 1.1 c	11.2 ± 0.6 b	4.4 ± 1.0 a	—	8.9 ± 0.9 b
10	68.2 ± 8.7 c	23.5 ± 7.0 b	6.9 ± 1.4 ab	7.0 ± 0.6 ab	24.5 ± 7.8 b	—	3.3 ± 0.5 a
11	—	—	—	—	—	—	20.8 ± 3.1
12	183.9 ± 7.1 b	110.0 ± 19.1 ab	527.2 ± 68.3 c	—	63.1 ± 7.3a	97.9 ± 4.0 ab	125.0 ± 13.5 ab
13	—	135.9 ± 24.6 a	667.7 ± 55.1 b	—	47.5 ± 9.3 a	—	—

<sup>z</sup>Phenolic acid and its derivative: 1 = methyl gallate hexoside; 2 = *p*-coumaric acid hexoside; 3 = 3-caffeoylquinic acid; 4 = caffeoyl hexose; 5 = *cis*-5-caffeoylquinic acid; 6 = 4-caffeoylquinic acid; 7 = sinapic acid hexoside; 8 = *trans*-5-caffeoylquinic acid; 9 = *cis*-5-*O*-*p*-coumaroylquinic acid; 10 = *trans*-5-*O*-*p*-coumaroylquinic acid; 11 = ellagic acid hexoside; 12 = sum of ellagic acid pentoside 1, 2; 13 = ellagic acid; — = compound not present in samples.

<sup>y</sup>Different letters (a–c) within individual phenolic compound denote statistically significant differences among *Rosa* species and cultivars by Duncan's multiple range test at *P* < 0.05.

FW = fresh weight.



Table 7. Hydrolysable tannins in leaves of *Rosa* species and cultivars.

Hydrolysable tannins <sup>2</sup>	<i>Rosa</i> species/cultivar						
	<i>R. canina</i>	<i>R. glauca</i>	<i>R. sempervirens</i>	<i>R. rubiginosa</i>	Rosarium Uetersen	Ulrich Brunner Fils	Schwanensee
	[mean $\pm$ SE ( $\mu\text{g}\cdot\text{g}^{-1}$ FW)]						
1	109.7 $\pm$ 8.3 bcd <sup>y</sup>	92.1 $\pm$ 13.6 bc	155.4 $\pm$ 14.3 d	104.5 $\pm$ 6.6 bcd	67.6 $\pm$ 18.0 ab	137.2 $\pm$ 32.4 cd	31.0 $\pm$ 2.0 a
2	266.3 $\pm$ 18.1 d	197.0 $\pm$ 46.7 bcd	210.1 $\pm$ 27.0 cd	692.0 $\pm$ 44.2 e	110.8 $\pm$ 19.0 ab	154.1 $\pm$ 14.4 bc	41.0 $\pm$ 3.4 a
3	289.2 $\pm$ 38.0 b	288.6 $\pm$ 75.9 b	66.5 $\pm$ 9.7 a	117.3 $\pm$ 6.4 a	324.9 $\pm$ 91.5 b	97.9 $\pm$ 26.8 a	20.1 $\pm$ 2.1 a
4	92.3 $\pm$ 9.8 ab	171.5 $\pm$ 40.9 c	—	527.7 $\pm$ 21.8 d	121.8 $\pm$ 29.4 bc	35.4 $\pm$ 7.4 a	32.6 $\pm$ 6.1 a
5	381.0 $\pm$ 52.5 ab	313.8 $\pm$ 80.3 ab	670.9 $\pm$ 58.5 b	3375.5 $\pm$ 288.0 c	119.4 $\pm$ 29.2 a	156.9 $\pm$ 34.8 a	129.6 $\pm$ 17.6 a
6	354.8 $\pm$ 22.9 b	172.8 $\pm$ 30.6 a	395.4 $\pm$ 41.8 b	655.0 $\pm$ 29.4 c	161.8 $\pm$ 28.0 a	153.7 $\pm$ 26.5 a	115.2 $\pm$ 14.5 a
7	—	—	695.1 $\pm$ 53.0 b	313.5 $\pm$ 20.0 a	—	—	—
8	—	—	—	328.8 $\pm$ 21.3 b	—	95.3 $\pm$ 13.7 a	—
9	—	38.5 $\pm$ 5.4 a	114.9 $\pm$ 8.0 b	138.4 $\pm$ 7.3 c	37.2 $\pm$ 9.4 a	—	—
10	—	—	—	—	—	31.0 $\pm$ 7.4	—

<sup>2</sup>Hydrolysable tannin: 1 = sum of di-HHDP glucose 1, 2; 2 = sum of HHDP digalloyl glucose isomer 1, 2, 3; 3 = sum of vescalagin isomer 1, 2, 3, 4; 4 = galloyl bis HHDP glucose; 5 = sum of trigalloyl HHDP glucose 1, 2; 6 = galloyl quinic acid; 7 = digalloyl quinic acid; 8 = sum of digalloyl glucose isomer 1, 2, 3; 9 = sum of trigalloyl hexose 1, 2; 10 = digalloyl pentose; — = compound not present in samples.

<sup>y</sup>Different letters (a–e) within individual phenolic compound denote statistically significant differences among *Rosa* species and cultivars by Duncan's multiple range test at  $P < 0.05$ .

FW = fresh weight; HHDP = hexahydroxydiphenyl.

distinct of all species analyzed and grouped in the third cluster along with rose cultivars. However, 'Schwanensee' appeared most dissimilar of all cultivars analyzed as a result of low levels of phenolic constituents measured in its leaves (Tables 4 to 7). This could potentially be linked to its known susceptibility to diseases because phenolic composition and their antioxidant

effects reportedly play a role in plants' defense against various stressors (Dixon and Paiva, 1995; Osbourn, 1996; Shetty et al., 2011; Sroka, 2005).

It seems that species are more suitable as a potential source of leaf phenols with antioxidative activity. Traditional practice of *R. canina* selective use for medicinal purposes also appears

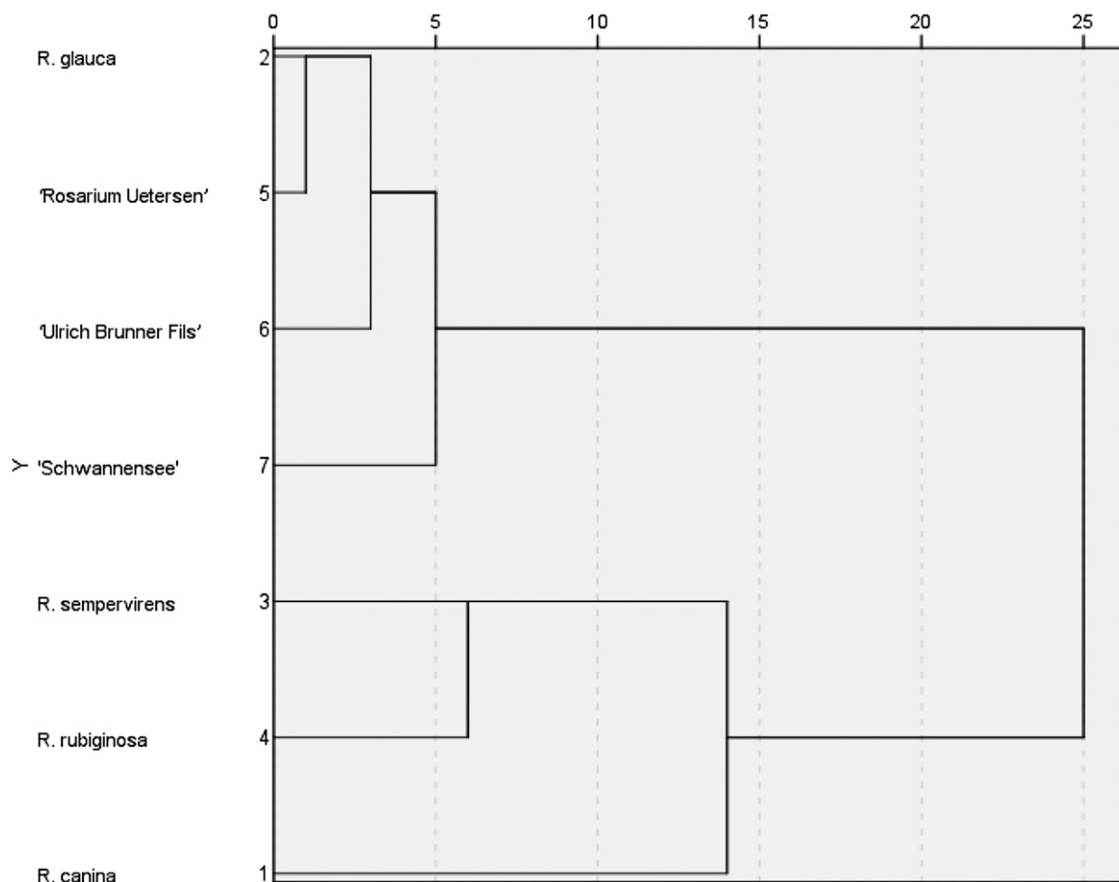


Fig. 2. Dendrogram of *Rosa* species and cultivars using Ward's method based on square Euclidian distance combining biochemical data on petal and leaf phenolic composition.

scientifically justified because it contained significantly more phenolic antioxidants compared with other naturally occurring rose species of the region. Moreover, because specific phenolic compounds display different biological functions in plants themselves, the data on phenolic content in rose species and cultivars may be important in breeding for further research of resistance and susceptibility to plant disease.

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