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Research Article

Profile of the Phenolic Compounds of *Rosa rugosa* Petals

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Rosa rugosa petals are a rich source of phenolic compounds, which determined their antioxidant properties. The aim of this study was to determine the polyphenolic composition of not processed petals of *Rosa rugosa* collected from the commodity crops and to determine the variability of the contained therein polyphenols between harvesting seasons. Twenty polyphenols were identified by UPLC-ESI-MS. The main fraction of polyphenols was ellagitannins, which are 69 to 74% of the total polyphenols of the petals. In the petals of *Rosa rugosa*, four anthocyanins have been identified: cyanidin 3,5-di-O-glucoside, peonidin 3-O-sophoroside, peonidin 3,5-di-O-glucoside, and peonidin 3-O-glucoside, of which the predominant peonidin 3,5-di-O-glucoside represented approx. 85% of all the determined anthocyanin compounds. It was found that the petals of *Rosa rugosa* are a valuable source of bioactive compounds and can be considered as a healthy valuable resource.

1. Introduction

Polyphenols are present in a variety of plants utilized as important components of both human and animal diets. Polyphenols are products of the secondary metabolism of plants. The expression "phenolic compounds" embraces a considerable range of substances that possess an aromatic ring bearing one or more hydroxyl substituents. Flavonoids represent the most common and widely distributed group of plant phenolics. Their common structure is that of diphenylpropanes (C6-C3-C6) and consists of two aromatic rings linked through three carbons that usually form an oxygenated heterocycle. The flavonoid variants are all related by a common biosynthetic pathway, incorporating precursors from both the shikimate and the acetate-malonate pathways [1]. Phenolic compounds act as antioxidants with mechanisms involving both free radical scavenging and metal chelation. Diets rich in fruits and vegetables, such as vegetarian and Mediterranean diets, contain a large quantity of polyphenols [2]. There is no accurate information available on the dietary intake of polyphenols because their content in plant foods varies greatly, even among cultivars of the same species. The presence of polyphenols in plant foods is largely influenced by genetic factors and environmental conditions. Other factors, such as germination, degree of ripening, variety, processing, and storage, also influence the content of plant phenolics [2–5].

Rose petals of *Rosa rugosa* are characterized by a high content of various biologically active compounds, such as anthocyanins (glycosides, such as cyanidins, pelargonidins, and peonidins), flavonols (including derivatives of kaempferol and quercetin), flavan-3-ols, and their derivatives, procyanidins and proanthocyanidins, a large group of ellagitannins and phenolic acids, such as gallic, ellagic, quinine, and essential oils [6–8]. The content of these compounds is very important for health reasons.

The anthocyanin compounds seal the capillaries, prevent edema, and have anti-inflammatory activity [9]. By inhibiting free radicals, they prevent lipid peroxidation and are important in the prevention of cancer [9]. They inhibit the development of colon cancer, as well as proanthocyanidins [10]. The most important feature of flavonoids is their antioxidant activity [11], which shows many pharmacological applications and, above all, the tumor growth inhibition. These effects have, inter alia, quercetin, genistein, 5,7-dimethoxyflavone, and 5,7,4′-trimethoxyflavone [12], apigenin, and tricine [13]. Quercetin, apigenin, and kaempferol inhibit cytochrome P-450, subfamily CYP1A, an enzyme involved in the activation of a number of carcinogens such as polycyclic aromatic or heterocyclic amines. Flavonoids also play a role in the prevention of cardiovascular diseases, such as atherosclerosis [14]. Quercetin and other flavones, flavanones, inhibit the growth of *Helicobacter pylori* agent of stomach ulcers [15]. Flavonoids also act against other microorganisms, which belong to a small group of compounds that selectively inhibit the proliferation of viruses such as herpes virus *Herpes simplex* and *Polio* virus [16]. One of the more interesting phenolic acids is the ellagic acid, which exhibits antitumor, antioxidant, antimutagenic, antibacterial, and antivirus features [14, 16–18]. A significant amount of ellagic acid is found in plants in the form of multimolecular ellagitannins and in combination with molecules of glucose, rhamnose, and xylose in the form of glycosides [19]. It is believed that some health promoting properties of ellagitannin are related to the ability to release from its molecules the free ellagic acid and its subsequent metabolism in humans and animals. The consequence of ellagitannin metabolism by the bacterial flora of the gastrointestinal tract is a sustained release of the ellagic acid into the blood. Free ellagic acid is converted to dimethylellagic acid glucuronide. This compound is easily metabolized by the microflora of the large intestine to hydroxyl-6H-dibenzopyrene derivatives. These derivatives called A and B urolithins have a proven biological activity [20].

Positive results of studies on the effectiveness of healthy action of polyphenolic compounds, including ellagitannins and free ellagic acid, tend to seek new natural sources of these valuable compounds. Therefore, particular attention should be paid to petals rich in the bioactive substances. Ellagitannins contained therein are present in high concentrations, and their profile is very broad. In fresh *Rosa rugosa* petals was confirmed the presence of hydrolyzable tannins such as tellimagrandin I, tellimagrandin II, rugosines A, B, and C, and rugosines D, E, F, and G. The presence of two simple gallotannins, 1,2,3-tri-galloilo-β-D-glucose, and 1,2,6-tri-O-galloilo-β-D-glucose was also demonstrated [21].

The aim of the study was to determine the polyphenolic composition of the fresh petals of *Rosa rugosa* collected from the commodity crops and to determine the variability of the contained therein polyphenols between harvesting seasons.

2. Materials and Methods

2.1. Chemicals. Formic acid (98–100%), acetonitrile (gradient grade for HPLC), methanol (gradient grade for HPLC), acetic acid (≥99.5%), ascorbic acid (≥99%), and phloroglucinol (≥99.0%) were purchased from Sigma-Aldrich (Steinheim, Germany). (+)-Catechin (≥99%), (−) epicatechin (≥99%), ellagic acid (≥95%), isorhamnetin 3-O-glucoside (≥99%), kaempferol 3-O-galactoside (≥99%), myricetin 3-O-glucoside (≥99%), quercetin 3-O-glucoside (≥99%), sanguine H-2 (≥90%), cyanidin 3-O-glucoside (≥96%), and procyanidins A1 (≥99%), B1 (≥90%), B2 (≥90%), and C1 (≥99%) standards were purchased from Extrasynthese (Lyon, France). All reagents were of analytical grade.

2.2. Plant Material. Fresh petals of *Rosa rugosa* were collected from the industrial-scale plantation of the company "Polska Róża" located in Kotlina Kłodzka (16°39′E 50°27′N, Poland) in June 2011, June 2012, and June 2013. The collected petals were completely dyed (flower was fully open) without signs of deteriorations and mechanical damage. The temperature during the vegetative period was close to the annual averages. There were no extremes that could affect normal development of *Rosa rugosa*. Seedlings of *Rosa rugosa* were planted in October 2008 into a loose loam type soil mixture. The soil was slightly moist with added manure. The pH of the soil was about 6–6.5. Before planting and during the first year after the planting no mineral fertilizers were used. In early spring plants were trimmed 20 cm from root collar. Compound fertilizers (NaturalCrop® SL; Herbagreen® Z20) were used during following years of plants growth. Fertilization was performed until the end of April. Longer fertilization period would have caused longer vegetation period which in turn results with worse adaptation to the winter conditions.

Fresh raw material was stored under refrigeration (6 ± 2°C), until the time of analytical determinations, but no longer than 3 days. A detailed profile of polyphenolic compounds was carried out in rose petals from two harvesting seasons, that is, over the period 2012-2013.

2.3. Identification of Polyphenols by the UPLC-PDA-Q/TOF-MS Method. Determination of polyphenolic compounds was performed by UPLC method described by Kolniak-Ostek et al. [22] and Teleszko et al. [23].

Identification of polyphenolic compounds of tests was carried out with the use of an ACQUITY Ultra-Performance LC system equipped with photodiode array detector with a binary solvent manager (Waters Corporation, Milford, MA) series with a mass detector G2 Q/TOF Micro Mass Spectrometer (Waters, Manchester, UK) equipped with an electrospray ionization (ESI) source operating in negative and positive modes. Separations of individual polyphenols were carried out using a UPLC BEH C18 column (2.1 mm × 100 mm × 1.7 μm; Waters Corporation, Milford, MA) at 30°C. The samples (10 μl) were injected, and the elution was completed in 15 min with a sequence of linear gradients and isocratic flow rates of 0.45 ml/min. The mobile phase consisted of solvent A (4.5% formic acid, v/v) and solvent B (100% of acetonitrile). The program began with isocratic elution with 99% solvent A (0-1 min), and then a linear gradient was used until 12 min, lowering solvent A to 0%; from 12.5 to 13.5 min, the gradient returned to the initial composition (99% A), and then it was held constant to reequilibrate the column. The analysis was carried out using

full-scan, data-dependent MS scanning from m/z 100 to 1500. Leucine enkephalin was used as the reference compound at a concentration of 500 pg/μl, at a flow rate of 2 μl/min, and the $[M - H]^-$ ion at 554.2615 Da was detected. The $[M - H]^-$ and $[M + H]^+$ ions were detected during 15 min analysis performed in ESI–MS accurate mass experiments, which were permanently introduced via the LockSpray channel using a Hamilton pump. The effluent was led directly to an electrospray source with a source block temperature of 130°C, desolvation temperature of 350°C, capillary voltage of 2.5 kV, and cone voltage of 30 V. Nitrogen was used as a desolvation gas at flow rate of 300 L/h.

The characterization of the single components was carried out based on the retention time and the accurate molecular masses. Each compound was optimized to its estimated molecular mass $[M - H]^-$ or $[M + H]^+$ in the negative and positive (for anthocyanins) mode before and after fragmentation. The data obtained from UPLC/MS were subsequently entered into the Mass-Lynx™ 4.0 ChromaLynx Application Manager software. On the basis of these data, the software is able to scan different samples for the characterized substances. The runs were monitored at the following wavelength: ellagitannins at 254 nm, flavan-3-ols at 280 nm, phenolic acid at 320 nm and flavonol glycosides at 360 nm, and anthocyanins at 520 nm. The PDA spectra were measured over the wavelength range of 200–800 nm in steps of 2 nm. The retention times and spectra were compared to those of the pure standards.

2.4. Quantification of Polyphenols by UPLC-PDA Method. Quantification of phenolic compounds in the samples was determined using ultra-performance liquid chromatography UPLC (Waters Corporation, Milford, MA) and a diode array detector in the analysis conditions (column, mobile phase) identical to the identification of polyphenolic compounds. Flavonols were monitored at 360 nm, phenolic acids at 320 nm, flavan-3-ols at 280 nm, and ellagitannins at 254 nm. The calibration curves were run at 254, 280, 320, and 360 nm for the standard at concentrations ranging from 0.05 to 5 mg/ml ($r^2 \leq 0.9998$). Limits of detection (LOD) and limits of quantification (LOQ) were estimated by obtaining the average height at the appropriate retention time for each compound on blank runs ($n = 6$), and the average height was converted to a concentration (employing calibration curves). LOD and LOQ were determined by multiplying the concentration by 3 and 9, respectively. LOD and LOQ were from 0.01 to 0.18 mg/ml and from 0.02 to 0.55 mg/ml, respectively. The results were expressed in mg/100 g of fresh weight (FW).

2.5. Analysis of Proanthocyanidins by Phloroglucinolysis. Polymer content of proanthocyanidins in the samples was determined by phloroglucinolysis method using ultra-performance liquid chromatography UPLC (Waters Corporation, Milford, MA). Lyophilized samples were weighed into Eppendorf tube, subsequently adding 0.8 ml of a methanolic solution of phloroglucinol (75 g/l) and ascorbic acid (15 g/l) and 0.4 ml of methanol acidified with HCl (0.3 M). Vials with reaction mixture were capped and shaken in a thermo shaker-type device (BIOSAN, Latvia) for 30 min at 50°C. The reaction was stopped by putting the tubes after shaking to a 2°C water bath and adding 0.6 ml of acetate buffer (0.2 M), then the samples were centrifuged immediately for 10 min at 4°C at 20,000 rpm (MPW, Poland). The identification was carried out using the gradient reverse phase technique using Acquity BEH Shield C 18 column (100 × 2.1 mm × 1.7 μm; Waters Corporation, Milford, MA). In the mobile phase was used a 2.5% solution of acetic acid (reagent A) and 100% acetonitrile (reagent B), under flow rate of 0.45 ml/min. Analysis time took 7.5 minutes according to the program: 0–2 min, 2 to 9% B; 2–5 min from 9 to 60% B; 5–7.50 min—stabilization of the column—2% B. The chromatographic analysis was carried out in the temp. 4°C. Identification by means of a fluorescence detector (Acquity TM, Waters, USA) was performed at excitation 278 nm and emission 360 nm. The calibration curves which were based on peak area were established using (+)-catechin, (−)-epicatechin and (+)-catechins, (−)-epicatechin-phloroglucinol adducts from procyanidins standards. Quantification (mg/100 g of FW) of the (+)-catechin, (−)-epicatechin, (+)-catechin, and (−)-epicatechin-phloroglucinol adducts was achieved by using the calibration curves of the corresponding standards.

2.6. Quantitative Determination of Anthocyanins Monomers by HPLC. For the determination of anthocyanins monomers was applied high-performance liquid chromatography (HPLC), consisting of a Shimadzu set equipped with a chromatography pump LC-10AT VP, diode array detector SPD-M10A VP, oven CTO-10AS VP, degasser DEGASEXTM model DG-400, loops 20 μl capacity, and computer Chromax 2003 with a program to collect data. In the research was used column Luna 5 μm C18 (250 × 4.6 mm × 5 μm; Phenomenex, Torrance, USA). The separation of anthocyanins has been carried out in an isocratic system at a temperature of 25°C and at a flow rate of 1 ml/min. In the mobile phase were used water, acetonitrile, and formic acid (79 : 11 : 10 *v/v/v*). The quantitative analysis was performed at the wavelength $\lambda = 520$ nm. Anthocyanins quantity was calculated from HPLC-DAD peak at 520 nm against cyanidin-3-O-glucoside as the external standards. The calibration curve (the range of 0.05–5 mg/ml) was linear with correlation coefficient of 0.998. The results were expressed in mg/100 g of fresh weight (FW).

2.7. Statistical Analysis. All results are expressed as the mean ± standard deviation (SD) of three replicates. Statistical analysis was conducted using Statistica version 12.0 (StatSoft, Tulsa, OK, USA). Significant differences ($p < 0.05$) between average responses were evaluated with the use of one-way ANOVA with Duncan test.

3. Results and Discussion

In Table 1 are shown the results of identification and the quantification of polyphenolic compounds in the tested *Rosa rugosa* petals. In the *Rosa rugosa* petals were identified polyphenolic compounds belonging to the phenolic acid,

Table 1: The results of identification and the quantification of polyphenolic compounds in the tested *Rosa rugosa* petals.

Peak number	Compound	t_R UPLC-MS [min]	$[M - H]^-$ (m/z)	$[MS^2]$ (m/z)	Harvesting season [year] 2012	2013
					(mg/100 g fresh weight of rose petals)	
12	Myricetin 3,5-di-O-glucoside	4.07	641.1755	479.1206; 317.0670	82.00 ± 2.54[a]	79.00 ± 1.81[a]
13	Quercetin 3,4-di-O-glucoside	5.85	625.1386	300.0277	158.62 ± 13.80[a]	164.62 ± 1.10[a]
15	Kaempferol 3,4-di-O-glucoside	6.75	609.1432	284.0348	40.56 ± 0.58[a]	45.99 ± 4.38[a]
16	Quercetin 3-O-glucosyl-xyloside	6.84	595.1262	300.0277	100.63 ± 1.12[b]	59.93 ± 0.30[a]
17	Isorhamnetin 3-O-glucoside	7.36	477.1022	315.0476; 271.0413	6.85 ± 0.05[b]	6.23 ± 0.02[a]
18	Unknown quercetin derivatives	7.58	1087.0920	301.0354	10.28 ± 0.18[a]	14.16 ± 0.67[b]
19	Kaempferol 3,7-di-O-rhamnoside	7.96	579.1329	284.0313	6.42 ± 0.00[a]	8.52 ± 1.05[b]
20	Quercetin 3-O-rhamnoside	8.5	447.0916	300.0277	7.33 ± 0.20[a]	9.00 ± 1.17[b]
11	(+)-catechin	3.19	289.0688	169.0136	177.60 ± 6.22[a]	185.20 ± 4.78[a]
5	Sanguine H-2	5.33	1103.0829	935.0815; 300.9999	165.45 ± 4.60[a]	209.65 ± 2.40[b]
6	Ellagitannin[1]	5.42	860.0810	785.0868; 300.9999	72.57 ± 2.60[a]	91.57 ± 1.20[b]
7	Ellagitannin[2]	5.79	860.0870	785.0868; 300.9999	21.41 ± 1.61[a]	51.41 ± 0.82[b]
8	Ellagitannin[3]	5.89	937.0917	465.0684; 300.9999	1071.81 ± 29.30[a]	1271.81 ± 8.90[b]
9	Ellagitannin[4]	6.19	1105.1028	1061.1268; 300.9999	185.77 ± 1.30[a]	283.33 ± 2.70[b]
10	Isomer galloilo-bis-HHDP glucose	6.36	935.0815	433.0352; 300.9999	20.83 ± 7.58[a]	26.83 ± 1.91[a]
14	Ellagic acid	6.63	300.9999		47.30 ± 6.58[a]	50.90 ± 2.85[a]
	Procyanidins polymer				33.10 ± 0.75[a]	32.10 ± 1.34[a]
	SUM				2208.53 ± 79.01[a]	2590.25 ± 37.40[b]

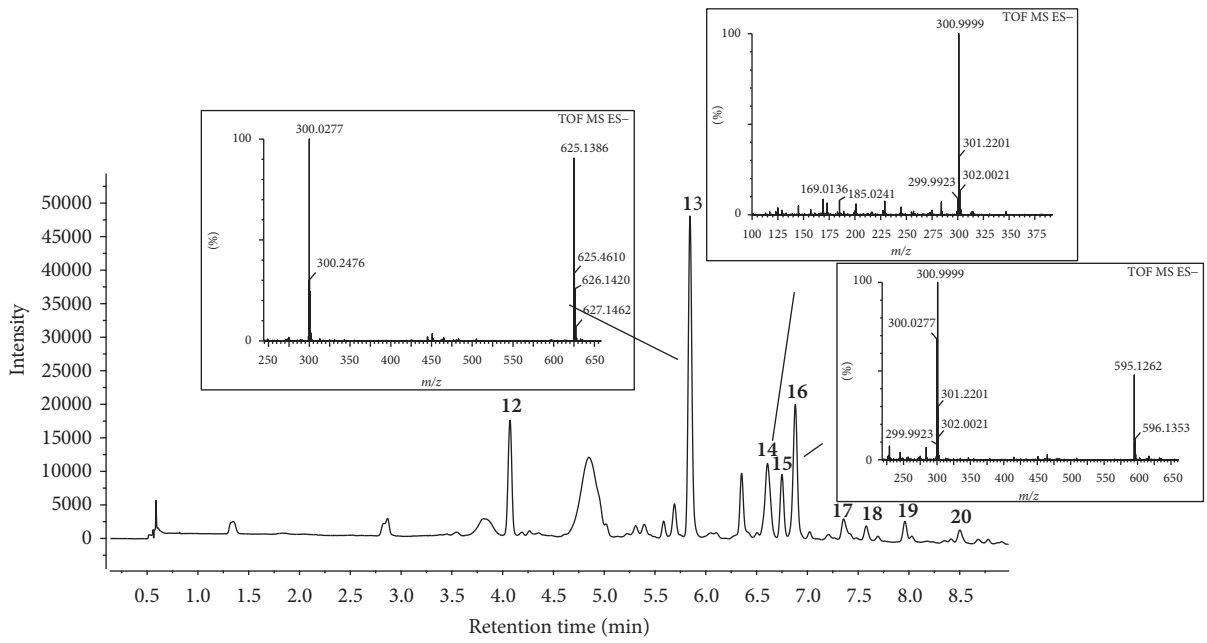
Averages marked with the same letters in the row do not differ significantly statistically at $p < 0.05$; [1,2,3,4] unidentified ellagitannins.

flavonols, flavan-3-ols, and ellagitannins (Figure 1). The total content of these compounds stood depending on the harvesting season at the levels 2208.53 mg/100 g FW (season 2012) and 2590.25 mg/100 g FW (season 2013) and the differences found were statistically significant. In the group of phenolic acids was revealed the presence of free ellagic acid. The content of free ellagic acid was not different significantly between harvesting seasons and averaged 49.1 mg/100 g FW (Table 1). Most of the ellagic acid is present in the plant material in form of glycosides and macromolecular ellagitannins.

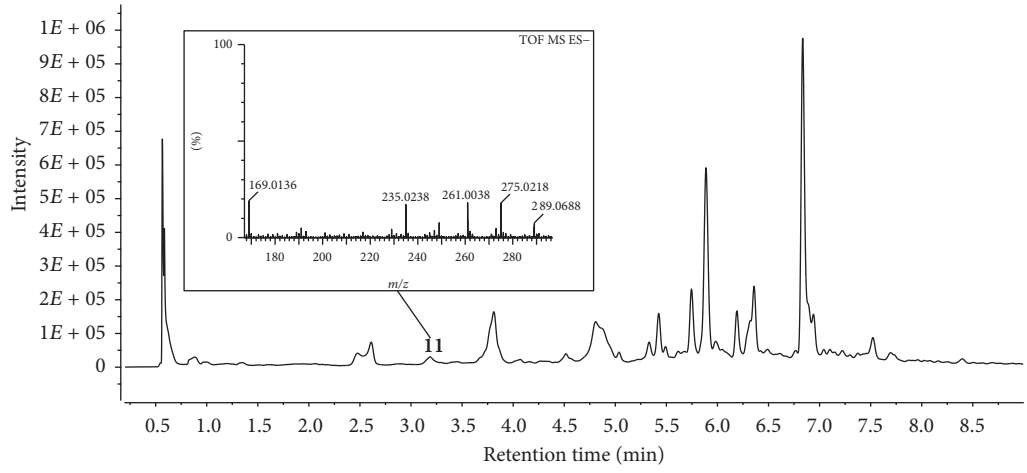
The literature data indicate that the free ellagic acid is only a few percent of the total number of its derivatives [7, 8, 23, 24]. A similar proportion was found in the tested *Rosa rugosa* petals, wherein the proportion of free ellagic acid in a total amount of designated ellagitannins ranged from 2.7% (season 2013) to 3.1% (season 2012). In their studies Nowak et al. [7, 24] did not confirm the presence of free ellagic acid in rose petals. For example, Nowak et al. [7] in the research studies on other anatomical parts of roses (false fruits) designated free ellagic acid in an amount of 10.1 to 63.1 mg/100 g DW. However according to Teleszko et al. [23] in false fruits of selected rose species the level of ellagic acid ranged between 40.31 mg/100 DW and 124.75 mg/100 g DW.

From the data presented by Nowak et al. [7], it is apparent that the *Rosa rugosa* petals also contain other phenolic acids, including caffeic acid, gentisic acid, protocatechuic acid, gallic acid, salicylic acid, sinapinic acid, and p-coumaric acid, which in the studied petals were not found.

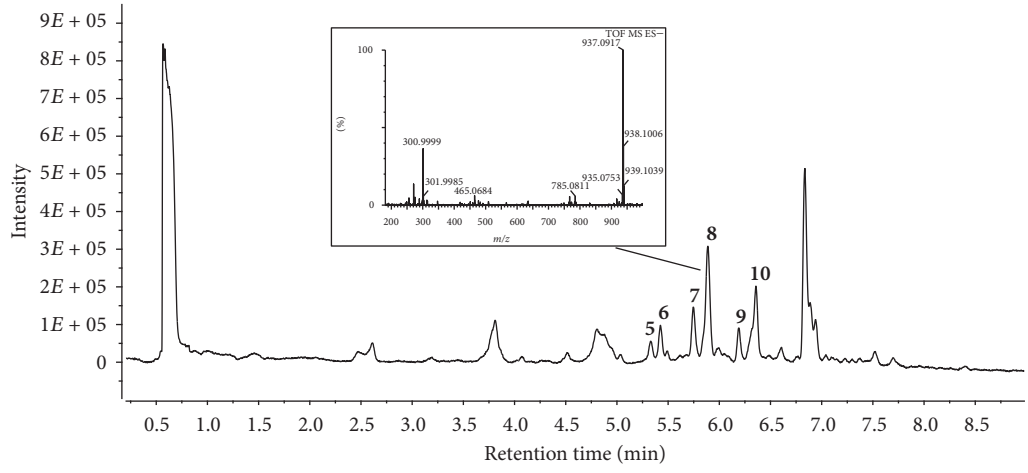
Additionally, as described by Cendrowski et al. [8], in tested rose petals were identified the following flavonols: one myricetin glycoside, four quercetin glycosides (including one unidentified), two kaempferol glycosides, and one isorhamnetin glycoside. The total content of this group of compounds in two successive harvesting seasons was 412.69 (season 2012) and 387.46 mg/100 g FW (season 2013). Among the identified flavonols studied in rose petals predominated quercetin 3,4-di-O-glucoside content of which did not differ from the two harvesting seasons and averaged 161.62 mg/100 g FW, while, for the petals from harvesting season 2013, almost 2 times lower content of quercetin 3-O-glucosyl-xyloside (approx. 59.93 mg/100 g FW) was found compared to the 2012 season (approx. 100.63 mg/100 g DW). Nowak et al. [7] identified in



(a)



(b)



(c)

Figure 1: Sample chromatograms of UPLC-ESI-MS and the mass spectrum of selected polyphenol compounds of *Rosa rugosa* petals: flavonols (a), flavan-3-ols (b), and ellagitannins (c) (numbers in the chromatogram correspond to the compounds listed in Table 1).

rose petals nine derivatives of flavonoids, but only quercetin 3-O-rhamnoside appeared both in the examined petals and in petals described by the above-mentioned authors.

From the group of flavonols, Kumar et al. [25] identified by UPLC-ESI-MS/MS in methanol extracts of *Rosa damascena*, *Rosa bourboniana*, and *Rosa brunonii* petals several different flavonoids, including quercetin derivatives, kaempferol derivatives, and myricetin. Also they have identified gallic acid, caffeic acid, two galloilo tannins, and tetra-O-galloilo-hexoside and di-O-galloilo-hexoside.

Similarly Velioglu and Mazza [26] confirmed by HPLC with diode detector in the methanol extracts of the *R. damascena* Mill. petals the presence of flavan-3-ols.

Ochir et al. [6] showed a varied profile for designated three kaempferol derivatives and three quercetin derivatives in rose petals of Mei-gui (*Rosa rugosa* Thunb.), *Rosa maikwai* Hara, and *Rosa rugosa* Thunb. varieties grown under different conditions of cultivation in China and Japan. Schmitzer et al. [27] examining the petals of *Rosa x hybrida* L. KORcrisett cultivation variety denoted at every stage of flower development different amounts of flavonols. The buds contained much more quercetin derivatives, catechins, and phenolic acids than flowers in the subsequent stages of development. The most significant differences were observed in the content of gallic acid. The buds contained almost six times higher content than after flowering. Also the content of catechin underwent significant change during flower development. In the bud the catechin content was 442.5 mg/100 g FW whereas in overripe flower it was 204.1 mg/100 g FW.

In this study, in the *Rosa rugosa* petals, neither quercetin and kaempferol derivatives nor the phenolic acids were identified as described by Kumar et al. [25], Velioglu and Mazza [26], and Ochir et al. [6]. In the examined petals only was confirmed the presence of catechin, whose content was lower (181.4 mg/100 g FW) than that denoted by Schmitzer et al. [27] for an overripe flower.

Among ellagitannins were identified sanguine H-2 and four unknown ellagitannins. At this stage of examination, it was not possible to find out more about the unknown ellagitannins on the basis of the mass spectra and on the fragmentation, among others, due to the lack of standards [8]. It has been shown that the examined petals were the most abundant in ellagitannins and their share in the total polyphenol content was, depending on the harvesting season, 68.7% (season 2012) and 73.6% (season 2013). The content of predominant ellagitannin with t_R = 5.89 min varied and amounted to 1071.81 mg/100 g (season 2012) and 1271.81 mg/100 g (2013 season). In the petals of *Rosa rugosa* analyzed by spectrophotometric methods by Nowak et al. [7], the total content of phenolic compounds (flavonoids, phenolic acids, and tannins) was 107.44 mg/g DW including the share of tannins at 42.9% and was very similar to that determined in this work. Other authors testing the rose petals also confirmed the high content of ellagitannins [6, 21]. However, in their study, these authors did not confirm the presence of sanguine H-2. On the other hand, Hashidoko [21] confirmed in the *Rosa rugosa* petals the presence of tellimagrandin I, tellimagrandin II, rugosines A, B, and C, rugosines D, E, F, and G, and rugosine 1,2,3-tri-galloilo-β-D-glucose and 1,2,6-O-trigalloilo-β-D-glucose. Also Ochir et al. [6] determined in the dry *Rosa rugosa* petals from Japan, Korea, and China the hydrolyzing tannins. In 50% water-ethanol extracts of rose petals have been determined quantitatively only five tannins (tellimagrandin I, tellimagrandin II, rugosine A, rugosine D, and casuarictin). The predominant tannin in rose petals *Rosa rugosa* in eight out of the eleven regions studied was tellimagrandin I (10.4–41.6 mg/g FW) and the total content for the five determined tannins ranged from 31.2 to 100.8 mg/g FW, that is, from 2 to 5 times lower than determined in the examined *Rosa rugosa* petals. According to Ochir et al. [6], the qualitative and quantitative profile of hydrolyzing tannins depends on the climate and growing conditions of roses and different flower structure (single flowers and hybrids with double flowers). Also it seems very important while comparing the test results between different authors to specify how the sample was prepared and which type of extraction was used.

The amount of polymerized procyanidins determined by floroglucinolisis method for the examined petals remained at the same level for the petals of the two harvesting seasons and averaged 32.6 mg/100 g FW (Table 1). For comparison, according to Teleszko et al. [23], the content of polymerized procyanidins for false fruits of *Rosa canina*, *Rosa pomifera* Karpatia, *Rosa rugosa*, and *Rosa rugosa* Plowid was at the level of 2122.1 mg/100 g FW to 4471.6 mg/100 g FW and was several times higher than in the studied petals of *Rosa rugosa*. The differences present in the profile of phenolic compounds between the examined *Rosa rugosa* petals and the described by Nowak et al. [7] may also be associated with the method of determination and with using different extraction during sample preparation. This is also confirmed by research of Olech and Nowak [28], which showed the impact of the extractant used on the flavonoid content obtained (from 1.35 to 5.05 mg/g of dry extract) in extracts from *Rosa rugosa* petals. The identification of anthocyanin monomers in the petals of *Rosa rugosa* based on the analysis of spectra and retention times comparative to the retention times of available standards and also to literature data enabled the identification of four anthocyanin monomers (Figure 2). Mass spectrometry results before and after the fragmentation of the anthocyanins tested in *Rosa rugosa* petals are shown in Table 2.

The carried out identification of the anthocyanins' monomers allowed the unambiguous identification of two anthocyanins monomers, namely, cyanidin 3,5-di-O-glucoside and peonidin 3-O-glucoside. On the basis of the obtained retention times and mass spectra for the other two peaks, it can not be excluded that the compounds present in the tested petals were peonidin 3-O-sophoroside with retention time of 4.45 min and peonidin 3,5-di-O-glucoside with retention time of 4.87 min. This understanding of the matter is supported by the results of many other authors, who consider as dominant from the group of anthocyanins of the rose petals the peonidin 3,5-di-O-glucoside. In addition, in order to confirm the point of attachment of sugar to the aglycone molecule, the ratio of absorbance at the wavelength λ = 440 nm to the absorbance at the maximum wavelength in

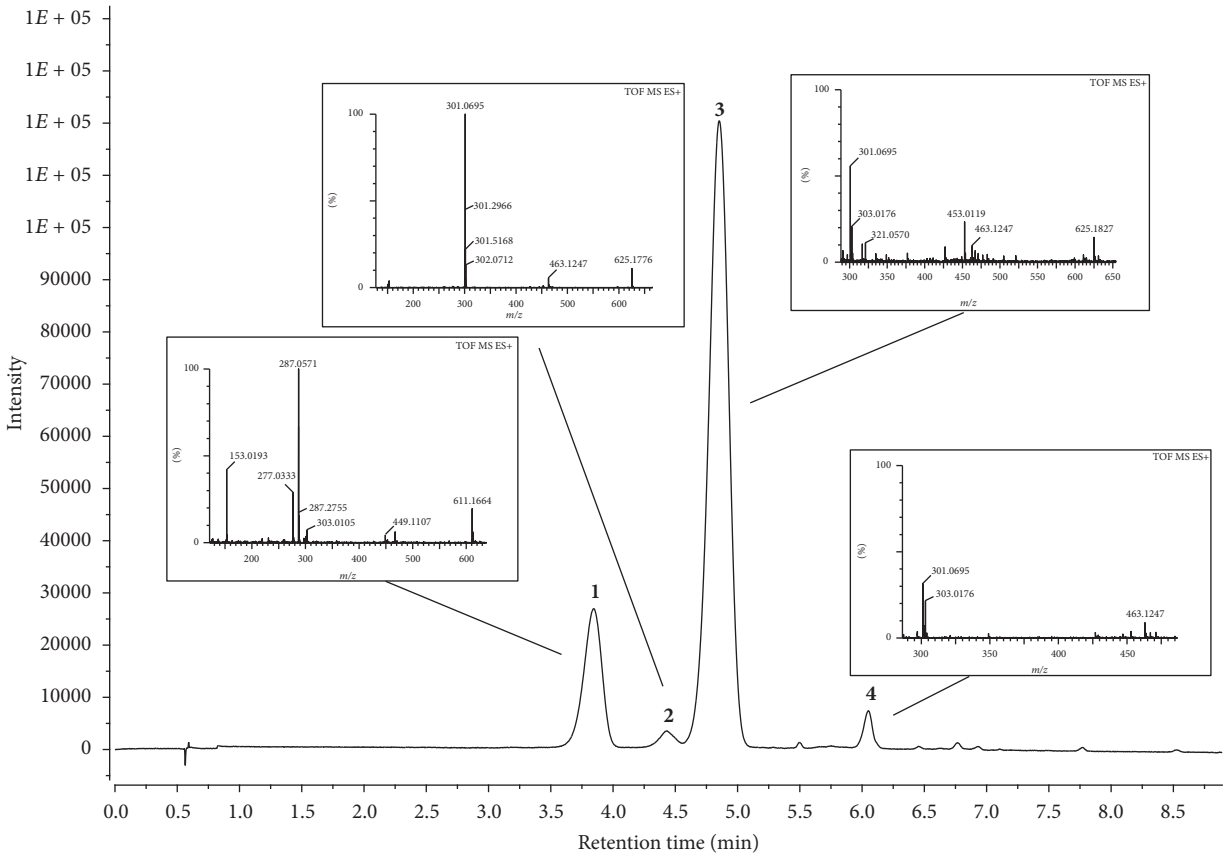


Figure 2: Chromatogram UPLC-ESI-MS and the mass spectra of ions in a positive ionization mode of the anthocyanins in petals of *Rosa rugosa* (the numbers in the chromatogram correspond to the compounds listed in Table 2).

the visible range for the tested anthocyanin compound was determined. It is known that anthocyanins attached in the C-3 position have this ratio two or three times higher than anthocyanins with the glycosylation in the C-5 position or simultaneously in the positions C-3 and C-5 [7]. High values $Abs_{440}/Abs_{\lambda max}$ of 36% and 45%, respectively, for peaks 2 and 4 (Table 2) confirm that the attachment of sugar takes place in the C-3 position. The low value $Abs_{440}/Abs_{\lambda max}$ for peaks 1 and 3 (Table 2) suggests that the attachment of the sugar was at the same time in positions C-3 and C-5. For comparison, in the tests conducted by Mikanagi et al. [29] in the taxa of section *Rosa*, the ratio of $Abs_{440}/Abs_{\lambda max}$ was for the peonidin 3-O-glucoside, peonidin 3,5-di-O-glucoside, and cyanidin 3,5-di-O-glucoside, respectively, 28, 13, and 16%.

The statistical analysis of the test results showed significant differences regarding the content of anthocyanins in rose petals from seasons 2011, 2012, and 2013 (Table 2), which probably can be explained by different growing conditions and the difference in maturity of the collected petals in different seasons. The sum of the contents of four anthocyanins present in the fresh petals of *Rosa rugosa* averaged approx. 172.23 mg/100 g for three harvesting seasons. The percentages of each anthocyanin have been calculated in comparison to its total quantity. In the rose petals dominated peonidin 3,5-di-O-glucoside, which represented depending on the harvesting season 83.2% (2011 season), 82.9% (season 2012), and 82.6% (season 2013), respectively, of the sum of total anthocyanins.

The results of identification of anthocyanins were compared with the literature data [30, 31]. The authors identified anthocyanins using different methods: HPLC-DAD-ESI/MS and NMR, HPLC, HPLC-DAD [7]. The research results of the above-mentioned researchers mostly coincide with the results of the anthocyanins' analysis of the studied *Rosa rugosa* petals. Similarly as in *Rosa rugosa* petals, the predominant anthocyanins in the tested petals by Mikanagi et al. [29] twenty-six taxa of the Rosa section were peonidin 3,5-di-O-glucoside and cyanidin 3,5-di-O-glucoside. The determined content of peonidin 3-O-glucoside was several dozen times higher in comparison to the determined in the tested petals.

The content of Cy 3,5-glu in the garden variety *Rosa rugosa* cv. Roseraie del'Hay was very similar to the content of Cy 3,5-glu determined in the *Rosa rugosa* petals tested in this paper. According to Ge and Ma [31], the predominant anthocyanin in the Chinese *Edible rose* petals was cyanidin 3,5-di-O-glucoside, which constitutes approx. 94.9% of the sum of anthocyanins content. The sum of anthocyanins content in the petals of the Chinese *Edible rose* was determined at the level of 353.56 mg/100 g FW and was similar to the determined by Lee et al. [30] in *Rosa hybrida* petals (375 mg/100 g) and at the same time two times higher in

Table 2: The results of identification and the quantification of anthocyanins monomers in the tested *Rosa rugosa* petals.

Peak number	Compound	t_R UPLC-MS [min]	$[M-H]^+$ (m/z)	$[MS^2]$ (m/z)	Peak area [%]	$Abs_{440}/Abs_{\lambda max}$ [%]	Harvesting season [year]		
							2011	2012	2013
							(mg/100 g fresh weight of rose petals)		
1	*Cyanidin 3,5-di-O-glucoside (Cy 3,5-glu)*	3.85	611.1664	449.1107; 287.0571	13.6	19.0	23.61 ± 0.39^a	25.78 ± 0.45^b	27.44 ± 0.99^b
2	*Peonidin 3-O-sophoroside (Pn 3,5-sop)*	4.45	625.1776	463.1247; 301.0695	1.0	36.0	1.97 ± 0.22^a	1.89 ± 0.05^a	1.62 ± 0.51^a
3	*Peonidin 3,5-di-O-glucoside (Pn 3,5-glu)*	4.87	625.1827	463.1247; 301.0695	83.6	11.0	136.45 ± 2.48^a	142.81 ± 1.17^b	149.09 ± 1.22^c
4	*Peonidin 3-O-glucoside (Pn 3-glu)*	6.08	463.1247	434.2115; 301.0695	1.9	45.0	1.97 ± 0.75^a	1.72 ± 0.26^a	2.35 ± 0.43^a
	SUM						164.00 ± 3.84^a	172.20 ± 1.93^b	180.50 ± 3.15^c

Averages marked with the same letters in the row do not differ significantly statistically at $p < 0.05$.

comparison to the content determined in the tested *Rosa rugosa* petals.

Schmitzer et al. [32] for petals of eight varieties of cultivated roses *Rosa x hybrida* have identified five anthocyanins, including two predominant pelargonidin 3,5-di-O-glucoside and cyanidin 3,5-di-O-glucoside. It should be noted that earlier Schmitzer et al. [27] examining the petals of cultivated variety *Rosa x hybrida* L. KORcrisett have identified the same anthocyanins. These authors found that during flower development the content of the two main anthocyanins increased, and then a slight decrease followed after flowering. This trend was observed in majority of species, but it was not always statistically significant.

A similar trend was observed by Sood et al. [33] in the roses flowers of *R. damascena* and *R. bourboniana* showing an increase in the content of anthocyanins in the first stage of flower development and then a decrease in half and in fully open flowers. The above-mentioned reports about the decrease in content of the anthocyanins in the roses petals during development and then flower aging may indicate the reason of the relatively low anthocyanin content determined in the studied *Rosa rugosa* petals. The petals used in the study were collected at the final stage of full development of flowers, when the amount of anthocyanins in petals decreases.

4. Conclusion

The main polyphenol fraction in *Rosa rugosa* petals was ellagitannins constituting from 69 to 74% of the total petals' polyphenols. Among ellagitannins sanguine H-2 was identified, and other four unidentified compounds were determined in this group. Among the eight identified flavonols, in the highest amount was present the quercetin 3,4-O-diglucoside (161 mg/100 g FW). The petals tended to have high content of (+)-catechin (181 mg/100 g FW) and ellagic acid (49 mg/100 g FW). In the *Rosa rugosa* petals, four anthocyanins have been identified: peonidin 3,5-di-O-glucoside, constituting approx. 85% of all the determined anthocyanin compounds and cyanidin 3,5-di-O-glucoside, peonidin 3-O-sophoroside, and peonidin 3-O-glucoside. Due to the high content of bioactive compounds, especially polyphenolic compounds, including anthocyanins, flavonols, and ellagitannins, *Rosa rugosa* petals can be a valuable raw material for the production of prohealth preparations.

Conflicts of Interest

The authors have declared that no conflicts of interest exist.

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