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Content of oil and main fatty acids in hips of rose species native in Poland

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Abstract: Rose hips are a valuable source of many pharmacologically active compounds and they also contain nutrients, including high quality fatty oil. The aim of the study was to determine the variability in the content of oil and main fatty acids in hips of all native rose species of the section *Caninae*. An attempt was also made to evaluate the taxonomic value of the compounds under consideration. In the investigations, 48 samples representing 11 taxa were used. These species were as follows: *Rosa agrestis* Savi, *R. canina* L., *R. dumalis* Bechst., *R. inodora* Fries, *R. marginata* Wallr. (= *R. jundzillii* Besser), *R. micrantha* Borrer ex Sm. in Sowerby, *R. rubiginosa* L., *R. sherardii* Davies, *R. tomentosa* Sm., *R. villosa* L. and *R. zalana* Wiesb. The fatty oil was extracted with *n*-hexane in a Soxhlet apparatus. The fatty acid profile was determined by gas chromatography (GC-FID). The obtained results show a high range of differentiation in the levels of the investigated compounds in rose hips. The average oil content was from 2.9% in *R. tomentosa* to 5.9% in *R. sherardii*. The oil was characterized by a high average content of polyunsaturated fatty acids – PUFA (59.5%), and a low level of erucic acid (0.3%). The erucic acid content was strongly negatively correlated with the amount of linoleic acid. Cluster analysis of the levels of oils, fatty acids and relative ratios of fatty acids gave a structure of phytochemical similarity of the roses, which was to some extent consistent with that obtained on the basis of their morphological characters.

Additional key words: *Rosa*, section *Caninae*, medicinal plants, taxonomy

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Introduction

Wild growing rose species are a source of herbal raw material rich in vitamin C, flavonoids, and carotenoids, as well as essential oils and fatty oil (Olsson et al. 2005; Nowak 2006; Buchwald et al. 2007; Kozłowski et al. 2009). 14 native rose species are found in Poland, including as many as 11 species that belong to the critical section *Caninae* DC. em. Christ. (Zieliński 1987). This is the most polymorphic group within the genus *Rosa* L., which causes great taxonomic problems (Zieliński 1985; Popek 1996; Henker 2000; Werlemark and Nybom 2001; Ritz et al. 2005). The multitude of identified lower-order taxonomic units is evidence of the range of variability among the species under discussion, not to mention the controversies regarding the delineation of species themselves. In a monographic study on the genus *Rosa* in Poland and in neighbouring countries, Popek (1996) reports as many as 72 varieties and forms for 11 species of the section *Caninae*.

Based on their morphological characters, the native taxa from the section in question can be grouped in 4 subsections (Małecka et al. 1990; Wissemann 2000; de Cock et al. 2008): *Caninae* (*R. canina* and *R. dumalis*), *Vestitae* (*R. tomentosa*, *R. sherardii* and *R.*

villosa), *Rubiginosae* (*R. agrestis*, *R. inodora*, *R. micrantha*, *R. rubiginosa* and *R. zalana*) and *Trachyphyllae* (*R. marginata*). But the boundaries between the groups so designated are not distinct. The most common species – *R. canina*, is morphologically linked to both *R. dumalis* and the species representing other subsections: *R. tomentosa*, *R. agrestis*, and *R. marginata* (Zieliński 1987). In this context, it would be interesting to use various phytochemical data as an additional taxonomic criterion.

Due to the great importance of roses in phytotherapy as well as in the food and cosmetic industries, numerous studies have been undertaken to describe 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 of these species (Krzaczek and Krzaczek 1979; Tarnoveanu et al. 1995; Nowak and Gawlik-Dziki 2007). In the rose hips, the level of vitamin C has been most frequently determined (Demir and Özcan 2001; Strålsjö et al. 2003; Uggla et al. 2003; Erentürk et al. 2005; Novruzov and Shamsizade 2005; Nojavan et al. 2008; Nueleanu et al. 2008). The research has covered less frequently other groups of compounds, such as carotenoids (Hodisan et al. 1997; Böhm et al. 2003; Olsson et al. 2005), flavonoids and anthocyanins (Nowak and Hawrył 2005; Pirone et al. 2007; Adamczak et al. 2010), total phenols (Olsson et al. 2005; Ercisli 2007; Kilicgun and Altiner 2010) and sugars (Kovács et al. 2000; Uggla et al. 2005; Pirone et al. 2007). The largest amount of data in this regard can be found in the monographic study of Nowak (2006).

Articles devoted to the determination of oil and fatty acid content in rose fruits have been relatively numerous (Cisowski et al. 1995; del Valle et al. 2000; Özcan 2002; Szentmihályi et al. 2002; Nowak 2005; Machmudah et al. 2007; Barros et al. 2011). But they are most often related to only one species: *Rosa canina*. It was only Nowak (2005) who included a larger number of taxa in her study: 5 species of the section *Caninae* (*R. canina*, *R. dumalis*, *R. inodora*, *R. rubiginosa* and *R. villosa*) and 1 species of the section *Cinnamomeae* (*R. rugosa*). It would be interesting to extend phytochemical analysis by including the other rose taxa widely growing in Poland.

The aim of the present study was to determine the variation in the content of oil and main fatty acids in hips of all native rose species of the section *Caninae* (11 taxa). An attempt was also made to evaluate the taxonomic value of the compounds under consideration.

Material and methods

Plant material

The present study covered native rose species of the section *Caninae* DC. em. Christ. that are found in Poland (48 samples from 11 taxa). These species were as follows: *Rosa agrestis* Savi, *R. canina* L., *R. dumalis* Bechst. (= *R. glauca* Vill., *R. vosagiaca* Desp.), *R. inodora* Fries (= *R. elliptica* Tausch), *R. marginata* Wallr. (= *R. jundzillii* Besser), *R. micrantha* Borrer ex Sm. in Sowerby, *R. rubiginosa* L., *R. sherardii* Davies, *R. tomentosa* Sm., *R. villosa* L. and *R. zalana* Wiesb. Samples were collected in a period of three years 2007–2009 in the area of Greater Poland, Lower Silesia, Lubusz Land, Ponidzie region, Kraków-Częstochowa Upland and Podlasie region. Plant material was sampled mainly from wild growing specimens, but in several cases from the shrubs that are in the collection of the Institute of Dendrology of the Polish Academy of Sciences in Kórnik as well as from the shrubs growing in the Adam Mickiewicz University Botanical Garden in Poznań and in the Garden of Medicinal Plants of the Institute of Natural Fibres and Medicinal Plants. The obtained plant material was dried in a closed room at room temperature (up to 23°C) and with a relative humidity of 50–60%. The phytochemical analysis were performed in the year of sample collecting.

Species identification and taxon nomenclature generally followed by Zieliński (1987). In this systematic approach the broad species concept was used. All samples were documented in voucher specimens that are deposited in the herbarium of Department of Botany and Agronomy of Medicinal Plants in Plewiska near Poznań (Institute of Natural Fibres and Medicinal Plants).

Fatty oil extraction and GC-FID

The dried and powdered rose hips (10 g) were extracted with *n*-hexane in a Soxhlet apparatus for 6 hours. The solvent was evaporated off under reduced pressure (Cisowski et al. 1995; Ercisli 2007). The obtained fatty oil was subjected to saponification, derivatization, and *n*-heptane extraction. This extract was saturated with NaCl solution and dried with anhydrous sodium sulphate.

The content of the main fatty acids (Nowak 2005) in rose oil was determined on a Clarus 500 gas chromatograph (Perkin Elmer) with a flame ionization detector (FID). The fatty oil components were subjected to separation on an Elite-FFAP column (30 m × 0.32 mm × 0.25 μm). The operating temperature of detector and oven was respectively 260°C and 240°C. The injection temperature was 220°C with a hydrogen flow rate of 45 ml/min. The carrier gas flow rate was: 2.0 ml/min, air: 450 ml/min.

The fatty oil content was determined in whole rose hips (hypanthia and achenes), expressing it in percentage of dry matter. In the extracted oil, the relative amounts of the following 7 fatty acids was determined (Table 1): linoleic (C18:2 Δ9,12), α-linolenic (C18:3 Δ9,12,15), oleic (C18:1 Δ9), palmitic (C16:0), stearic (C18:0), erucic (C22:1), and arachidic acids (C20:0). The level of polyunsaturated fatty acids (PUFA) was determined as the sum of the relative contents of linoleic and linolenic acids (Szentmihályi et al. 2002).

Statistical analysis

Statistica 7.1 (StatSoft 2005) software was used in statistical calculations. The Kruskal-Wallis test and post-hoc multiple comparisons of mean ranks for all groups were used to determine the statistical significance of the differences in oil and fatty acid content. Pearson coefficient of correlation was used to evaluate correlations between variables. The Shapiro-Wilk test was applied to assess the normality of variable distribution. A logarithmic transformation was performed for the right-skewed distribution of erucic acid content. In the cluster analysis of phytochemical similarity of the rose species, the Euclidean distance was used as a measure of distance, and UPGMA as the clustering method. Dendrograms were constructed based on the standardized means of oil and fatty acid content in the hips of particular rose species (8 variables for 11 taxa) and based on the standardized means of relative ratios (proportions) of these acids (21 variables).

Results

In the present study, a wide range of differentiation was found in terms of the level of fatty oil and the main fatty acids in the hips of rose species of the section *Caninae*. However, the variability coefficient was relatively low (Table 1). The highest interspecific differentiation was found in the case of the content of polyunsaturated fatty acids: linolenic and linoleic acids (Table 2). The level of linoleic acid was strongly negatively correlated with the amount of erucic acid

Table 1. Variability of the oil content [%] and fatty acid composition [%] in rose hips (n=48)

Variables	Mean ± SD	Min.	Max.	V [%]
content of oil	4.33 ± 1.39	2.13	8.90	32
PUFA	59.53 ± 7.29	36.09	69.18	12
linoleic acid (C18:2)	40.63 ± 5.71	26.89	52.88	14
α-linolenic acid (C18:3)	18.90 ± 5.29	9.20	29.17	28
oleic acid (C18:1)	13.03 ± 2.48	9.18	19.89	19
palmitic acid (C16:0)	3.18 ± 0.71	1.67	6.03	22
stearic acid (C18:0)	1.95 ± 0.43	1.13	2.92	22
erucic acid (C22:1)	0.32 ± 0.25	0.00	1.13	76
arachidic acid (C20:0)	0.29 ± 0.15	0.13	0.75	51

PUFA – polyunsaturated fatty acids: linoleic and linolenic acids; SD – standard deviation; V – variability coefficient.

Table 2. Oil content [%] and fatty acid composition [%] in hips of different rose species of the section *Caninae* (mean ± SD)

Species\Variables	Ocont.	PUFA	C18:2	C18:3	C18:1	C16:0	C18:0	C22:1	C20:0
R. agrestis (n=6)	3.37 ±0.55	55.91 ±5.72	41.36 ±4.11	14.54 ±2.09	16.28 ±2.96	3.45 ±0.31	2.16 ±0.35	0.21 ±0.09	0.24 ±0.05
R. canina (n=5)	4.07 ±1.10	62.40 ±2.17	47.01 ±2.58	15.39 ±0.85	13.17 ±2.20	2.72 ±0.62	2.12 ±0.26	0.18 ±0.06	0.23 ±0.05
R. dumalis (n=7)	4.34 ±0.93	63.81 ±4.71	42.11 ±5.93	21.71 ±2.95	12.44 ±1.60	3.08 ±0.45	1.96 ±0.38	0.39 ±0.37	0.24 ±0.06
R. inodora (n=3)	3.45 ±0.77	57.99 ±4.56	37.62 ±3.38	20.37 ±1.45	13.53 ±2.02	3.43 ±0.15	2.01 ±0.02	0.26 ±0.02	0.34 ±0.25
R. marginata (n=5)	4.39 ±1.02	60.06 ±5.88	46.62 ±4.72	13.45 ±2.93	14.76 ±3.04	2.96 ±0.31	2.37 ±0.57	0.17 ±0.15	0.32 ±0.25
R. micrantha (n=1)	4.78	60.78	44.48	16.30	17.03	3.24	2.24	0.22	0.34
R. rubiginosa (n=6)	4.89 ±1.33	62.91 ±3.64	37.25 ±2.40	25.65 ±1.92	11.12 ±0.83	2.72 ±0.25	1.53 ±0.18	0.49 ±0.18	0.25 ±0.02
R. sherardii (n=5)	5.91 ±1.12	64.86 ±3.42	40.19 ±1.82	24.67 ±1.95	12.02 ±0.88	2.77 ±0.31	1.74 ±0.38	0.25 ±0.24	0.20 ±0.04
R. tomentosa (n=5)	2.85 ±0.67	46.26 ±9.48	33.54 ±6.46	12.72 ±3.08	11.21 ±1.74	4.08 ±0.85	2.06 ±0.37	0.51 ±0.34	0.51 ±0.22
R. villosa (n=4)	5.48 ±2.50	59.73 ±7.57	37.00 ±4.94	22.73 ±2.99	12.19 ±0.55	3.70 ±1.56	1.41 ±0.19	0.48 ±0.24	0.30 ±0.10
R. zalana (n=1)	4.84	56.40	41.66	14.74	11.81	2.87	2.35	0.15	0.22
p-value	0.0157	0.0325	0.0046	0.0000	0.0187	0.0059	0.0104	0.0576	0.4631
	*	*	**	***	*	**	*	N.S.	N.S.

Ocont. – oil content; PUFA – polyunsaturated fatty acids: linoleic and linolenic acids; C18:2 – linoleic acid; C18:3 – α-linolenic acid; C18:1 – oleic acid; C16:0 – palmitic acid; C18:0 – stearic acid; C22:1 – erucic acid; C20:0 – arachidic acid; n – number of samples; SD – standard deviation; p-value – Kruskal-Wallis test probability of interspecific differences in the content of the investigated compounds: N.S. – not significant; * – $p<0.05$; ** – $p<0.01$; *** – $p<0.001$.

(Fig. 1). The average oil content in rose hips was from 2.9% in *Rosa tomentosa* to 5.9% in *R. sherardii* (Table 2), reaching its maximum value of 8.9% (Table 1) in a shrub of *R. villosa* growing in the collection of the Garden of Medicinal Plants in Plewiska. The obtained oil was characterized by a high average content of polyunsaturated fatty acids – PUFA (59.5%), and a low level of erucic acid (0.3%) (Table 1). The highest average and maximum content of PUFA was found in *R. sherardii*, 64.9% and 69.2%, respectively (Table 2).

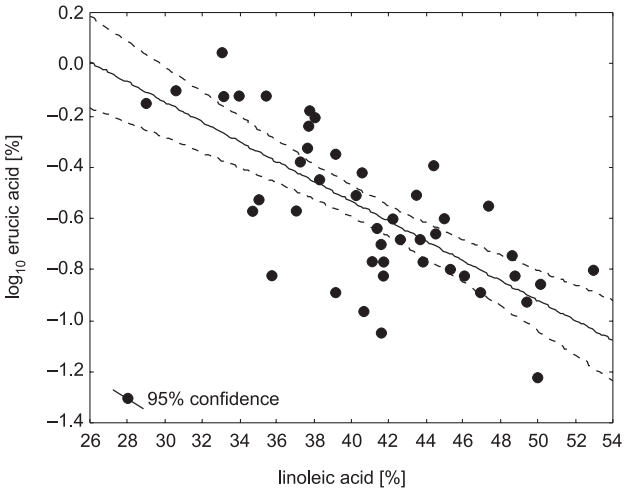

Fig. 1. Correlation between erucic and linoleic acid contents in rose hips

Pearson coefficient of correlation: –0.72; p<0.001; n=46.

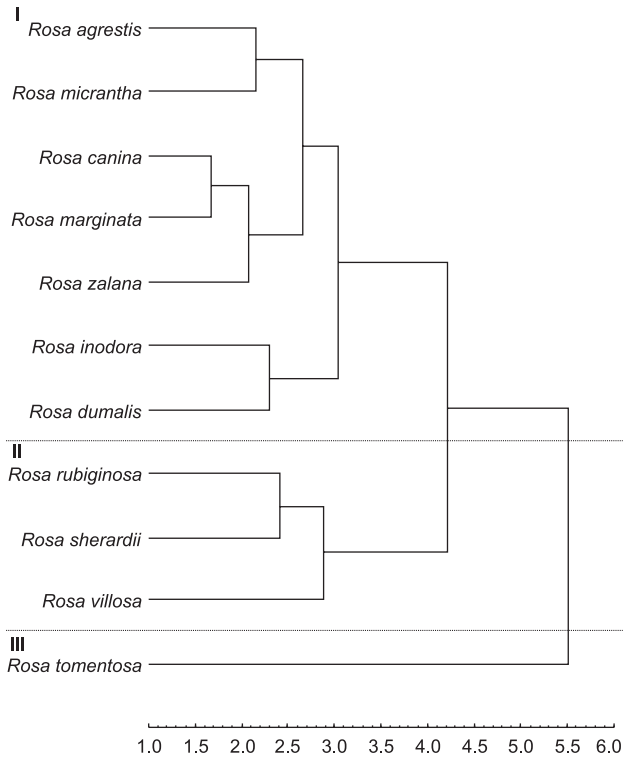

Fig. 2. UPGMA cluster analysis based on Euclidean distance of the mean contents of oil and main fatty acids in hips of different rose species of the section *Caninae*

The analysis of oil and fatty acid contents in rose hips allowed the construction of a dendrogram of similarity between the taxa under discussion (Fig. 2). Two distinct groups of species were distinguished in the diagram: (I) *Rosa agrestis – R. dumalis*, and (II) *Rosa rubiginosa – R. villosa*. (III) *Rosa tomentosa* was clearly separated from them. The first group included most of the native representatives of the subsection *Rubiginosae* (*R. agrestis*, *R. micrantha*, *R. zalana* and *R. inodora*) as well as the species of the subsection *Caninae* (*R. canina* and *R. dumalis*) and *Trachyphyllae* (*R. marginata*). *Rosa rubiginosa* – a species belonging to the subsection *Rubiginosae* – was present in the second group, alongside two taxa of the subsection *Vestitae* (*R. sherardii* and *R. villosa*). The hips of *R. tomentosa* were found to have a low content both of fatty oil (on average 2.9%) and of the sum of unsaturated fatty acids: linoleic, linolenic, and oleic acids (57.5%). In the other two groups, the average amount of these acids was at a level of more than 74%, while the average oil content was, respectively, 4.0% (the group *Rosa agrestis – R. dumalis*), and 5.4% (the group *Rosa rubiginosa – R. villosa*). [Table 2, Figs 3–4].

The cluster analysis of the relative ratios of the investigated fatty acids produced an interesting view of phytochemical similarity of the species in question (Fig. 5). The arrangement of taxa in the dendrogram was basically in agreement with that obtained based on raw data (Fig. 2): it also indicated high phytochemical divergence of *R. tomentosa* and a significant distance of *R. rubiginosa* from the other representatives of the subsection *Rubiginosae*.

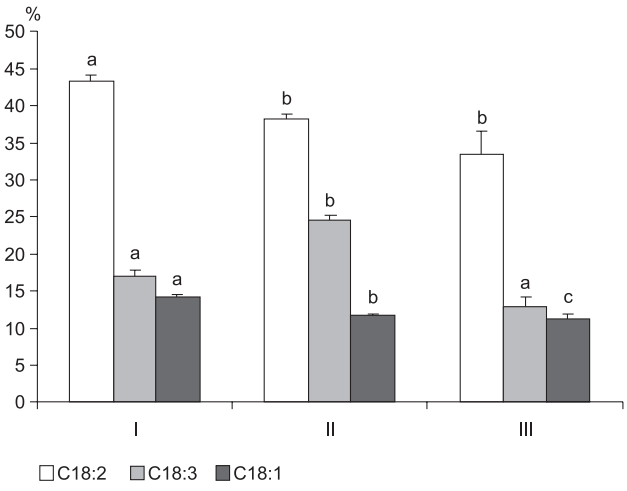

Fig. 3. Differentiation of the content of main unsaturated fatty acids in hips of three groups of rose species: (I) *Rosa agrestis – R. dumalis*, (II) *Rosa rubiginosa – R. villosa* and (III) *Rosa tomentosa*

Kruskal-Wallis test for linoleic acid (C18:2): 14.65146, p=0.0007; α-linolenic acid (C18:3): 27.42475, p=0.0000; oleic acid (C18:1): 12.28501, p=0.0021. Different letters indicate significant differences between means (mean ± SE, p<0.05). Groups of rose species – like in Fig. 2.

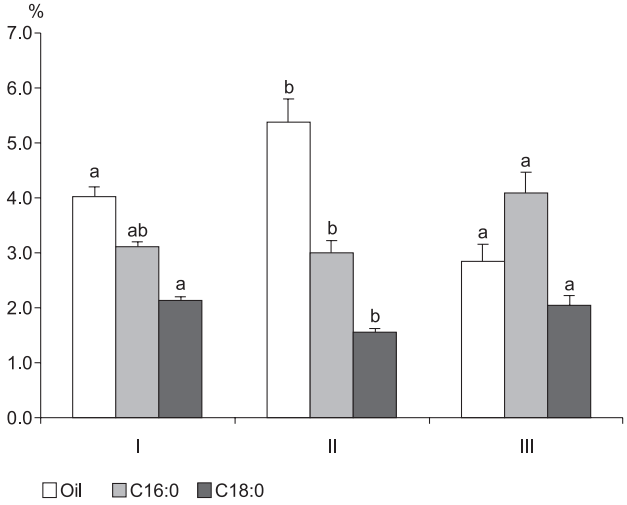

Fig. 4. Differentiation of the content of oil and main saturated fatty acids in hips of three groups of rose species: (I) *Rosa agrestis – R. dumalis*, (II) *Rosa rubiginosa – R. villosa* and (III) *Rosa tomentosa*

Kruskal-Wallis test for oil content: 15.07822, p=0.0005; palmitic acid (C16:0): 12.85472, p=0.0016; stearic acid (C18:0): 18.69836, p=0.0001. Different letters indicate significant differences between means (mean ± SE, $p<0.05$). Groups of rose species – like in Fig. 2.

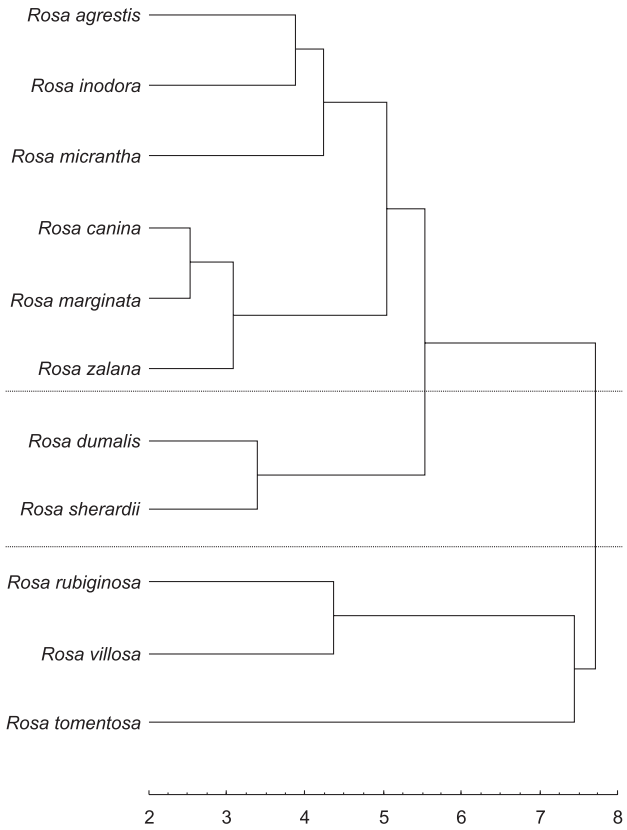

Fig. 5. UPGMA cluster analysis based on Euclidean distance of the relative rations of investigated fatty acids in hips of different rose species of the section *Caninae*

Discussion

In the presented study, fatty oil content in rose hips was from 2.1% to 8.9% of dry matter. The percentage of polyunsaturated fatty acids ranged from 36.1% to 69.2%, and the dominant component was linoleic acid: 26.9–52.9% (Table 1). The literature data show that oil accounts for nearly 2% of the rose hypanthium (Ercisli 2007) and ca. 6–18% of achene weight (Cisowski et al. 1995; del Valle et al. 2000; Özcan 2002; Nowak 2005). The oil pressed from the rose fruits (achenes) is characterized by a high content of polyunsaturated fatty acids – PUFA (58–89%), including in particular linoleic acid that is at a level of 36–61% (Cisowski et al. 1995; Özcan 2002; Szentmihályi et al. 2002; Cinar and Colakoğlu 2005; Nowak 2006; Machmudah et al. 2007; Barros et al. 2011). In the hypanthia, PUFA account for 34–64% of oil and its dominant component is linolenic acid that makes up 34–50% (Ercisli 2007). The oil extracted from rose achenes is characterized by a very low level of erucic acid: 0.03–0.17% (Cisowski et al. 1995; Nowak 2005), which was confirmed in the present study on whole rose hips (Tables 1–2). This is important, since erucic acid is considered to be an anti-nutritional component that may cause increased incidence of myocarditis (Somerville 1993; Carlson et al. 1997; Gandhi et al. 2009). In this context, it is interesting that a strong negative correlation was found between the content of an important nutritional component – linoleic acid, and erucic acid that is unwanted in the diet (Fig. 1).

Analysis of the fatty acid profile of oils has been earlier used in chemotaxonomic studies of various plant groups (Velasco and Goffman 1999; Mayworm and Salatino 2002; Sanina et al. 2004; Gören et al. 2006; da Silva et al. 2010). In the opinion of Nowak (2006), it is also of great importance in the case of the genus *Rosa*, but of lesser significance within the section *Caninae* that comprises species being at an early stage of divergence. The presented study indicates the possibility of using the analysis of the oil and fatty acid contents, in particular proportions of fatty acids, also in chemotaxonomy of the section *Caninae* (Figs 2, 5).

The above cited author (Nowak 2006) shows, among others, significant phytochemical dissimilarity between *Rosa canina* and *R. dumalis* as well as high divergence of *R. rubiginosa*. This is also confirmed by the present study (Figs 2, 5). In the case of *R. canina* and *R. dumalis*, large differences were found in the average content of the two main components of oil: linoleic and linolenic acids (Table 2). The cluster analysis of oil content as well as of the composition of fatty acids and their ratios shows that the native rose species of the section *Rubiginosae* constitute a quite phytochemically homogeneous group. Only *R. rubiginosa* maintains its high divergence and unexpectedly

comes close to the taxa of the subsection *Vestitae* (Figs 2, 5). The inclusion of the relative ratios of particular fatty acids present in rose hips allows one to highlight the great phytochemical similarity of three species of the subsection *Rubiginosae*: *Rosa agrestis*, *R. inodora,* and *R. micrantha* (Fig. 5). But *R. zalana* shows a certain distance from this group, which could speak for its hybrid origin from *R. rubiginosa* and *R. agrestis* (Větvička and Zieliński 1981; Zieliński 1987).

In spite of a relatively low content of oil in rose hips (Table 1), pressing of rose oil can be economically justified, since fruits (achenes), which make up 30% of the total weight of rose hips and which are easily available waste material in the food industry, contain more oil (Cisowski et al. 1995; Szentmihályi et al. 2002). By using modern extraction methods, e.g. supercritical CO_2 extraction, as much as 18% of high quality oil, containing almost 90% of PUFA: linoleic and linolenic acids, is obtained from rose achenes (Machmudah et al. 2007). The above mentioned fatty acids are an essential component of the diet, since they are not synthesized in human body but perform many important functions in it, regulating, i.a., blood pressure, blood viscosity as well as immune and inflammatory responses (Ercisli 2007). The high content of PUFA in consumed oils is also important in the prevention of cardiovascular diseases (Cisowski et al. 1995; Das 2000; Djoussé et al. 2001, 2003).

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