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COMPARATIVE STUDY OF PHENOLIC ACIDS IN PSEUDOFRUITS OF SOME SPECIES OF ROSES

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Abstract: A free and liberated by acid and alkaline hydrolysis phenolic acids from the hips of fourteen species of wildly growing roses were identifed and determined using SPE RP HPLC method. Eleven major phenolic acids (gallic, protocatechuic, gentisic, *p*-hydroxybenzoic, vanillic, caffeic, syringic, *p*-coumaric, ferulic, *p*-hydroxyphenylacetic, salicylic) were quantitatively investigated. The amount of individual compounds ranged from 0.2 mg/g to 303.2 mg/g of dry material. Conjugated forms of phenolic acids were predominated in the fruits and they were hydrolyzed mainly to gallic acid (93-303 mg/g in dry plant material). The total amount of phenolic acid after hydrolyses was from 186.4 mg/g (*R. inodora*) to 466 mg/g (*R. rugosa*) of dry weight of plant material.

Keywords: Phenolic acids composition, *Rosa* L. hips, HPLC analysis

Pseudofruits of wild roses are valuable dietary medicament and vitamins supplement (mainly vitamin C). They are often applied in colds, flu, at the reduced organism immunity and in the case of vitamin C deficiency (1). Recently, some new medicinal drugs from rose hips appeared such as Litozin (Dansk Droge, Denmark) and Langelands-Hyben (Hyben Vital International ApS, Denmark). They possess well documented anti-inflammatory action (2-5). Another studies have shown that the extracts of rose hips and leaves have antioxidative and anticarcinogenic effects *in vitro*, which are partly due to phenolic compounds in this material (6-8).

Roses are a rich source of phenolic compounds (9-12). The polyphenols of the *Rosa* L. have been studied earlier. In particular, many of kaempferol and quercetin glycosides and a number of anthocyanidin glycosides have been detected (13). The variations in the contents of phenolic acids in leaves of some *Rosa* L. species and their chemotaxonomic significance have been reported previously by Krzaczek and Krzaczek (14). However, the qualitative composition and the amount of phenolic acids in rose hips have not been studied. In this work a combined liquid-liquid extraction (LLE), solid phase extraction (SPE) and RP HPLC method were applied for this purpose.

EXPERIMENTAL

Plant material

The study material – whole pseudofruits – Fructus Rosae cum semine Polish Pharmacopoeia IV (15) was collected from bushes growing in their natural environment in eastern Poland (Lubelszczyzna Region), in September 2004 at the optimum stage of ripeness.

The investigated rose species (16, 17) and places of their collection are given in Table 1.

The plants were authenticated by Prof. Dr. T. Krzaczek and voucher specimens were deposited in the herbarium of the Department of Pharmaceutical Botany, Medical University, in Lublin (Poland).

Chemicals

Standards of phenolic acids were purchased from Sigma (St. Louis. MO, USA) and from Roth (Karlsruhe, Germany). Compounds were dissolved in methanol to obtain a stock solution (0.5 mg×mL^{-1}). All solvents used were of analytical or HPLC grade (Merck, Darmstadt, Germany).

Extraction and isolation of free phenolic acids fractions

The dried and powdered samples (10 g each) of plant material were extracted three times for 30 min in water bath with portions of 80% methanol (v/v, first 100 mL and twice 50 mL) at 90^{o}C. The methanolic extracts were filtered, mixed and the solvents were evaporated in vacuum to the volume of 30 mL. The water residues were portioned into two parts. The first one was diluted with water to 50 mL, adjusted to pH 1,5 (with 1 M HCl) and extracted three times with diethyl ether/ethyl acetate mixture (1:1, v/v; 30 mL each). The combined extracts were evaporated to dryness in vacuum (35^{o}C) and the

Table 1. Plant material and place of their collection.

Taxa no.	Sections, species, varieties	Synonymous names	Place of collection	Data of collection
		Sectio *Cinnamomea DC*.		
1.	*R. rugosa* Thunb.		Lublin	04-09-28
		Sectio Caninae DC. Em. Christ.		
	R. rubiginosa L.= *R. eglanteria* L.			
2.	*R. rubiginosa* L. var. *rubiginosa*	*R. comosa* Ripart in Schultz *R. rubiginosa* var. *typica* Heinr. Braun in Beck	Męćmierz	04-09-19
3.	*R. rubiginosa* L. var. umbellata (Leers) Dumort	*R. umbellata* Leers	Panasówka	04-09-23
4.	*R. villosa* L. subsp. *mollis* R. Keller et Gams	*R. mollis* Sm. var. *ciliato-petala* (Besser) Popek syn. *R. pomifera var. ciliato-petala* (Bess.) Chrshan.	Lublin	04-09-07
5.	*R . tomentosa* Smith	*R. cinerascens* Dum	Józefów	04-09-29
6.	*R. inodora* Fries	*R. inodora* Fries var. *inodora* *R. agrestis* Savi var. *inodora* (Fries) Borbás	Kazimierz Dolny	04-09-19
	R. canina L.:			
7.	*R. canina* L. var. *canina*	*R. canina* L. var. *typical* Braun	Kazimierz Dolny	04-09-19
8.	*R. canina* L. var *corymbifera* (Borkh) Boulenger	*R. dumetorum* Thuill. *R. corymbifera* Borkh.	Męćmierz	04-09-23
9.	*R. canina* L. var *dumalis* Baker	*R. canina* var. *transistoria* R. Keller	Józefów	04-09-29
	R. dumalis Bechst.:			
10.	*R. vosagiaca* Desportes	*R. dumalis* var. *afzeliana* (Fr.) Boulenger	Bochotnica	04-09-19
11.	*R. caryophyllacea* Besser pro parte	*R. dumalis* var. *besseriana* Popek	Męćmierz	04-09-23
12.	*R. subcanina* (Christ) Keller		Lublin	04-09-14
13.	*R. coriifolia* Fries	*R. dumalis* var. *coriifolia* (Fr.) Boulenger *R. caesia* Smith	Bochotnica	04-09-19
14.	*R. subcollina* (Hayek) R. Keller	*R. dumalis* var. *coriifolia* for. *tristis* (A. Kerner) Popek	Kazimierz Dolny	04-09-19

residues were dissolved in 10 mL of 30% methanol in 0.1% HCl. The solutions were passed through a C_{18} solid-phase extraction cartridges (Octadecyl, 500 mg, J.T. Baker, Phillipsburg, NJ, USA) which have been preconditioned with methanol and 30% methanol in 0,1% HCl, respectively. Vacuum manifold processor (system Baker SPE-12G, J.T. Baker, Phillipsburg. NJ, USA) was used. The collected eluates with free phenolic acids (fraction A) were analyzed directly by RP-HPLC.

Isolation of bound phenolic acids fractions

Bound phenolic acids were liberated from water extracts (15 mL of second parts) using first alkaline and then acid hydrolysis. The method of Schmidtlein and Herrmann (18) and Świątek (19) with some modifications was applied. Samples were hydrolyzed with 1% $Ba(OH)_2$ with $NaBH_4$ in the amount of 0.2 g per 30 mL (pH 12-13, 100°C, 15 min), then acidified to pH 2-3 with conc. H_2SO_4 and heated (100°C) during 40 min. The mixtures were cooled, filtered, diluted with water to 50 mL and extracted with diethyl ether/ethyl acetate (1:1) as described above. Then fractions B of phenolic acids released after alkaline and acidic hydrolyses were isolated in the analogous way to fractions A using solid phase extraction and were used for HPLC analyses.

Validation of LLE-SPE extraction and isolation

0.1 mg of each standard (200 mL of each stock solution) were added to 10 mL of *R. rugosa* crude extract, extracted using described above methods and then analyzed. This procedure was repeated in triplicate.

Table 2. Retention times of analyzed phenolic acids (t_R [min] ± SD, n=3).

No.	Compound	Commonly used name	t_R [min]	
			A	B
1	3,4,5-Trihydroxybenzoic acid	Gallic acid	4.0 ±0.09	3.6 ±0.06
2	3,4-Dihydroxybenzoic acid	Protocatechuic acid	6.4 ±0.12	4.6 ±0.08
3	2,5-Dihydroxybenzoic acid	Gentisic acid	10.8 ±0.14	8.9 ±0.09
4	4-Hydroxybenzoic acid	*p*-Hydroxybenzoic acid	11,2 ±0.09	7.8 ±0.1
5	3-Methoxy-4-hydroxybenzoic acid	Vanillic acid	13.8 ±0.08	9.3 ±0.09
6	3,4-Dihydroxycinnamic acid	Caffeic acid	14.8 ±0.16	10.4 ±0.14
7	3,4,5-Trimethoxybenzoic acid	Syringic acid	15.4 ±0.18	6.9 ±0.08
8	4-Hydroxycinnamic acid	*p*-Coumaric acid	27.5 ±0.22	16.5 ±0.16
9	3-Methoxy-4-hydroxycinnamic acid *trans*	Ferulic trans acid	32.6 ±0.3	17.5 ±0.21
9’	3-Methoxy-4-hydroxycinnamic acid *cis*	Ferulic cis acid	36.9 ±0.32	25.4 ±0.12
10	4-Hydroxyphenylacetic acid	*p*-Hydroxyphenylacetic acid	34.0 ±0.29	30.7 ±0.20
11	2-Hydroxybenzoic acid	Salicylic acid	45.7 ±0.43	34.7 ±0.33

t_R – retention time; A, B – HPLC eluents
HPLC analysis was carried out on Zorbax SBC 18 (200 x 4,6mm,I.D., 5 mm) column under isocratic conditions; mobile phases A: methanol-water-acetic acid (23:77:1, v/v) and B: acetonitrile-water-trifluoroacetic acid (17:82,5:0,5, v/v); flow-rate 1 mL/min; detection, 320, 254 nm.

Quantification of phenolic acids using HPLC analysis

Equipment

All separations were performed with an HPLC system (Knauer, Berlin, Germany) consisting of a HPLC Pump K-1001, Solvent Organizer K-1500, UV-VIS Detector Fast Scanning Spectrophotometer K-2600, Degasser K-5004, Column Thermostat and 20 mL sample injector (Rheodyne, Cotati, CA, USA). Chromatographic data were collected and recorded using a computer program Eurochrom 2000.

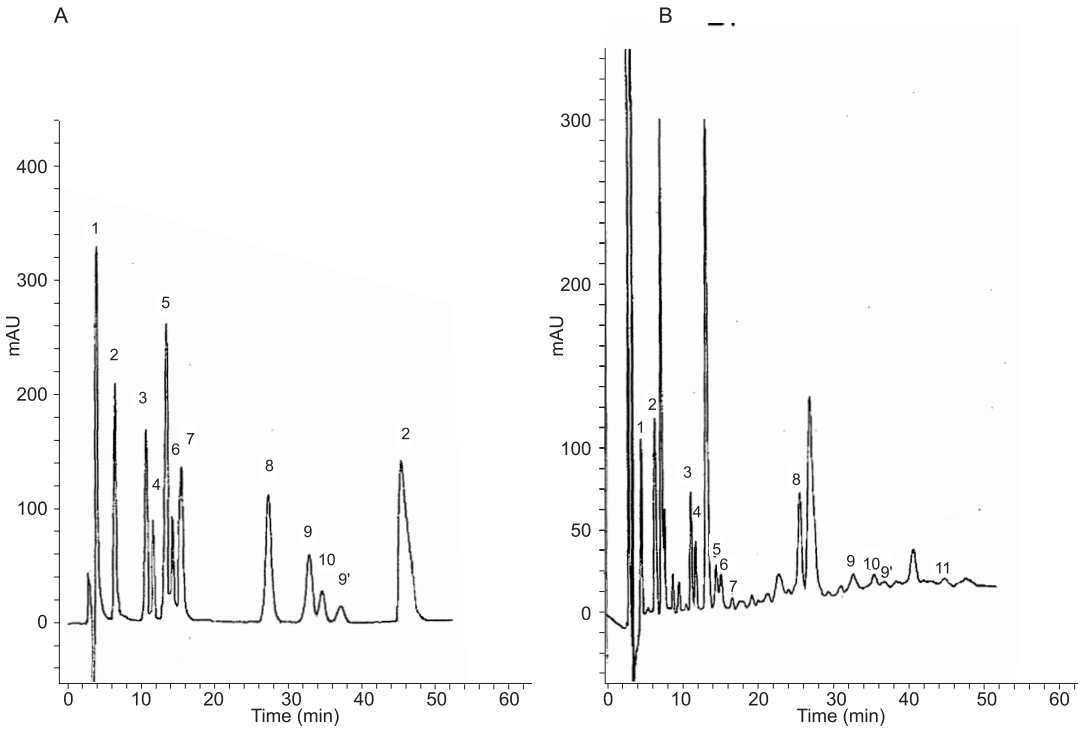

Figure 1. HPLC chromatograms of the standards mixture of phenolic acids (A) and the fraction of free phenolic acids from pseudofruits of *R. rugosa* (B). Conditions of HPLC analysis and number of compounds as in Table 2.

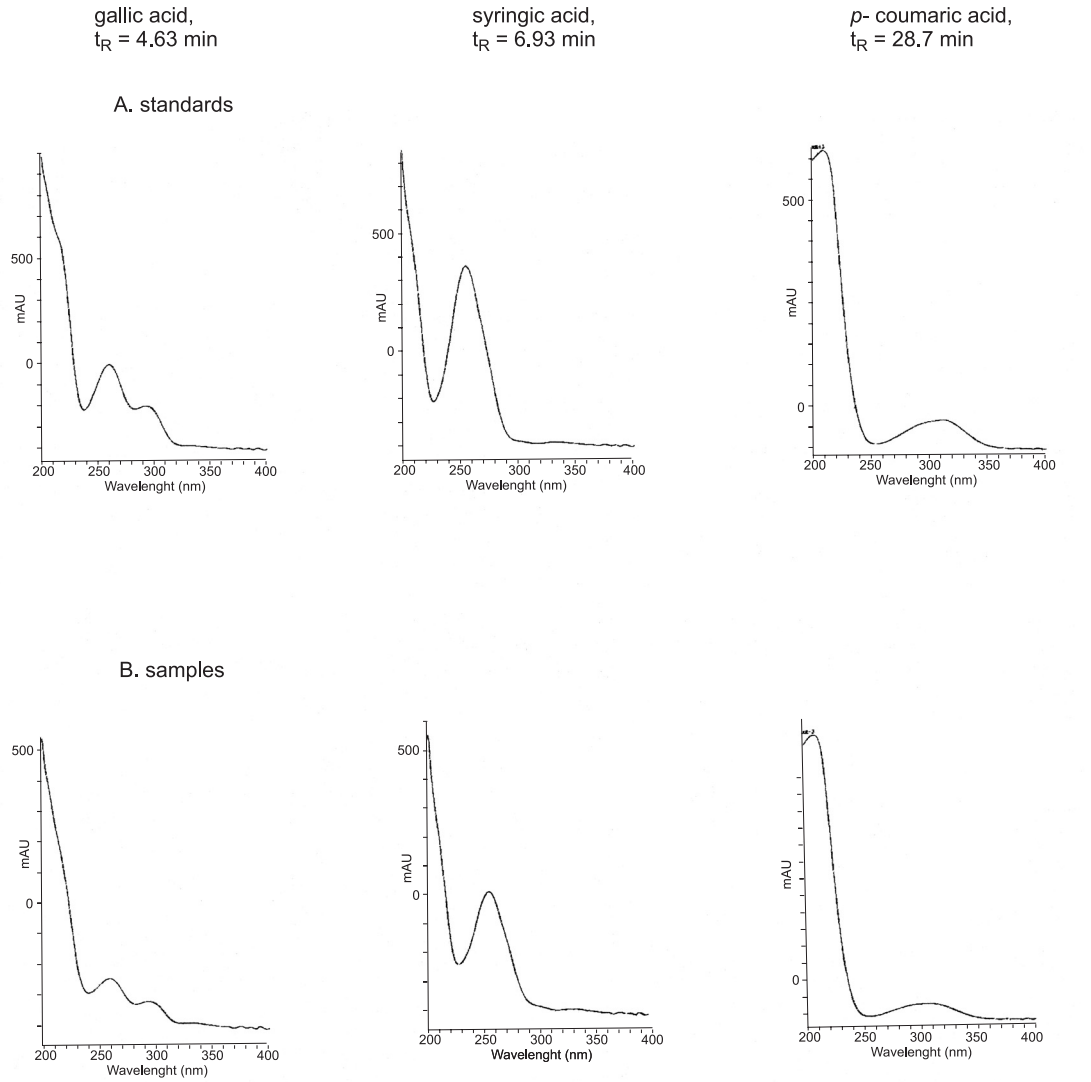

Figure 2. Comparison of some UV spectra of standards (A) and HPLC peaks of phenolic acids in sample of free phenolic acids from pseudofruits of *R. rugosa* (B).

The analytical column was Hypersil (200 x 4,6 mm I.D., 5 µm; Agilent Technologies, Germany) with guard column (5 µm, 125 mm x 4.6 mm I.D., Agilent Technologies, Germany).

HPLC analysis

Phenolic acids were separated by isocratic elution with two solvent systems: A, methanol – water – acetic acid (23:76:1, v/v/v) and B, acetonitrile – water – trifluoroacetic acid (17:82.9:0.1, v/v/v), at 20°C and a flow-rate of 1.0 mL/min. The compounds were detected at λ = 254 nm and λ = 320 nm.
After preparation, the mobile phases were filtered through 0.45 mm filter (J.T. Baker, Phillipsburg, NY, USA). Quantitative analysis of phenolic acids was performed using system elution A.

Stock solutions of phenolic acids were prepared by dissolving 5 mg of each compound in 10 mL of methanol. Standard solutions of phenolic acids were prepared in methanol over the concentration range 0.0125-0.1 mg/mL. The volumes injected amounted 10 mL.

Calibration curves were obtained by plotting the peak area (y) against the concentration of standard solutions (x) and showed linear relationships. All phenolic acids were quantified using the external standard method. The equations for the experimental calibration curves and the detection limits for all compounds were determined (Table 2, Table 3).

The identification of phenolic acids was accomplished by comparison of their retention times

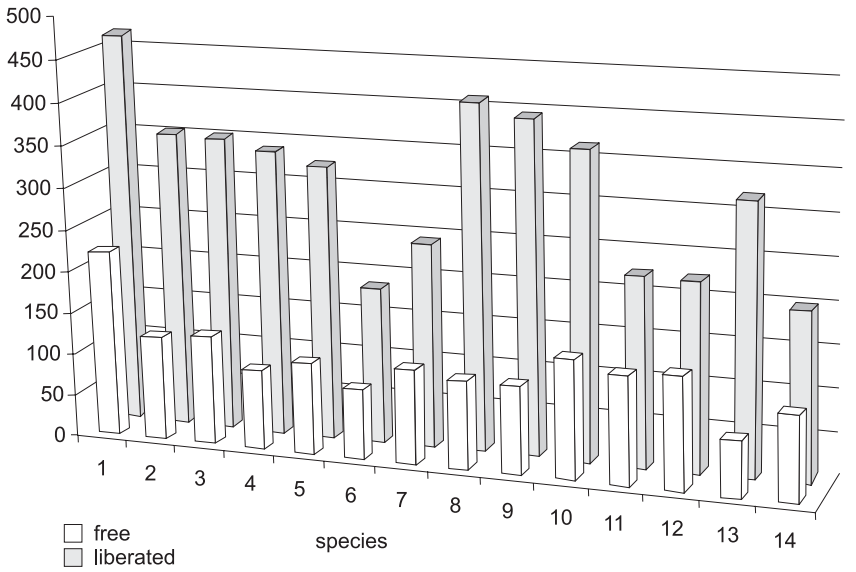

Figure 3. The comparison of the content of free and bound phenolic acids in pseudofruits of investigated rose species [in µg/g of dry weight].

and UV spectra with those of appropriate standard compounds (Figure 1, Figure 2).

The results of this quantitative analysis are shown in Tables 4-6 and Figure 3.

Statistical analysis

Statistical analysis was done by one-way analysis of variance (Statistica 6.0). Values of $p < 0.05$ were considered statistically significant.

Thin layer chromatography

Fractions A and B remaining after RP HPLC analysis were evaporated under vacuum with the rotary evaporator (at 35°C) to dryness and dissolved in 1 mL of methanol before the TLC and 2D TLC analysis. The horizontal sandwich DS-chambers (Chromdes, Lublin, Poland) were used (20).

The 2D-TLC-analysis was performed on cellulose plates (DC-Fertigplatten, Merck, Cellulose 100 x 100 x 0.1 mm) using method described before (21, 22).

RESULTS AND DISCUSSION

The present investigation is the first attempt at revealing the qualitative and quantitative composition of phenolic acids in hips of some *Rosa* L.

Table 3. Linear equations of calibration curves and determination limits of analyzed phenolic acids.

No.	Phenolic acid	Linear equation of curve	R	DL (mg × L^{-1})
1	Gallic acid	C = 0.0043 A – 0.00759	0.9915	0.05
2	Protocatechuic acid	C = 0.00183 A + 0.00167	0.9999	0.15
3	Gentisic acid	C = 0.0338 A – 0.0223	0.9955	0.25
4	*p*-Hydroxybenzoic acid	C = 0.000733 A – 0.0027	0.9983	0.05
5	Vanillic acid	C = 0.00016 A + 0.00157	0.9992	0.05
6	Caffeic acid	C= 0.000432A+0.000504	0.9998	0.15
7	Syringic acid	C = 0.00049 A – 0.00175	0.9929	0.05
8	*p*-Coumaric acid	C = 0.0026 A – 0.00147	1.0	0.15
9	Ferulic *trans* acid	C = 0.0409 A +1.2	0.9875	0.25
9’	Ferulic *cis* acid	C = 0.0207 A – 0.00883	0.9971	0.25
10	*p*-Hydroxyphenylacetic acid	C = 0.00966 A – 0.0121	0.9999	0.50
11	Salicylic acid	C= 0.000768 A – 0.00465	0.9899	0.50

C- concentration (mg × l^{-1}); A- pick area

HPLC mobile phase A, another conditions of analysis see Table 2.

Table 4. The content of free phenolic acids in the investigated rose pseudofruits [in mg/g of dry weight].

No.	Phenolic acid	*R. rugosa*	*R. rubiginosa* var. *rubiginosa*	*R. rubiginosa* var. *umbellata*	*R. villosa*	*R. tomentosa*	*R. inodora*	*R. canina* var.	*R. canina var. corymbifera*	*R. canina* var. *dumalis*	*R. vosagiaca*	*R. caryophyllacea*	*R. subcanina*	*R. corifolia*	*R. subcollina*
1	Gallic	94.3 ±9.3	57.9 ±8.9	94.4 ±11.7	41.0 ±5.8	64.7 ±4.7	55.6 ±7.6	43.5 ±4.2	46.7 ±5.7	57.0 ±7.2	58.1 ±7.3	62.8 ±10.1	59.5 ±9.7	27.2 ±3.6	60.1 ±7.6
2	Protocatechuic	20.7 ±1.7	17.0 ±1.3	5.9 ±1.1	13.9 ±0.2	10.9 ±1.2	5.5 ±0.7	14.5 ±0.8	13.5 ±1.1	12.2 ±2.1	15.2 ±2.4	6.0 ±0.81	19.9 ±1,4	12.2 ±1.4	7.8 ±0.8
3	Gentisic	64.2 ±13.7	26.6 ±1.7	10.8 ±0.3	17.3 ±1.4	16.5 ±0.9	8.3 ±0.6	27.6 ±4.2	22.3 ±0.7	16.8 ±2.3	38.9 ±7.6	29.4 ±1.1	31.2 ±2.9	10.3 ±0.95	17.3 ±1.2
4	*p*-Hydroxy-benzoic	1.3 ±0.1	0.6 ±0.1	0.2 ±0.1	0.4 ±0.1	0.4 ±0.1	0.3 ±0.1	0.4 ±0.1	0.4 ±0.1	0.2 ±0.1	0.5 ±0.1	0.5 ±0.08	0.5 ±0.1	0.3 ±0.1	0.2 ±0.1
5	Vanillic	0.9 ±0.08	0.8 ±0.1	0.7 ±0.1	0.5 ±0.1	0.5 ±0.1	0.5 ±0.1	0.6 ±0.1	0.6 ±0.1	0.6 ±0.1	0.7 ±0.5	0.5 ±0.1	0.5 0.1	0.7 ±0.1	0.8 ±0.1
6	Caffeic	8.3 ±0.95	4.8 ±0.6	1.9 ±0.5	3.6 ±0.2	3.3 ±0.2	2.2 ±0.2	5.9 ±0.6	3.5 ±0.6	3.3 ±0.2	6.9 ±0.7	6.4 ±0.9	6.4 ±0.5	2.1 ±0.1	2.3 ±0.3
7	Syringic	5.8 ±0.3	2.2 ±0.1	4.8 ±0.2	6.7 ±0.4	4.5 ±0.2	2.4 ±0.2	3.5 ±0.7	6.9 ±0.5	2.9 ±0.4	3.5 ±0.4	7.6 ±0.9	3.0 ±0.3	5.7 ±0.7	3.6 ±0.6
8	*p*-Coumaric	6.4 ±1.4	2.3 ±0.4	0.9 ±0.1	1.7 ±0.1	2.2 ±0.1	1.5 ±0.4	3.7 ±0.3	1.9 ±0.2	2.2 ±0.1	2.8 ±0.1	4.2 0.4	2.8 ±0.25	1.4 ±0.2	1.5 ±0.5
9	Ferulic *trans*	0.5 ±0.1	2.0 ±0.3	0.6 ±0.1	1.4 ±0.1	0.6 ±0.1	0.5 ±0.1	3.3 ±0.26	1.1 ±0.1	2.1 ±0.2	1.8 ±0.1	1.9 ±0.1	1.8 ±0.2	0.6 ±0.1	0.7 ±0.1
9’	Ferulic *cis*	0.7 ±0.15	0.5 ±0.1	1.3 ±0.2	tr	0.7 ±0.1	1.6 ±0.3	3.0 ±0.2	0.7 ±0.1	2.6 ±0.1	4.6 ±0.6	tr	tr	0.6 ±0.1	0.5 ±0.1
10	*p*-Hydroxy-phenylacetic	16.9 ±2.6	6.5 ±0.9	6.3 ±0.4	6.6 ±0.8	3.6 ±0.2	3.0 ±0.3	5.6 ±0.3	5.4 ±0.7	4.2 ±0.5	7.8 ±0.8	7.1 ±0.7	6.7 ±0.8	3.5 ±0.6	4.0 ±0.3
11	Salicylic	1.5 ±0.2	2.5 ±0.2	2.2 ±0.4	1.6 ±0.1	1.5 ±0.3	2.3 ±0.2	1.3 ±0.1	2.3 ±0.2	2.1 ±0.3	0.8 ±0.1	1.2 ±0.25	0.7 ±0.1	2.9 ±0.6	3.7 ±0.2
	TOTAL	221.5 ±30.6	123.7 ±14.7	130.0 ±15.2	94.7 ±9.3	109.3 ±8.2	83.7 ±10.8	112.7 ±11.9	105.1 ±10.1	106.2 ±13.6	141.5 20.7	127.6 ±15.4	133.1 ±16.4	67.2 ±8.55	102.4 ±11.9

Concentrations below 0.01 are marked as „–” and those between 0.01 and 0.05 as „tr” (traces).
Values are mean ± SD of six replicates (two extractions and three injections of each one).

species, used as the main raw material in the Polish pharmaceutical and food industry.

Recently, phenolic acids have drawn more attention because of their biological properties (23). Different chromatographic techniques have been developed for the analysis of these compounds (24, 25). HPLC is preferred to the others, because it offers high sensitivity, great efficiency and enables the analysis of these phenols without the derivatization necessary for GC. However, achieving a satisfactory separation of these compounds in crude plants extracts using HPLC based methods is considered a major analytical problem, due to possible interference of other constituents with similar polarity and UV absorption.

The aim of this work was the analysis of the sets of free and bound phenolic acids in rose hips and to find the best analysis method for this purpose.

For isolation and purification of fractions of free phenolic acids and acids liberated after alkaline and acidic hydrolysis the combined liquid-liquid extraction (LLE with diethyl ether/ethyl acetate mixture 1:1, v/v) and solid phase extraction (SPE) method was elaborated.

The study of the repeatability of the method and its reproducibility was performed. The results for repeatability showed a relative standard deviation (n=3) ranging from 2.5 to 5%. The recovery ranged from 94% to 99.8%.

As a result of the performed studies, good resolution between the different peaks detected in rose fractions by HPLC for two used mobile phases was obtained (Table 2 and Figure 1). Good response lin-

earity was achieved for all of the compounds studied and the detection limits of the method were established as 0.05-0.5 mg/L (Table 3).

Rich qualitative composition of phenolic acids was observed in the fourteen examined species. 11 phenolic acids were identified in the materials. All these compounds detected in rose hips have been found early in the leaves of roses (14). The composition and contents of phenolic acids in rose pseudofruits were stated in the investigated species for the first time.

The phenolic acids appeared in rose hips both in the free and in the bound states (the occurrence in A and B fractions). The content of the compounds was determined by RP-HPLC method (Table 4 and 5). The amount of particular compounds ranged from 0,2 mg/g to 303,2 mg/g of dry material. Conjugated forms of phenolic acids were predominating in the fruits and they were hydrolyzed mainly to gallic acid (93-303 mg/g in dry plant material). A large quantity of gentisic acid (to 152,8 mg/g in dry plant material) was present in the investigated fractions and it seemed to be typical of this plant material.

The acquired results of TLC analysis of every fraction confirmed the HPLC data. However, a big amount of ellagic acid was detected. The methods used for determination of phenolic acids were unsuccessful for ellagic acid, because it could not be well released during applied hydrolysis and not well eluted under adopted HPLC conditions. For these purpose, elaboration of a new method is needed, which would be the aim of the next paper.

Table 5. The content of liberated phenolic acids after hydrolyses in the investigated rose pseudofruits [in mg/g of dry weight].

No.	Phenolic acid	*R. rugosa*	*R. rubiginosa* var. *rubiginosa*	*R. rubiginosa* var. *umbellata*	*R. villosa*	*R. tomentosa*	*R. inodora*	*R. canina* var.	*R. canina var. corymbifera*	*R. canina* var. *dumalis*	*R. vosagiaca*	*R. caryophyllacea*	*R. subcanina*	*R. corrifolia*	*R. subcollina*
1	Gallic	256.9 ±17.4	256.4 ±26.5	268.5 ±33.4	146.6 ±21.3	224.8 ±14.8	124.2 ±23.1	303.2 ±12.9	157,6 ±14.1	241.1 ±26.3	141.6 ±7.7	133.9 ±29.3	93.0 ±9.7	240.6 ±26.3	124.9 ±5.9
2	Protocatechuic	23.7 ±4.2	17.2 ±2.1	9.1 ±0.9	14.1 ±1.2	13.3 ±4.2	7.7 ±1.7	14.8 ±1.8	15.0 ±0.7	20.7 ±1.7	15.7 ±2.4	8.6 ±1.1	20.4 ±4.1	14.7 ±1.7	7.6 ±0.9
3	Gentisic	101.8 ±4.9	32.8 ±1.8	17.9 ±2.1	137.7 ±15.4	54.4 ±3.8	23.9 ±2.1	39.2 ±1.7	152.8 ±9.3	48.7 ±0.8	40.3 ±5.3	31.9 ±0.8	69.2 ±6.2	26.4 ±2.4	30.2 ±1.7
4	*p*-Hydroxy benzoic	1.5 ±0.1	1.2 ±0.5	0.5 ±0.1	1.1 ±0.2	0.2 ±0.1	0.2 ±0.1	0.7 ±0.1	1.0 ±0.1	1.2 ±0.2	0.4 ±0.1	1.0 ±0.1	1.0 ±0.2	0.4 ±0.1	0.5 ±0.1
5	Vanillic	1.0 ±0.1	0.5 ±0.1	1.0 ±0.1	0.6 ±0.1	0.5 ±0.1	0.7 ±0.1	1.0 ±0.1	2.2 ±0.2	0.6 ±0.1	0.7 ±0.2	0.4 ±0.1	0.5 ±0.1	1.2 ±0.2	1.3 ±0.3
6	Caffeic	10.5 ±0.6	6.9 ±0.2	4.1 ±0.2	6.1 ±0.6	3.3 ±0.3	2.6 ±0.2	4.4 ±0.3	14.1 ±0.4	6.6 ±0.2	5.4 ±0.7	8.0 ±0.2	5.4 ±0.8	4.0 ±0.3	3.9 ±0.4
7	Syringic	8.0 ±0.5	8.2 ±1.4	4.8 ±0.3	12.1 ±0.9	4.9 ±0.3	2.0 ±0.4	14.0 ±0.2	24.5 ±1.4	15.4 ±0.3	4.2 ±0.9	9.0 ±0.2	4.9 ±0.7	6.5 ±0.3	5.3 ±0.6
8	*p*-Coumaric	12.7 ±0.9	3.3 ±0.2	1.1 ±0.1	3.2 ±0.2	3.0 ±0.2	1.8 ±0.1	13.0 ±0.6	3.9 ±0.9	5.6 ±0.2	4.9 ±0.5	16.0 ±0.5	3.1 ±0.5	1.9 ±0.6	1.8 ±0.2
9	Ferulic *trans*	0.6 ±0.1	2.6 ±0.7	1.3 ±0.1	0.7 ±0.2	0.4 ±0.1	1.2 ±0.2	2.6 ±0.1	1.3 ±0.1	4.3 ±0.3	5.4 ±0.2	3.6 ±0.6	4.6 ±0.6	1.3 ±0.3	1.2 ±0.2
9’	Ferulic *cis*	0.5 ±0.1	tr	2.6 ±0.4	0.7 ±0.1	0.7 ±0.1	tr	3.3 ±0.2	0.7 ±0.1	0.5 ±0.1	tr	tr	0.8 ±0.2	tr	0.5 ±0.1
10	*p*-Hydroxy-phenylacetic	46.7 ±1.8	17.6 ±1.3	22.3 ±1.5	22.3 ±0.2	13.9 ±1.7	16.2 ±1.55	10.0 ±2.2	19.1 ±0.9	18.5 ±1.1	20.4 ±2.8	9.6 ±0.7	17.1 ±1.6	16.4 ±1.1	18.3 ±1.4
11	Salicylic	2.1 ±0.1	5.9 ±0.8	4.6 ±0.4	3.6 ±0.5	5.3 ±0.7	5.9 ±0.2	3.6 ±0.2	3.1 ±0.2	3.1 ±0.2	3.5 ±0.7	2.3 ±0.3	2.8 ±0.1	5.6 ±0.8	5.0 ±0.7
	TOTAL	466.0 ±30.8	352.7 ±36	348.9 ±40.9	337.8 ±40.5	324.4 ±26.4	186.4 ±29.8	242.5 ±21.5	409.8 ±20.4	395.3 ±28.4	366.2 ±31.5	224.3 ±33.9	223.0 ±24.8	318.9 ±35	200.4 ±12.5

Concentrations below 0.01 are marked as „–” and those between 0.01 and 0.05 as „tr” (traces).
Values are mean ± SD of six replicates (two extractions and three injections of each one).

In this study, it was observed that the qualitative composition of phenolic acids in the examined pseudofruits from some rose species is generally similar, but there exist some differences in quantitive proportions and in the mode of compound occurrence. For example, gentisic acid was determined in a great quantity in *R. rugosa* in the pseudofruits of A fraction (in the free state) and in the B fraction (in the glycoside and ester-bound form) while in *R. inodora* it was found to be present in a small amount. However, it seems that the set of phenolic acids in rose pseudofruits have very little chemotaxonomic value in this genus.

Phenolic acids are widespread in medicinal plants and plant foods. They contain important biological and pharmacological properties, some of which were shown to be effective as antioxidants and in preventing cancer (26, 27).

High level of gallic acid and gentisic acid in investigated roses seems to be especially interesting. Gallic acid has anti-fungal and anti-viral properties, acts as an antioxidant and helps to protect our cells against oxidative damage. It was found to show cytotoxicity against cancer cells, without harming healthy cells. Gentisic acid has antioxidant and immunostimulating activity (28, 29).

In the view of the above data, considering large contents of phenolic acids in the examined plant materials, one should assume that these compounds are, to a great extent, responsible for the antioxidant, anti-inflammatory, antibacterial and immunostimulating effect of plant material examined in this study.

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