

FLAVONOID AND ORGANIC ACID CONTENT IN ROSE HIPS (*ROSA* L., SECT. *CANINAE* DC. EM. CHRIST.)

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We determined the level of flavonoids, citric acid and ascorbic acid in hips of rose species from the *Caninae* section occurring in Poland. We performed phytochemical analyses of 75 samples representing 11 species: *Rosa agrestis* Savi, *R. canina* L., *R. dumalis* Bechst., *R. glauca* Pourret, *R. inodora* Fries, *R. jundzillii* Besser, *R. rubiginosa* L., *R. sherardii* Davies, *R. tomentosa* Sm., *R. villosa* L. and *R. zalana* Wiesb. Flavonoid content was determined spectrophotometrically, and organic acid concentrations by HPLC. The content of the studied compounds varied greatly. Interspecific differences in the amount of flavonoids and ascorbic acid were highly significant. The most common species, *Rosa canina*, showed low average content of vitamin C (0.51 g/100 g of dry matter) and flavonoids (41 mg/100 g DM) and high content of citric acid (3.48 g/100 g DM). Ascorbic acid was highest in *R. villosa* hips (avg. 2.25 g/100 g DM), flavonoids were highest in *R. rubiginosa* (72 mg/100 g DM), and citric acid was highest in *R. tomentosa* (4.34 g/100 g DM). Flavonoid level correlated negatively with the amount of citric acid ($r=-0.47$, $p<0.001$). Cluster analysis of rose species based on the content of the investigated compounds confirmed the validity of the division of sect. *Caninae* into three subsections: *Rubiginosae*, *Vestitae* and *Rubrifoliae*. The phytochemical variation of these roses reflects their probable phylogenetic relationships as determined from morphology.

Key words: *Rosa*, *Caninae*, rose hips, medicinal plants, flavonoids, vitamin C, phylogenetic relationships, taxonomy.

INTRODUCTION

Rose hip extracts have antibacterial, antifungal and antiinflammatory properties (Trovato et al., 2000; Kumarasamy et al., 2002; Larsen et al., 2003), and antioxidative activity has also been shown (Gao et al., 2000; Moure et al., 2001; Ochmańska et al., 2001; van Rensburg, 2005). They contain large amounts of pharmacologically active compounds such as organic acids (including ascorbic acid – vitamin C), flavonoids, carotenoids and tannins (Gao et al., 2005; Novruzov and Shamsizade, 2005; Olsson et al., 2005; Nowak, 2006; Buchwald et al., 2007; Kozłowski et al., 2009).

A large majority of European rose species belong to the section *Caninae* DC. em. Christ. This is the most polymorphic section of the genus, posing great difficulties in their taxonomy. It comprises

polyploids of hybrid origin, distinguishable by a specific process of meiosis (so-called balanced heterogamy). Presumably this section is at a relatively early stage of divergence, since the differences between species are sometimes smaller than their intraspecific variability. Frequently it is only possible to separate particular taxa on the basis of a whole set of morphological characters. Other problems with identification of species are related to the fact that they undergo secondary hybridization, producing numerous transitional forms (Zieliński, 1985, 1987; Popek, 1996; Henker, 2000; Werlemark and Nybom, 2001; Ritz and Wissemann, 2003; Lim et al., 2005; Ritz et al., 2005; Wissemann et al., 2007). Phytochemical data, which may serve as an additional taxonomic criterion, are an important supplemental tool for differentiation within this plant group (Stace, 1993).

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The published phytochemical studies of roses include analyses of the chemical composition of petals (Biolley and Jay, 1993; Jay et al., 1994; Raymond et al., 1995), hypanthia (Ercisli, 2007; Nojavan et al., 2008; Adamczak et al., 2010), achenes (Özcan, 2002; Kumarasamy et al., 2003; Nowak, 2005) and leaves (Krzaczek and Krzaczek, 1979; Tarnoveanu et al., 1995; Nowak and Gawlik-Dziki, 2007). In the hips of roses from the *Caninae* section, the level of ascorbic acid has been determined most frequently (Halášová and Jičínská, 1988; Gao et al., 2000; Kovács et al., 2000; Demir and Özcan, 2001; Strålsjö et al., 2003; Uggla et al., 2003; Erentürk et al., 2005; Kovács et al., 2005; Novruzov and Shamsizade, 2005; Pirone et al., 2007; Nojavan et al., 2008; Nueleanu et al., 2008). Also measured were carotenoids (Hodisan et al., 1997; Gao et al., 2000; Böhm et al., 2003; Novruzov and Shamsizade, 2005; Olsson et al., 2005; Pirone et al., 2007), flavonoids and anthocyanins (Nowak, 1994; Nowak and Hawrył, 2005; Pirone et al., 2007; Adamczak et al., 2010), total phenols (Gao et al., 2000; Nowak and Gawlik-Dziki, 2004; Olsson et al., 2005; Ercisli, 2007; Kilicgun and Altiner, 2010), sugars (Kovács et al., 2000; Uggla et al., 2005; Pirone et al., 2007) and nutrients (Kovács et al., 2000; Demir and Özcan, 2001; Ercisli, 2007). The largest amount of such data is in a monographic study by Nowak (2006). However, these studies most often relate only to *Rosa canina* (Hodisan et al., 1997; Demir and Özcan, 2001; Hvattum, 2002; Erentürk et al., 2005; Novruzov and Shamsizade, 2005; Nojavan et al., 2008; Nueleanu et al., 2008; Kilicgun and Altiner, 2010). There are few comparative studies shedding light on intra- and interspecific phytochemical variability within sect. *Caninae* (Krzaczek et al., 1970; Halášová and Jičínská, 1988; Nowak, 2006).

Here we determined the levels of flavonoids and organic (citric and ascorbic) acids in hips of rose species from the *Caninae* section found in Poland. We assessed the taxonomic value of these groups of compounds based on a large number of samples and species.

MATERIALS AND METHODS

PLANT MATERIAL

Phytochemical analysis was performed on 75 samples of rose hips representing 11 species from sect. *Caninae* DC. em. Christ.: *Rosa agrestis* Savi, *R. canina* L., *R. dumalis* Bechst. (= *R. glauca* Vill., *R. vosagiaca* Desp.), *R. glauca* Pourret, *R. inodora* Fries (= *R. elliptica* Tausch), *R. jundzillii* Besser (= *R. trachyphylla* Rau.), *R. rubiginosa* L., *R. sherardii* Davies, *R. tomentosa* Sm., *R. villosa* L. and

R. zalana Wiesb. Samples were collected in 2007–2008 in Greater Poland (Wielkopolska), Lower Silesia (Dolny Śląsk), the Lubusz region (Ziemia Lubuska), the Poniżdie region (Ponidzie) and the Kraków-Częstochowa Upland (Wyżyna Krakowsko-Częstochowska). Rose hips were harvested from the end of August to mid-October depending on the ripening period (Zieliński, 1987; Kovács et al., 2005). They were picked at full maturity as judged by their color (Erentürk et al., 2005; Gao et al., 2005; Nojavan et al., 2008). The material was taken mainly from wild specimens, and in several cases from shrubs in the garden collection of the Institute of Dendrology, Polish Academy of Sciences in Kórnik near Poznań. The obtained rose hips were lyophilized at -50°C and 0.5 hPa (Heto Drywinner DW 30).

Species identification and taxon nomenclature follow Zieliński (1987). All samples are documented by voucher specimens deposited in the herbarium of the Department of Botany, Breeding and Agronomy, Institute of Natural Fibres and Medicinal Plants in Poznań.

FLAVONOID ANALYSIS

Phytochemical screening of total flavonoids in lyophilized plant material (2.00 g sample) was done using the methodology described in the 6th *Farmakopea Polska* (2002). The spectrophotometric method we used to quantify total flavonoids, expressed as quercetin equivalent, is the standard Christ-Müller's procedure described for medicinal plants (Vladimir-Knežević et al., 2011). This method involves acid hydrolysis of flavonol glycosides and then the formation of colored complexes of these flavonoid compounds with AlCl₃. Absorbance was measured at λ=425.0 nm with a Cintra 20 UV-VIS spectrometer (GBC). All solvents (analytical grade) were purchased from POCH S.A., Poland.

CITRIC AND L-ASCORBIC ACID ANALYSIS

The freeze-dried and powdered rose hips (0.40–0.50 g) were extracted twice for 30 min with 2.5 ml 4.0% (m/V) L-cysteine and 10.0 ml water by sonification. All aqueous extracts were combined and diluted with water to 25 ml. The samples were analyzed using HPLC (PN-EN 14130:2004).

HPLC analyses were done with an Agilent 1100 HPLC system with a photodiode array detector (DAD), and all separations were on a Lichrospher 100 RP18 column (250.0×4.0 mm, 5.0 μm; Merck). The mobile phase consisted of 0.0272 g/l KH₂PO₄ adjusted to pH 2.40 with H₃PO₄, applied in isocratic elution for 30 min. The flow rate was adjusted to 1.0 ml/min. The detection wavelength was set to DAD at λ=215.0 nm for citric acid and λ=254.0 nm

TABLE 1. Content of flavonoids, citric acid and ascorbic acid in freeze-dried hips of roses from sect. *Caninae*

Variable	Mean ± SD	Min	Max	V [%]	N
Flavonoids [mg/100 g DM]	52 ± 20	20	98	38	74
Citric acid [g/100 g DM]	3.16 ± 1.12	0.20	5.37	35	75
Ascorbic acid [g/100 g DM]	1.06 ± 0.58	0.08	2.67	55	75

DM – dry matter of freeze-dried hips; content of flavonoids as quercetin equivalent; SD – standard deviation; V – variability coefficient; N – number of samples.

for ascorbic acid (L-ascorbic acid). 20.0 µl samples were injected. All separations were performed at 24.0°C. Peaks were assigned by spiking the samples with standard compounds and comparing the UV spectra and retention times (ascorbic acid 5.66 min, citric acid 8.90 min). Calibration curves were obtained from 5 concentrations of each external standard (0.01–1.20 mg/ml). The regression coefficient (R^2) of the calibration curve for ascorbic acid ($Y = 83780x - 19.693$) was 0.9985, and for citric acid ($Y = 2003.6x + 12.795$) it was $R^2 = 0.9998$. The RSD values for the repeatability (n=4) of standard solution were 0.40% (0.01 mg/ml ascorbic acid) and 0.83% (0.41 mg/ml citric acid). The limits of quantitation (LOQ) and detection (LOD) of ascorbic acid were 0.18 and 0.06 mg/L, respectively, and 7.41 and 2.47 mg/L for citric acid. All solvents used were HPLC grade (Merck). Reference standards were obtained from Sigma-Aldrich.

STATISTICAL ANALYSIS

Statistica 7.1 (StatSoft, 2005) was used for statistical calculations. The Kruskal-Wallis test and the median test as well as post-hoc multiple comparisons of mean ranks for all groups were used to determine the statistical significance of interspecific differences in the content of the investigated compounds. Pearson's coefficient of correlation was used to evaluate correlations between variables. The Shapiro-Wilk test was applied to assess the normality of variable distribution. Root transformation was performed for the right-skewed distribution of flavonoids. The phytochemical similarity of rose species was determined based on cluster analysis of standardized mean content of the studied compounds in the hips of particular taxa. Euclidean distance was used as a measure of distance, and UPGMA as the clustering method.

The level of the compounds was determined per the total weight of rose hips (hypanthia and achenes). Flavonoid content is given as mg/100 g, and ascorbic and citric acid content as g/100 g rose hip dry matter (DM). The relative amount of a given compound in the hips of particular rose species is given as a percentage of the highest average content of it found in the investigated taxa.

RESULTS

Our results show large variability of the content of the studied compounds in the hips of roses from sect. *Caninae* (Tab. 1). The observed interspecific differences in the amount of flavonoids (Fig. 1) and ascorbic acid (Fig. 2) were highly significant. For example, in the most common species, for which the largest number of samples was collected, average flavonoid content in *R. canina* hips (41 mg/100 g DM) was nearly half that of *R. rubiginosa* hips (72 mg/100 g DM). The average amount of vitamin C in *R. canina* hips (0.51 g/100 g DM) was nearly a third that of *R. dumalis* hips (1.44 g/100 g DM). For citric acid there were no such large interspecific differences between the taxa with the highest number of samples, hence there were no statistically significant differences (Fig. 3). The level of flavonoids in rose hips was negatively correlated with citric acid

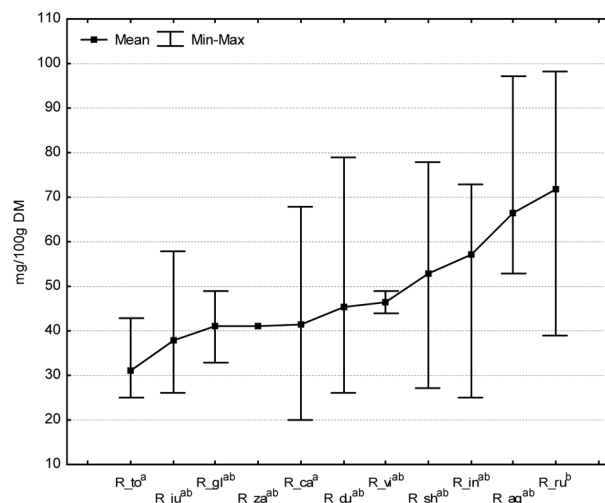


Fig. 1. Flavonoid content (as quercetin equivalent) in freeze-dried hips of rose species from sect. *Caninae* (n=74). Kruskal-Wallis test: 27.54, p=0.0021; median test: 28.02, p=0.0018; values with different letters differ significantly at p<0.05. R_ag – *Rosa agrestis* (n=7); R_ca – *R. canina* (n=12); R_du – *R. dumalis* (n=16); R_gl – *R. glauca* (n=2); R_in – *R. inodora* (n=5); R_ju – *R. jundzillii* (n=3); R_ru – *R. rubiginosa* (n=13); R_sh – *R. sherardii* (n=9); R_to – *R. tomentosa* (n=4); R_vi – *R. villosa* (n=2); R_zalana – *R. zalana* (n=1).

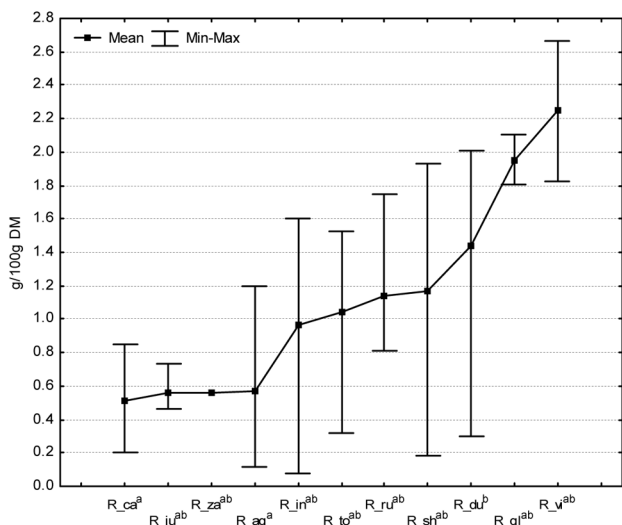


Fig. 2. Content of ascorbic acid (vitamin C) in freeze-dried hips of rose species from sect. *Caninae* (n=75). Kruskal-Wallis test: 37.76, $p < 0.001$; median test: 30.06, $p < 0.001$; values with different letters differ significantly at $p < 0.05$. R_ag - *Rosa agrestis* (n=7); R_ca - *R. canina* (n=12); R_du - *R. dumalis* (n=16); R_gl - *R. glauca* (n=2); R_in - *R. inodora* (n=5); R_ju - *R. jundzillii* (n=3); R_ru - *R. rubiginosa* (n=13); R_sh - *R. sherardii* (n=10); R_to - *R. tomentosa* (n=4); R_vi - *R. villosa* (n=2); R_za - *R. zalana* (n=1).

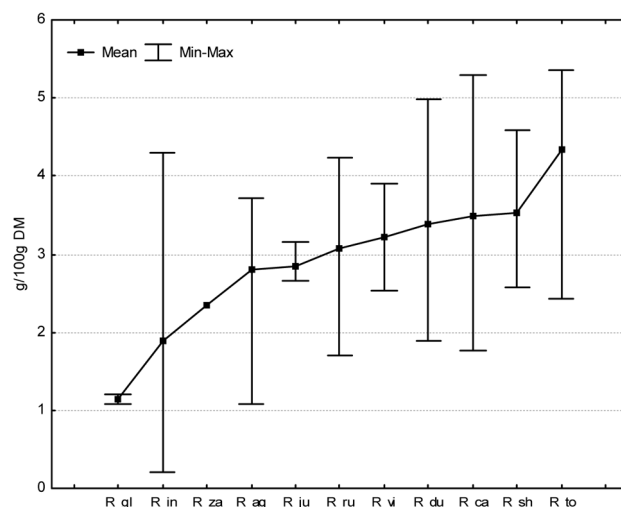


Fig. 3. Citric acid content in freeze-dried hips of rose species from sect. *Caninae* (n=75). Kruskal-Wallis test: 17.70, $p = 0.0603$ (N.S.); median test: 13.19, $p = 0.2131$ (N.S.); R_ag - *Rosa agrestis* (n=7); R_ca - *R. canina* (n=12); R_du - *R. dumalis* (n=16); R_gl - *R. glauca* (n=2); R_in - *R. inodora* (n=5); R_ju - *R. jundzillii* (n=3); R_ru - *R. rubiginosa* (n=13); R_sh - *R. sherardii* (n=10); R_to - *R. tomentosa* (n=4); R_vi - *R. villosa* (n=2); R_za - *R. zalana* (n=1).

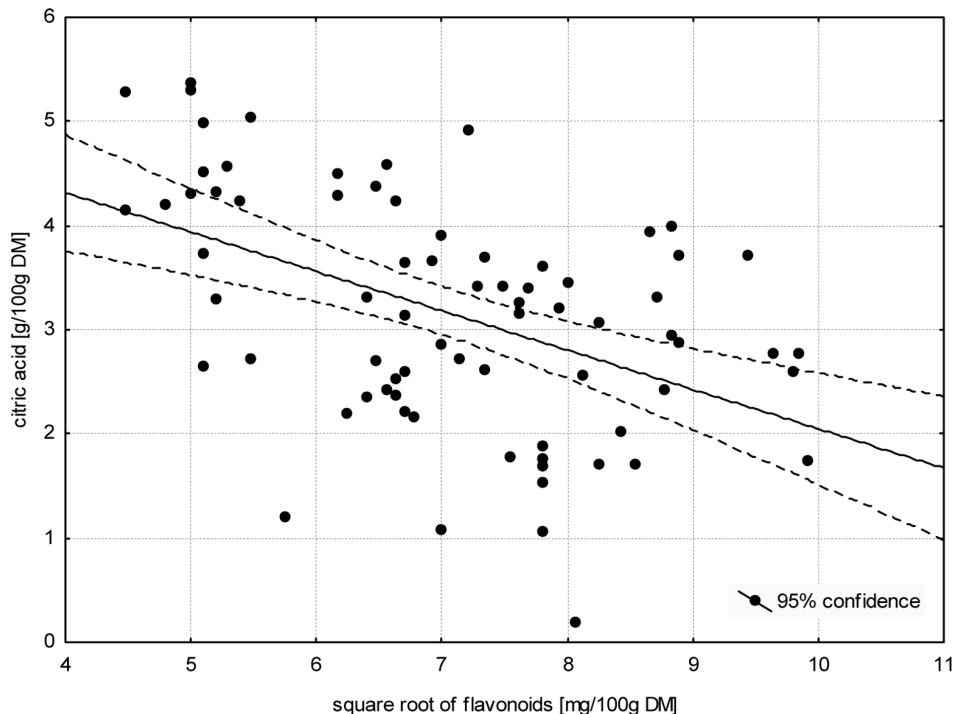


Fig. 4. Correlation between the citric acid and flavonoid content in freeze-dried rose hips. Pearson coefficient of correlation: -0.47; $p < 0.001$; n=74.

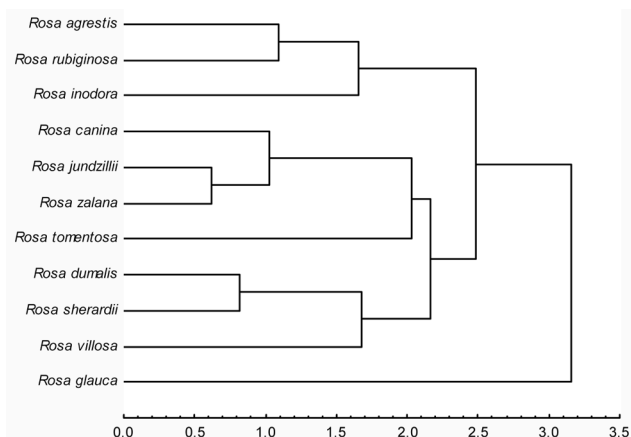


Fig. 5. UPGMA cluster analysis based on Euclidean distance of mean content of flavonoids, citric acid and ascorbic acid in freeze-dried hips of rose species from sect. *Caninae* (n=224).

content ($r=-0.47$, $p<0.001$; Fig. 4). Between the amounts of citric acid and ascorbic acid there was a statistically significant though distinctly weaker correlation ($r=0.28$, $p<0.05$).

Based on phytochemical analysis of the rose hips we constructed a dendrogram of similarity between the taxa (Fig. 5), which separated three groups of species: *Rosa agrestis*–*R. rubiginosa*–*R. inodora*; *Rosa canina*–*R. jundzillii*–*R. zalana*; and *Rosa dumalis*–*R. sherardii*–*R. villosa*. The first group was distinguished primarily by having the highest relative flavonoid content (avg. 91%), with moderate levels of citric acid (60%) and ascorbic acid (40%) (Fig. 6). The *Rosa canina* group was characterized by moderate content of flavonoids (56%) and citric acid (67%) as well as low ascorbic acid content (24%). The *Rosa dumalis* group showed moderate content of flavonoids (67%) and higher content of citric acid (78%) and ascorbic acid (72%).

DISCUSSION

The medicinal value of rose hips depends largely on the content of vitamin C and flavonoids (Kuźnicka and Dziak, 1987). This plant material is generally considered to be the most abundant natural source of vitamin C (Erentürk et al., 2005; Kobus et al., 2005; Nowak, 2006). The flavonoids and organic acids found in rose hips inhibit oxidation of vitamin C, which additionally increases its stability and bioavailability in humans (Ochmańska et al., 2001; Padayatty and Levine, 2001; Kobus et al., 2005). From the point of view of phytotherapy it is important to determine the content of vitamin C but also

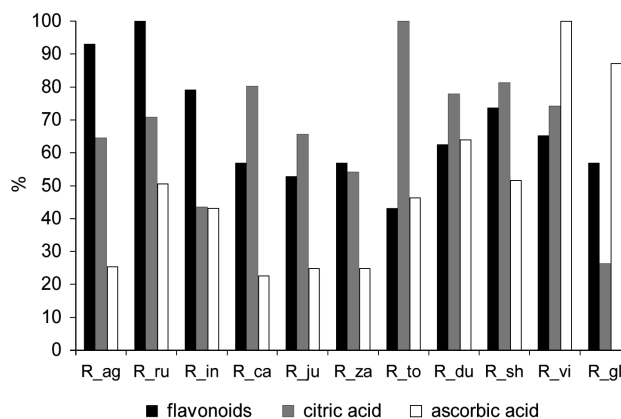


Fig. 6. Relative content* of flavonoids, citric acid and ascorbic acid in freeze-dried hips of rose species from sect. *Caninae* (n=224). *Species with the highest average content of a given compound (group of compounds) were taken as 100%. R_ag – *Rosa agrestis* (n=21); R_ca – *R. canina* (n=36); R_du – *R. dumalis* (n=48); R_gl – *R. glauca* (n=6); R_in – *R. inodora* (n=15); R_ju – *R. jundzillii* (n=9); R_ru – *R. rubiginosa* (n=39); R_sh – *R. sherardii* (n=29); R_to – *R. tomentosa* (n=12); R_vi – *R. villosa* (n=6); R_zal – *R. zalana* (n=3).

other organic acids (e.g., citric acid, commonly used in food processing as an acidity regulator and antioxidant) and flavonoids. Among the flavonoids in plants, quercetin and kaempferol are important and most common. These active compounds usually occur as glycosides with the sugar moiety bound at the C-3 position. In rose hips there are mainly glycoside derivatives of quercetin: quercitrin (quercetin-3-O-rhamnoside), isoquercitrin (quercetin-3-O-glucoside) and hyperoside (quercetin-3-O-galactoside) (Gao et al., 2000; Hvattum, 2002; Nowak, 2006). There may be interspecific differences between roses in the composition and concentration of individual flavonol compounds, but this is not yet well documented (Novruzov, 2005; Nowak and Tuzimski, 2005).

The literature data show large differences in the content of these compounds in the hips of roses from sect. *Caninae* (Kovács et al., 2005; Nowak, 2006; Adamczak et al., 2010). *Rosa villosa* is the richest source of vitamin C, while *R. canina* usually shows low content of it (Krzaczek et al., 1970; Gao et al., 2000); our results support that. Average ascorbic acid content in *R. villosa* was 2.25 g/100 g DM, and in *R. canina* only 0.51 g/100 g DM. *R. canina* showed a low range of variability as compared to other species we studied (e.g., *R. inodora*, *R. sherardii*, *R. dumalis*). Halášová and Jičinská (1988) found great variation of ascorbic acid content within *R. canina* L. [in the broad view of this species adopted here, following Zieliński (1987) and Popek

(1996)], ranging from 0.62 g/100 g DM in *R. andegavensis* Bast. (forms with glandular pedicels) to 4.57 g/100 g DM in air-dried hips without achenes in *R. corymbifera* Borkh. (forms with hairy leaves). Those authors stressed that the level of vitamin C in rose hips depends largely on soil and climatic conditions, but Krzaczek et al. (1970) maintained that the taxonomic assignment level lower than species (i.e., subspecies and variety) plays a greater role. We found high citric acid content in *R. canina* hips (Fig. 3), as did Kovács et al. (2005). *R. rubiginosa* hips showed the highest average flavonoid content, as in a preliminary study (Adamczak et al., 2010); usually it was low in *R. canina* (Fig. 1).

Owing to difficulties in the classification and identification of individual rose species, herbal raw material is obtained from different taxa; this is permitted by both *Farmakopea Polska* (1954, 1970) and the *European Pharmacopoeia* (2002). The one usually collected is the widespread dog rose (*Rosa canina* L.). Our results show that this species is not the most valuable herbal raw material, at least as a source of vitamin C and flavonoids. The use value of other rose species of the *Caninae* section is higher: *R. villosa* (for vitamin C) and *R. rubiginosa* (for flavonoids). We determined the content of the active compounds in whole rose pseudofruits (hips). The main part of the hip is fruit flesh. According to Kovács et al. (2000), achenes can constitute from 19% (*Rosa agrestis*) to 31% (*R. micrantha*) of rose hip weight, depending on the species. Uggla et al. (2003) reported a positive correlation between the percentage of fruit flesh and the weight of whole rose hips.

Based on their morphological characters, the rose species of sect. *Caninae* that occur in Poland can be classified in five subsections (Končalová and Klášterský, 1978; Małecka et al., 1990; Wissemann, 2000; de Cock et al., 2008): *Rubrifoliae* (*Rosa glauca*), *Caninae* (*R. canina*, *R. dumalis*), *Vestitae* (*R. tomentosa*, *R. sherardii*, *R. villosa*), *Rubiginosae* (*R. agrestis*, *R. inodora*, *R. micrantha*, *R. rubiginosa*, *R. zalana*) and *Trachyphyllae* (*R. jundzillii*). The boundaries between these groups are not sharp, since the most common species, *Rosa canina*, is morphologically linked to both *R. dumalis* and species representing other subsections: *R. tomentosa*, *R. agrestis* and *R. jundzillii* (Zieliński, 1987). Nevertheless, genetic investigations provide at least partial confirmation of that classification (Olsson et al., 2000; Werlemark and Nybom, 2001; Nowak and Polok, 2005; de Cock et al., 2008), and it is largely in agreement with our results on phytochemical variability (Figs. 5, 6). Cluster analysis on the basis of flavonoid and organic acid content separated three subsections: *Rubiginosae* (with *R. agrestis*, *R. rubiginosa* and *R. inodora*), *Vestitae* (with *R. sherardii* and *R. villosa*) and *Rubrifoliae* (with

R. glauca). There were also certain deviations from the classification based on morphological characters. *Rosa zalana*, which is considered to be intermediate between *R. rubiginosa* and *R. agrestis* (Zieliński, 1987), was outside the group of species from the *Rubiginosae* subsection. *R. tomentosa* was not included with *R. sherardii* and *R. villosa*, whereas *R. dumalis* was grouped with these species. *R. dumalis* has certain features (persistent sepals, a wide orifice, a flat disc, erect stems) that make it more similar to *R. sherardii* of subsect. *Vestitae* than to *R. canina* (caducous sepals, narrow orifice, conical disc, arched stems) of subsect. *Caninae*. Morphologically, *R. tomentosa* is intermediate between *R. canina* (*Caninae*) and *R. sherardii* (*Vestitae*).

The variation of flavonoid and organic acid content in these roses (Fig. 5) well reflects the probable phylogenetic relationships between the taxa as determined from morphology. The results of flavonoid analyses (Fig. 1) are closest to the pattern of morphological data. Phenolics, especially flavonoids, are particularly important in chemotaxonomy (Stace, 1993; Novruzov, 2005; Nowak, 2006). Not only qualitative but also quantitative features have chemotaxonomic value, though they are more subject to environmental effects and are more difficult to interpret. Different groups of compounds give varying results on the level of intra- and interspecific variability within the *Caninae* section (Krzaczek et al., 1970; Olsson et al., 2005; Nowak, 2006). Hence the largest possible number of compounds should be included in such chemotaxonomic analyses, requiring us to expand and continue this study.

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