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PHENOLIC ACIDS FROM *SYMPHORICARPOS ALBUS* (L.) BLAKE (*CAPRIFOLIACEAE*)

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Abstract: The leaves, flowers and fruits of *Symphoricarpos albus* (L.) Blake (*Caprifoliaceae*) were analysed for the presence of phenolic acids. Eleven free and liberated by hydrolysis phenolic acids were identified by TLC, HPLC and spectral (UV) methods.

Moreover, the HPLC method was applied for the quantitative determination of phenolic acids in the analysed fractions.

Keywords: *Symphoricarpos albus* (L.) Blake, *Caprifoliaceae*, phenolic acids, isolation, determination, TLC, HPLC, UV–spectra

Symphoricarpos albus (L.) S.T.Blake [= *Symphoricarpus albus* (L.) Blake, *Symphoricarpus racemosus* Michx., *Symphoricarpos rivularis* Suksd., *Lonicera racemosa* Pers., *Symphoria racemosa* (Michx.) Pursh.] *Caprifoliaceae* is a shrub of 1–3 m height native of North America, cultivated in parks and gardens as an ornamental throughout Europe, and is found also in natural habitats. Previous chemical studies showed the presence of the flavonoids: quercetin, apigenin, luteolin and their glycosides, coumarins: aesculin and fraxetin, tannins, iridoids, saponins, triterpens, sugars, pectins also isoquinoline alkaloids and choline. Chlorogenic, quinic, aminobutyric as well as malic, tartaric and citric acids were found in the fruits (1–6).

Snowberry is used in traditional medicine as emeticum, laxans, antiphlogisticum and in homeopathy for the treatment of alimentary tract diseases, colds, and as an immunostimulating agent. The berries had been reported to be poisonous but later investigations did not confirm their toxicity to animals (7–10).

In the present work the qualitative and quantitative determination of phenolic acids in the fractions of *Symphoricarpos albus* is described.

EXPERIMENTAL

Plant material

The samples of leaves or flowers (collected in June 1995) and fruits (collected in September 1996) of *S. albus* (L.) Blake were obtained from the Botanic Garden of A. Mickiewicz University in Poznań. Their taxonomical identification was carried out by the Department of Plant Taxonomy, A. Mickiewicz University, Poznań and the voucher specimens were deposited in the Department of Pharmacognosy, K. Marcinkowski University of Medical Sciences, Poznań.

Extraction and isolation

The air–dried and powdered leaves (350 g), flowers (45 g) were extracted with 85% methanol, whereas fresh fruits (1500 g) with pure methanol by heating under reflux in a water bath. The extracts were concentrated under reduced pressure. The residues were diluted with hot water, left at room temperature for 24 h, filtered, extracted with petroleum ether and divided into fractions according to a general procedure elaborated for phenolic acids (11). The aqueous solutions were extracted with diethyl ether yielding fractions of free phenolic acids L–1, K–1, F–1.

The aqueous phases were divided in two parts. The first part was subjected to acid hydrolysis (HCl). Fractions containing phenolic acids liberated in this method were marked as L–2, K–2, F–2. The second one was exposed to alkaline hydrolysis [$Ba(OH)_2$, $NaBH_4$] and the fractions of liberated phenolic acids were marked as L–3, K–3, F–3.

Qualitative analysis

TLC on cellulose (Cellulose DC–Alufolien 20x20 cm, Merck) was carried out using the following solvent systems: S_1 – toluene:acetic acid:water (6:7:3, upper phase), S_2 – acetic acid:water (15:85), whereas for silica gel plates (Kieselgel $GF_{254}60$, DC–Fertigplatten 20x20 cm,

Table 1. The results of TLC separation of free phenolic acids isolated from various parts of *S. albus*.

Compound	Phenolic acid	R_f values in solvent system						Spot colouration			
		S_1		S_2		S_3		I	II	III	IV
		x	y	x	y	x	y				
A	gallic	0.00	0.00	0.51	0.52	0.08	0.09	v	dm	pdm	pbr
B	α–resorcylic	0.01	0.02	0.63	0.64	0.20	0.20	ybr	pv	p	gbr
C	protocatechuic	0.04	0.05	0.64	0.66	0.23	0.24	dv	dm	b	rbr
D	chlorogenic	0.01	0.02	0.72	0.73	0.05	0.06	lb	b	yg	yg
E	*p*–hydroxybenzoic	0.24	0.25	0.70	0.71	0.48	0.49	dm	pdm	b	y
F	vanillic	0.67	0.69	0.70	0.71	0.66	0.67	pdm	pdm	u	o
G	caffeic	0.07	0.08	0.46	0.48	0.30	0.31	lb	lb	lb	gbr
				0.64*	0.64*	0.62	0.64				
H	izovanillic	0.61	0.61	0.64	0.65	0.59	0.61	pv	pv	lb	po
I	*p*–coumaric	0.26	0.28	0.54	0.55			dm	pdm	v	c
				0.81*	0.81*	0.63	0.64				
K	salicylic	0.94	0.96	0.70	0.71	0.82	0.83	b	b	lb	lgbr
L	ferulic	0.73	0.75	0.42	0.43			b	b	db	v
				0.78*	0.78*						

Explanations:
x – sample, y – standards
* – R_f values of *cis* isomers
Spot colouration:
b – blue, br – brown, c – carmine, dm – damson, g – green, o – orange, r – red, v – violet, y – yellow, l – light, d – dark, p – pale.

Table 2. UV spectral analysis.

Phenolic acid	Values λ_{max}(nm)								Values λ_{min}(nm)							
	x				y				x				y			
protocatechuic	210	256.5	292		209	256	292.6		236	277			235	277		
chlorogenic	219	246.5	305	331.5	219	246.8	304	331	236	265	307		238	267	307	
p–hydroxybenzoic	205	249			205	249.5			225				225.5			
vanillic	209	255	287		210	255.5	287.5		232	276			232.5	276.5		
caffeic	216	240	289.5	320	216.5	240	285	319.5	228	261.5	302		228.5	260	302	
p–coumaric	209	288			210.5	288.5			247				244			
ferulic	216	228	288	315.5	216	289	313		259	297			257.5	296		

Explanations: x– sample , y – standard

Merck) another system S_3 – toluene:methanol:acetic acid (90:16:4) was employed. All fractions of phenolic acids were subjected to qualitative analysis by thin–layer chromatography against 23 phenolic acid standards. The chromatograms were developed by the one– or two–dimensional techniques and then analysed under UV light 254 nm (I) and 366 nm before (II) and after treatment with ammonia vapour (III). Besides, all chromatograms were sprayed with diazotized sulphanilic acid and 20% sodium carbonate solution (IV) and then observed in the daylight (12).

The results of the TLC analysis of the fractions from *S. albus* leaves, flowers and fruits are given in Table 1.

Preparative chromatography

The fraction K–2 from flowers was separated by preparative paper chromatography (Whatman No. 3) using S_2 as mobile phase and by 2D–TLC on a cellulose plate using S_1 (first direction) and S_2 (second direction) as mobile phases.The phenolic acids were eluted with methanol (12).

Spectral analysis in UV

UV spectra of phenolic acids isolated by means of preparative chromatography were recorded in methanol on a Specord UV/VIS Zeiss–Jena.

Qualitative and quantitative analysis using RP–HPLC method

A Hewlett–Packard 1050 liquid chromatograph (Palo Alto, CA), equipped with a UV–VIS detector set at 254 nm, a 20 µl sample loop as part of a Rheodyne injector, a stainless–steel column (200x4.6 mm I.D.), packed with ODS Hypersil (d_p = 5 µm; Shandon Cheshire, UK) was used for qualitative and quantitative analysis of phenolic acids. Chromatograms were recorded on a 3396A integrator (Hewlett–Packard), at a chart speed of 5 mm/min. Samples were analysed under isocratic conditions using methanol–water (24:76 v/v) with 1% (v/v) addition of glacial acetic acid as a mobile phase, at a flow rate of 1 ml/min and at ambient temperature.

Methanol and acetic acid were of chromatographic grade (Merck, Germany) and in all experiments bidistilled water was used. Standards of phenolic acids were purchased from Sigma (St. Louis, MO, USA). The standard solutions of 0.1

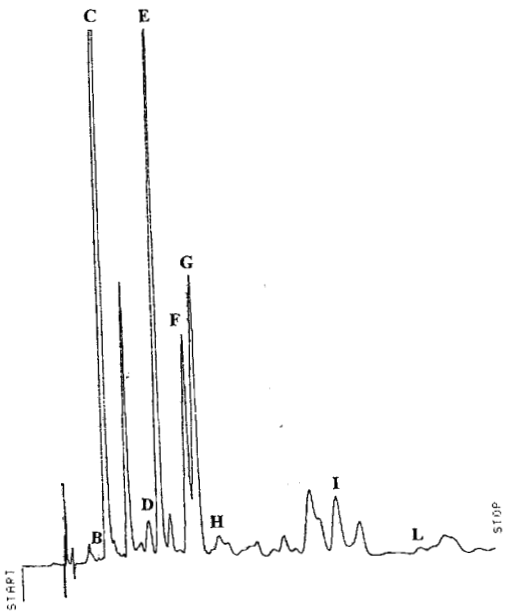

Figure 1. HPLC chromatogram of free phenolic acids from *S. albus* leaves (fraction L–1). The separation conditions – see Experimental. The symbols of peaks and corresponding names of phenolic acids are given in Table 3.

Table 3. Mean retention times (t_R) ± S.D. obtained for phenolic acids in the process of RP–HPLC analysis

Symbol	Phenolic acid	t_R [min]
A	gallic	3.040 ± 0.014
B	α–resorcylic	4.285 ± 0.192
C	protocatechuic	4.643 ± 0.195
D	chlorogenic	6.643 ± 0.236
E	*p*–hydroxybenzoic	7.535 ± 0.170
F	vanillic	9.147 ± 0.152
G	caffeic	9.564 ± 0.239
H	isovanillic	10.478 ± 0.322
I	*p*–coumaric	17.633 ± 0.303
K	salicylic	21.005 ± 0.178
L	ferulic	21.860 ± 0.170

Table 4. The results of quantitative analysis of free, acid–labile and alkali–labile phenolic acids obtained from *S. albus* leaves, flowers and fruits.

Fraction	Phenolic acid											
	Amount of compound (µg/g in dry material)											
	A	B	C	D	E	F	G	H	I	K	L	Total
L–1	–	0.03	5.39	1.80	1.27	1.26	3.24	0.17	2.55	–	0.24	15.95
L–2	–	0.34	0.73	5.00	0.09	–	84.33	1.67	11.43	–	1.18	104.77
L–3	–	–	0.07	0.75	0.03	–	0.68	–	–	1.70	–	3.23
K–1	–	0.34	13.67	71.20	3.90	12.75	41.67	1.07	3.70	6.36	1.88	156.54
K–2	5.57	1.88	13.60	7.20	–	59.16	1.67	2.80	4.44	–	–	96.32
K–3	–	–	1.45	11.20	0.23	29.21	–	–	0.74	–	–	42.93
F–1	–	–	0.555	0.926	0.185	0.741	0.370	–	–	–	–	2.776
F–2	–	–	0.185	–	0.019	–	4.630	–	–	–	–	4.834
F–3	–	–	0.185	–	0.019	–	–	–	–	–	–	0.244

Table 5. Percentage concentration of predominant phenolic acids in fractions examined.

Fraction	Phenolic acid			
	Protocatechuic	chlorogenic	vanillic	caffeic
L–1	33.80	11.28	7.90	20.30
K–1	8.73	45.48	8.14	26.62
F–1	19.19	33.36	26.69	13.33
L–2	0.70	4.77	–	80.49
K–2	14.12	7.47	61.00	1.73
F–2	3.83	–	–	95.78
L–3	2.16	23.22	–	21.05
K–3	3.38	26.08	68.04	–
F–3	90.69	–	–	–

mg/ml in methanol were prepared and the successive dilutions of mother aliquots (n=4) were used for the estimation of calibration curves, showing correlation coefficients not lower than 0.9985 for all compounds examined.

The methanolic extracts for HPLC analysis were prepared from *S. albus* leaves (45 g), flowers (12 g) and fruits (400 g), according to the extraction procedure described above. Fractions obtained from leaves (L–1 to L–3), flowers (K–1 to K–3) and fruits (F–1 to F–3) were evaporated under reduced pressure and diluted with methanol. Then, samples were filtered using 0.2 μm SRP syringe filters (Sartorius, Germany) and injected into the chromatographic column. The results of RP–HPLC analysis are presented in Figure 1 and in Tables 3 and 4.

RESULTS, DISCUSSION AND CONCLUSION

The plant material, i.e. the leaves, flowers and fruits of *S. albus*, afforded fractions of free phenolic acids (L–1, K–1, F–1), as well as phenolic acids liberated by acid (L–2, K–2, F–2), and alkaline (L–3, K–3, F–3) hydrolysis. The fractions were subjected to chromatographic analysis using TLC, 2D–TLC and HPLC techniques. By comparison with standards the quantitative analysis revealed the presence of 11 phenolic acids, namely the derivatives of benzoic (protocatechuic, *p*–hydroxybenzoic, vanillic, isovanillic, gallic, salicylic and α–resorcylic) and cinnamic (caffeic, *p*–coumaric, ferulic, chlorogenic) acids. The results of TLC analysis are shown in Table 2, one of HPLC chromatograms is depicted in Figure 1, and the mean retention times of phenolic acids present in plant material are given in Table 3.

Nine compounds were isolated from fraction K–2 using PC and 2D–TLC. The compounds were identified by co–chromatography with the reference samples and their UV spectra (Table 2).

Three of the phenolic acids were most widely distributed in the fractions examinated (Table 4). All fractions contained protocatechuic acid and *p*–hydroxybenzoic acid was not found only in fraction K–2. Chlorogenic and caffeic acids were present in every fraction apart from the fractions from fruits F–2 and F–3, containing acids liberated by acidic and alkaline hydrolyses (chlorogenic acid), and fractions from flowers and fruits K–2 and K–3, comprising acids liberated by alkaline hydrolysis (caffeic acid). α–resorcylic acid was found in the fractions from the leaves and flowers, containing free phenolic acids (L–1, K–1) or acids liberated by acidic hydrolysis (L–2, K–2). Besides fraction K–2, the above fractions included also ferulic acid, whereas gallic acid was found only in the fraction K–2 from the flowers after acidic hydrolysis. Salicylic acid was present in the fraction of free phenolic acids from the flowers K–1 and in the fraction L–3 from the leaves containing acids liberated by alkaline hydrolysis. Coumaric acid was absent in each fraction isolated from the leaves and also in the fraction L–2 from the leaves after acidic hydrolysis. Vanillic acid was not found in the fractions from the leaves L–2 and L–3, and fruits F–2 and F–3 after acidic and alkaline hydrolyses. The presence of isovanillic acid was not determined in all fractions from the fruits and also in fractions L–3 and K–3 from the leaves and flowers after alkaline hydrolysis.

The content of 11 phenolic acids present in all fractions examined was determined by RP–HPLC and the results are shown in Table 4. The total content of free phenolic acids was ten–fold higher in the flowers than in the leaves, i.e. 156.71 μg/g and 15.95 μg/g, whereas the fruits contained 2.777 μg/g calculated for the dried material. The amount of the acids liberated by acidic hydrolysis was similar in the leaves and flowers (104.17 and 96.32 μg/g) but was very low in the fruits (4.834 μg/g). The content of the phenolic acids determined in the fractions after alkaline hydrolysis varied significantly from 42.93 μg/g (K–3, flowers) to 3.23 μg/g (L–3, leaves) and 0.24 μg/g (F–3, fruits).

The fraction of free phenolic acids consisted mainly of chlorogenic acid, namely 45.49% in flowers (K–1) and 33.36% in fruits (F–1). Other principal constituents included protocatechuic acid, which constituted 33.8% and 19.19% of the fraction of free phenolic acids from leaves (L–1) and fruits (F–1), respectively, caffeic acid, which amoun-

ted to 20.5% in the fraction from leaves (L–1) and to 26.62% in the fraction from flowers (K–1). Free vanillic acid predominated in the fraction obtained from fruits (F–1). A relatively high concentration (15.99%) of free *p*–coumaric acid in the fraction from leaves (L–1) was also noteworthy.

Two phenolic acids were prevalent among the compounds liberated by acid hydrolysis: caffeic, in the fraction L–2 from leaves (80.49%) and F–2 from the fruits (95.78%), and vanillic in the fraction K–2 from flowers (61.0%). *p*–coumaric acid constituted 15.98% of the total amount of free phenolic acids in the fractions L–1 and 10.91% in L–2 (after acid hydrolysis) both obtained from leaves. On the other hand, protocatechuic and vanillic acids predominated in the fractions after alkaline hydrolysis, the first one in *S. albus* fruits (F–3, 90.69%), the second one in flowers (K–3, 68.04%). The percentages of chlorogenic acid, in the fractions from leaves and flowers, and caffeic acid, isolated from leaves, represented similar levels and amounted to 23.22% (L–3), 26.08% (K–3) and 21.05% (L–3), respectively. The content of salicylic acid was significant (52.63%) in the fraction of alkali–labile phenolic acids (L–3), obtained from leaves. The concentrations of the remaining phenolic acids in all fractions examined were low and variable. The results are given in Table 5.

The above described qualitative and quantitative determination of phenolic acids in the fractions obtained from the domestic species *S. albus* has been undertaken for the first time.

The presence of the phenolic acids in this plant material might justify its use in medicine. Literature data show that caffeic, protocatechuic and chlorogenic acids have immunostimulating activity, caffeic, *p*–coumaric and salicylic acids exhibit antiinflammatory action, whereas protocatechuic and caffeic acids determine choleretic and cholekinetic properties (13,14).

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REFERENCES

1. Hegnauer R: Chemotaxonomie der Pflanzen Vol. III, VIII, Birkhäuser Verlag, Basel 1964, 1989.
2. Hänsel R., Keller K., Rimpler H., Schneider G: Drogen P–Z, p. 853, Springer Verlag, Berlin 1994.
3. Szaufer M., Kowalewski Z., Phillipson J.D.: Phytochemistry 17, 1445 (1978).
4. Szaufer M., Kowalewski Z.: Herba Polon. 22, 205 (1976).
5. Bylka W., Kowalewski Z.: Herba Polon. 1, 11 (1980).
6. Bylka W., Matławska I.: Acta Pol. Pharm. 56, 249 (1999).
7. Braun H.: Heilpflanzen Lexicon für Arzte und Apotheker, Birkhäuser Verlag, Stuttgart 1968.
8. Tietze L.F., Henke S., Remberg G.: Liebigs Ann. Chem. 1413 (1986).
9. Willuhn G., Merfort I., Matthiesen U.: Phytochemistry 22, 137 (1983).
10. Merfort I., Willuhn G.: Pharmazeutische Ztg. 130, 2467 (1985).
11. Świątek L.: Herba Polon. 23, 201 (1977).
12. Drost–Karbowska K., Szaufer–Hajdrych M., Kowalewski. Z., Zgórka G.: Herba Polon. 42, 21 (1996).
13. Sawicka T., Drozd J., Prosińska J., Borkowski B.: Herba Polon. 40, 31 (1994).
14. Borkowski B.: Herba Polon. 39, 139 (1993).

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