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HPLC OF FLAVANONES AND CHALCONES IN DIFFERENT SPECIES AND CLONES OF *SALIX*

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Abstract: The SPE-HPLC method was developed to determine an isosalipurposide (**5**) and its derivative, 6”-*O*-*p*-coumaroyl ester (**6**) in the bark of eight taxa (**I**–**VIII**) belonging to three species of the genus *Salix* and originating from a natural habitat or cultivated for pharmaceutical purposes. The chalcones were separated by HPLC under gradient elution with the concentration of ACN increasing from 20% to 50% in 0.1% aqueous H_3PO_4 (t_G 15 min). The content of both compounds was determined by an external standardization with the use of isoliquiritigenin (**7**) as a reference substance – a commercially available chalcone, and also isosalipurposide (**5**) and its derivative, 6”-*O*-*p*-coumaroyl ester (**6**). The latter compound was isolated from the bark of *Salix daphnoides* (**IV**) by a CC and semi-preparative HPLC and its structure was elucidated by MS and NMR spectra. It was stated that 6”-*O*-*p*-coumaroylisosalipurposide (**6**), in addition to isosalipurposide (**5**), is a characteristic flavonoid for the *S. daphnoides* species. Moreover, the presence of these two chalcones was confirmed in the bark of *S. acutifolia* (**I**). Differences were observed in the results obtained from a quantitative analysis due to the type of reference substance used. The content of chalcones was varied and dependent on the species selected for analysis, namely from 22.01/21.08 mg/g in *S. daphnoides* clone 1095 (**III**) to 2.47/2.44 mg/g in *S. daphnoides* (**II**), collected from a natural habitat. Isosalipurposide (**5**) was determined in all the investigated species and clones of *Salix,* besides a number of naringenin derivatives. Separation of all flavonoids: flavanones – naringenin (**1**), naringenin (+)-5-*O*-glucoside (2), (–)-5-*O*-glucoside (**3**), 7-*O*-glucoside (**4**) and chalcones (**5** and **6**) was performed under gradient elution with the same solvents and changes in ACN concentration from 2% to 37% (t_G 60 min). The total amount of flavanones ranged from 4.69 mg/g in *S. purpurea* clone 1132 (**VII**) to 41.93 mg/g in *S. purpurea* (**VIII**) from Herbapol Wrocław.

Keywords: HPLC, SPE, chalcones, flavanones, *Salix*

The bark of a willow is a herbal remedy frequently used as an antiphlogistic, antipyretic and antirheumatic (1). Such pharmacological activity is due to a presence of salicin and its derivatives (1). According to a monograph of a willow bark, published in the European Pharmacopeia (2), different species of *Salix* are a source of medicinal plant material containing not less than 1.5% of salicin. However, some reports suggest that in addition to salicylic compounds also other constituents of bark, including polyphenols, may be responsible for the pharmacological effects (3, 4). In consideration of such data, a model based on a multivariate data analysis of HPTLC densitograms of willow extracts has been proposed in connection to their anti-inflammatory properties to predict the activity of a willow bark (5).

The chemical composition of polyphenols in willow bark, especially of the flavonoids, depends on the following species: flavonols are present in *S. alba* (6), but chalcones and flavanones occur only in *S. acutifolia, S. purpurea* and *S. daphnoides* (7, 8). It seems probable that the presence of dissimilar groups of flavonoids may change the type of a pharmacological activity in the bark – flavonols, from the increase of antioxidant effect (1), chalcones (isosalipurposide and its derivatives) and flavanones (naringenin and its glucosides) to an enhancement of spasmolytic and anti-inflammatory activity, respectively (1, 9, 10). Furthermore, an antiulcer activity of naringenin was revealed (11, 12). This compound has been reported to significantly reduce the intragastric concentration of histamine in rats (12). It was also found to be a specific 5-lipoxygenase inhibitor and that it can decrease

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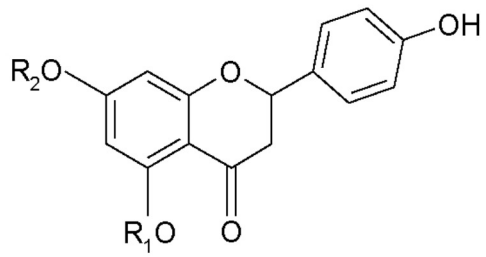
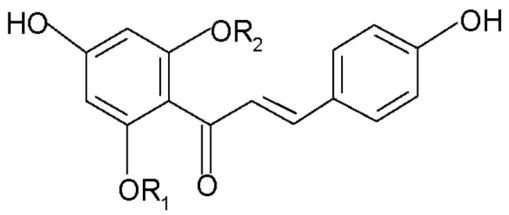
leukotriene formation, which has an influence on the integrity of gastric mucosa (12). It was also demonstrated that naringenin inhibits the growth of *Helicobacter pylori* (11). Chalcones are another group of flavonoids present in the genus *Salix*. Some chalcone derivatives have anti-inflammatory properties due to the inhibition of mast cells and neutrophil degranulation (10), inhibition of nitric oxide (NO) formation or the inhibition of interleukin-1 biosynthesis (9). For example, 4-methylchalcone showed an anti-inflammatory effect comparable to ibuprofen (9). In addition, chalcone and floretin inhibited the release of histamine from human basophils (9). It is worth to notice that some naturally occurring chalcones and their synthetic derivatives demonstrate antiulcer properties. Sophoradin inhibits pig gastric H^+, K^+-ATPase and Na^+, K^+-ATPase competitively with ATP at ATP site and, thus, blocks phosphorylation of this enzyme and leads to the inhibition of gastric juice secretion (9). 2',4'-Dihydroxychalcone and 2',4'-dihydroxy-3'-methoxychalcone from the leaf resin of *Zuccagnia punctata* (Fabaceae) showed a significant protective effect against stomach and duodenal mucosa damage (13).

Not many reports concern the estimation of flavonoid content in willow bark (7, 14–17).

The set of flavonoids in the bark of two species, namely of *S. daphnoides* and *S. purpurea*,



		R_1	R_2
1	Naringenin	H	H
2/3	Naringenin-5-*O*-glucoside	glucose	H
4	Naringenin-7-*O*-glucoside	H	glucose



		R_1	R_2
5	Isosalipurposide	glucose	OH
6	6"-*O*-*p*-coumaroylisosalipurposide	coumaroyl	OH
7	Isoliquiritigenin	H	H

coumaroyl =

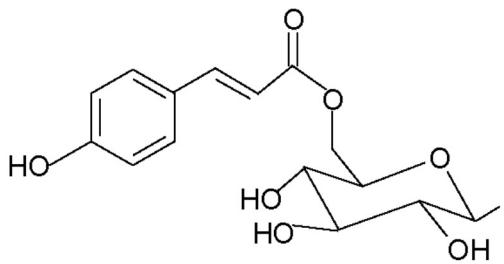


Figure 1. Chemical structures of the analyzed compounds

which are the constituents of some herbal drugs, such as Assalix (Bionorica, Germany), Salicortex (Labofarm, Poland), Pyrosal (Herbapol Wrocław, Poland), are very similar. Meier et al. (7) have confirmed, by means of HPLC method, the presence of the following flavonoids in both of the aforementioned species: isosalipurposide (**5**), (+)-naringenin-5-*O*-glucoside (**2**), (–)-naringenin-5-*O*-glucoside (**3**) and naringenin-7-*O*-glucoside (**4**). The occurrence of the above mentioned compounds in bark of *S. daphnoides* and *S. purpurea x daphnoides* was also confirmed by HPLC-MS/MS (8).

The aim of our work was to optimize the SPE-HPLC method for rapid and accurate determination of chalcones in some species and clones of *Salix,* cultivated for the purposes of pharmaceutical industry and originating from a natural habitat. Furthermore, the aim of our work was also to compare the amounts of other flavonoid groups, especially flavanones, in the investigated plant material.

EXPERIMENTAL

General

ACN was a HPLC grade while phosphoric acid was Baker analyzed reagent (Philipsburg, PA, USA). H_2O was prepared with a Milli-Q (Bedford, MA, USA) H_2O purification system. Polyamide for CC was from Roth (Karlsruhe, Germany). MeOH, $CHCl_3$ and H_3PO_4 were obtained from POCh (Gliwice, Poland). Standard flavonoids (Fig. 1), namely naringenin (**1**), naringenin-7-O-glucoside (**4**), isoliquiritigenin (**7**) were obtained from Extrasynthese (Genay, France). The other flavonoids, such as (+)-naringenin-5-O-glucoside (**2**), (–)-naringenin-5-O-glucoside (**3**), isosalipurposide (**5**) and 6”-O-p-coumaroylisosalipurposide (**6**), were isolated from the bark of *S. daphnoides* (**IV**) (Labofarm), as described below. HPLC was performed by means of a Merck-Hitachi HPLC System (Tokyo, Japan) consisting of a pump L-7100, DAD detector L-7455, UV-Vis detector L-7400, autosampler L-7200, thermostat L-7350, membrane degasser L-7612 and interface D-7000, controlled by LaChrom D-7000 HPLC System Manager (Merck-Hitachi) software. Knauer HPLC System (Berlin, Germany) was also employed for semi-preparative purposes, consisting of two pumps 64-00, detector UV 87-00, a dynamic mixing chamber, a manual injector 7000 (Rheodyne, Cotati, CA, USA), controlled by means of Eurochrom 2000 Software. ^{1}H- and ^{13}C-NMR spectra were obtained using a Bruker MSL 300 (Billerica, MA, USA) spectrometer, at 300 (^{1}H) and 75.5 MHz (^{13}C). TMS was used as an internal standard. FAB-MS spectra were obtained on Trio-3 VG (MassLab, Altrincham, UK) spectrometer.

Plant material

The barks of three species from genus *Salix* (Salicaceae), namely *of S. acutifolia* (I), *S. daphnoides* (**II**) and *S. purpurea* (**V** and **VI**) were collected from the plants growing in a natural habitat in Poland, in April 2004. The barks of the two clones of *Salix,* namely *S. daphnoides* clone 1095 (**III**) and *S. purpurea* clone 1132 (**VII**), were obtained in March 2003 from the experimental collection of willows at the University of Warmia and Mazury in Olsztyn (Poland). The sample of *S. daphnoides* (**IV**) was also obtained from the cultivation of Labofarm (Starogard Gdański, Poland). One more sample of *S. purpurea* (**VIII**) originated from the Herbapol Wrocław (Poland). The voucher specimens were deposited at the Department of Pharmacognosy of the Medical University of Gdańsk (Poland). The dried extracts (**IX** and **X**) were obtained from the Herbapol Wrocław (Poland) (**IX**) and Finzelberg (Germany) (**X**).

Isolation and identification of flavonoids.

The MeOH extract was obtained from the dried and pulverized bark of *S. daphnoides* (**IV**) from Labofarm (450 g), defatted earlier by extraction with $CHCl_3$ in a Soxhlet apparatus. After partial evaporation of the solvent *in vacuum*, the extract was fractionated in a polyamide column using H_2O/MeOH mixture with the increasing concentration of MeOH. Compound (**6**) precipitated in a crystalline form from eluates 107–125. Compounds (**2**), (**3**) and (**5**), present in eluates 36–42, were purified by a semi-preparative HPLC. The silica gel RP-18 (40–63 µm) (Merck, Germany) (800 mg) was covered with a mixture of separated flavonoids and was transferred into a stainless steel column (125 × 4 mm i.d.) after drying. The column was directly connected to a manual injector and used instead of a loop as a pre-column. Before separation, the pre-column was degassed with helium and next, was washed with ACN/H_2O mixture (2 : 98, v/v). When the first drops of a mobile phase showed, the pre-column was connected to a semi-preparative column. A semi-preparative separation was performed on a Lichrospher RP-18 column (7 µm, 250 × 10 mm i.d.; Merck) under gradient elution, according to program III: solvents A – ACN, B – H_2O, linear gradient from 2% to 60% A in (A+B) from 0 min to 150 min with a flow rate of 5.0 mL/min, at ambient temperature and re-equilibration period of 20 min between individual runs. The flavonoids were separated as follows: compound (**2**) – (+)-naringenin-5-

O-glucoside (4 mg) was eluted with t_R 63–67 min; compound (**3**) – (–)-naringenin-5-*O*-glucoside (5 mg) was eluted with t_R 68-71 min and compound (**5**) – isosalipurposide (15 mg) with t_R 81–90 min. Compound structures were confirmed by UV (18), MS and NMR data (7, 19, 20).

6'-*O*-(6''-*O*-(*E*)-*p*-coumaroyl)-β-*D*-glucopyranoside-2',4',4,-trihydroxychalcone (**6**)

FAB-MS (+) m/z (rel. int.): 582 $[M + H]^+$ (95), 309 $[M + A]^+$ (20), 273 $[A + H]^+$ (60). ^{13}C-NMR (75.5 MHz, DMSO-d_6, δ, ppm): 192.0 (C=O), 166.5 (C-9'''), 166.0 (C-4''), 164.7 (C-2''), 160.1 (C-6''), 159.9 (C-4'''), 159.9 (C-4), 144.9 (C-7'''), 142.9 (C-α), 130.8 (C-2, 6), 130.3 (C-2''', 6'''), 126.2 (C-1'''), 125.0 (C-β), 124.0 (C-1), 115.9 (C-3, 5), 115.7 (C-3''', 5'''), 113.8 (C-8'''), 105.5 (C-1''), 100.3 (C-1''), 97.0 (C-3''), 94.9 (C-5''), 76.5 (C-3''), 74.1 (C-5''), 73.6 (C-2''), 69.7 (C-4''), 63.2 (C-6'').

HPLC analysis

Sample preparation

Dried and pulverized bark (1 g) was extracted with MeOH (3 × 30 mL) (60°C, 45 min). The MeOH extracts were combined and concentrated under reduced pressure (5 mL). Eighty µL of the extract was evaporated to dryness and re-dissolved in the same volume of 20% ACN. Next, the sample was subjected to a solid phase extraction (SPE) procedure.

SPE

A glass column (42 × 4 mm i.d.) was packed with LiChroprep™ RP-18 (40–63 µm) (Merck) (300 mg). The adsorbent was capped at both ends with PTFE frits (J.T. Baker, Holland). Conditioning of the columns was performed with 6 mL ACN and next, with 6 mL 20% ACN/H_2O. Eighty mL of the analyzed sample was transferred into the column and, after rinsing with 3.2 mL 20% ACN/H_2O, the isosalipurposide (**5**) was eluted with 2 mL 20% ACN/H_2O (fraction A). Next, the column was washed with 2.7 mL 30% ACN/H_2O and 6''-*O*-*p*-coumaroylisosalipurposide (**6**) was eluted with 2.3 mL 30% ACN (fraction B). The obtained fractions A and B containing chalcones were combined and adjusted to 10 mL in a volumetric flask and analyzed by HPLC.

Chromatographic conditions

Analytical separations were performed on a Discovery C18 column (5 µm, 150 × 2.1 mm i.d.; Supelco). The gradient elution was performed according to the following programs: program I solvents A – ACN, B – H_3PO_4/H_2O (99.9 : 0.1, v/v), linear gradient 2–37% A in A + B from 0 to 60 min; flow rate, 0.4 mL/min; at temperature of 20°C, re-equilibration period of 10 min was conducted between individual runs; program II solvents A – ACN, B – H_3PO_4/H_2O (99.9 : 0.1, v/v), linear gradient 20–50% A in A + B from 0 to 15 min; flow rate, 0.4 mL/min; at temperature of 21°C, re-equilibration period of 15 min was conducted between individual runs. Chalcones and flavanones were identified with UV-Vis DAD detection at 280 nm.

Quantitative analysis of flavonoids

It was performed by means of the external or internal standardization. Standard solutions (Tab. 1) were prepared in MeOH and next, 20 µL of each standard solution was injected. Calibration graphs for all flavonoids were obtained by plotting flavonoid peak areas against concentrations (Tab. 1).

RESULTS AND DISCUSSION

In order to determine the presence of flavonoids in willow bark by means of HPLC method, two flavanones and two chalcones from the bark of *S. daphnoides* were isolated by CC on polyamide and semi-preparative HPLC and employed as standard compounds, namely (+)-naringenin-5-*O*-glucoside (**2**), (–)-naringenin-5-*O*-glucoside (**3**), and isosalipurposide (**5**) (Fig. 1). Moreover, the investigations we have conducted on

Table 1. Validation parameters of HPLC analysis.

Compound	LOQ (µg/mL)	Range (µg/mL) n = 6	Linear regression equation y = ax + b	Correlation coefficient
1	1.436	0.5 – 4.0	y = 0.37a + 0.08	0.9999
4	0.190	10.0 – 60.0	y = 1.12a + 1.10	0.9999
5	0.942	2.0 – 7.0	y = 4.02a – 0.26	0.9999
6	0.552	2.0 – 7.0	y = 0.96a + 0.26	0.9994
7	0.728	9.0 – 36.0	y = 2.58a – 1.09	0.9995

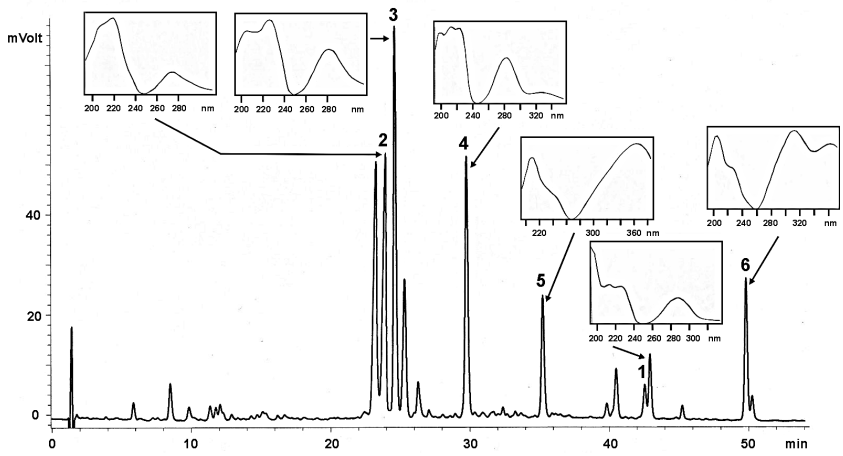


Figure 2. HPLC separation of the MeOH extract of *Salix daphnoides* (**IV**) bark (Discovery C18,5 µm, 150 × 2.1 mm i.d.; Supelco, program I). Detection at 280 nm. Peak numbers correspond to compounds **1** – **6**

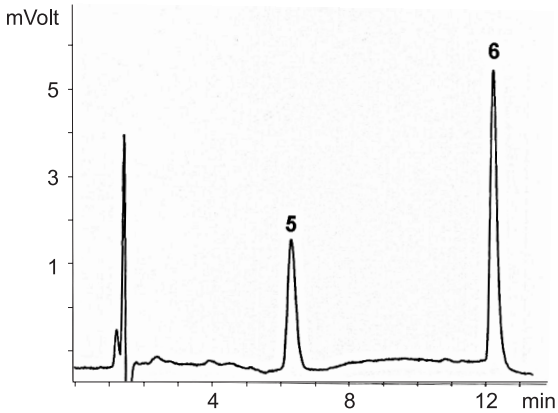


Figure 3. HPLC separation of the SPE purified MeOH extract from the *Salix daphnoides* (**IV**) bark (Discovery C18,5 µm, 150 × 2.1 mm i.d.; Supelco, program II): Detection at 280 nm. Peak numbers correspond to compounds **5** and **6**

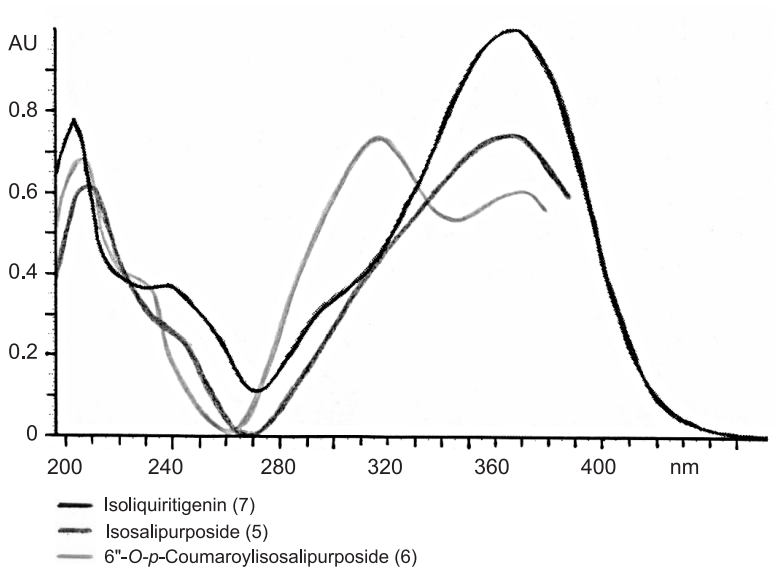


Figure 4. DAD spectra of chalcones

Table 2. The content of chalcones [mg/g] in the analyzed barks of willow and some of dried extracts used in pharmaceutical industry determined by SPE-HPLC.

No.	Sample	Content [mg/g]					
		5		**6**		Σ of **5** and **6**	
		[a]	[b]	[a]	[b]	[a]	[b]
I	*Salix acutifolia*	4.45 ± 0.09	2.76 ± 0.05	0.74 ± 0.05	1.61 ± 0.08	5.19 ± 0.09	4.37 ± 0.09
II	*S. daphnoides*	1.85 ± 0.005	1.19 ± 0.07	0.59 ± 0.03	1.28 ± 0.04	2.44 ± 0.07	2.47 ± 0.07
III	*S. daphnoides* clone 1095	17.54 ± 0.12	10.44 ± 0.09	4.47 ± 0.09	10.64 ± 0.12	22.01 ± 0.13	21.08 ± 0.11
IV	*S. daphnoides* (Labofarm)	5.28 ± 0.11	3.57 ± 0.09	2.49 ± 0.11	5.41 ± 0.14	7.77 ± 0.15	8.98 ± 0.12
V *S*	*S. purpurea*	1.04 ± 0.004	0.69 ± 0.04				
VI	*S. purpurea*	1.74 ± 0.05	1.12 ± 0.06				
VII	*S. purpurea* clone 1132	10.00 ± 0.13	5.92 ± 0.11				
VIII	*S. purpurea* (Herbapol Wrocław)	1.35 ± 0.02	0.90 ± 0.07				
IX	Dry extract (Herbapol Wrocław)	8.22 ± 0.11	5.25 ± 0.07			8.22 ± 0.13	5.25 ± 0.11
X	Dry extract (Finzelberg)	0.74 ± 0.02	0.83 ± 0.02			0.74 ± 0.07	0.83 ± 0.09

[a]) – the content determined by internal standard – isosalipurposide (**5**) or 6''-*O*-p-coumaroylisosalipurposide (**6**),
[b]) – the content determined by external standard – isoliquiritigenin (**7**).

flavonoids in violet willow have led us to isolate and identify a new chalcone in this species, namely 6'-*O*-(6''-*O*-(*E*)-*p*-coumaroyl)-*β*-D-glucopyranoside-2',4',4,-trihydroxychalcone (6"-*O*-*p*-coumaroylisosalipurposide) (**6**). The presence of ester bonding at C-6" of glucose was confirmed by HMBC spectrum and the correlated signals H-6"a/C-9'" at δ_H 4.42/δ_C 166.5 and H-6"e/C-9'" at δ_H 4.15/δ_C 166.5. It is worth to notice that 6"-*O*-*p*-coumaroylisosalipurposide (**6**) was previously identified only in the bark of *S. x rubra* (21) and *S. acutifolia* (19).

As a result of the composition optimization of the mobile phase, the mixture of ACN and 0.1% H_3PO_4 was chosen for RP-HPLC separation of chalcones and flavanones. The best resolution was obtained under a gradient elution with the concentration of ACN increasing from 2% to 37% in 0.1% H_3PO_4 (Fig. 2). All flavonoids were detected at 280 nm. Prior to this, the flavonoids in *S. purpurea* and *S. daphnoides* barks were analyzed, besides the salicylic derivatives, at a slightly lower wavelength, namely at 270 nm (7).

In the investigated plant material, the presence of the above mentioned set of flavonoids was confirmed, although with one exception; 6"-*O*-*p*-coumaroylisosalipurposide (**6**) was detected only in the barks of *S. daphnoides* (**II**, **III** and **IV**), and *S. acutifolia* (**I**) (Tab. 2). It was stated that the presence of 6"-*O*-*p*-coumaroylisosalipurposide (**6**) distinguishes violet (*Salix daphnoides*) and sharpleaf (*S. acutifolia)* willows from purple willow (*S. purpurea*). This feature could be helpful for the botanical identification of willows.

Naringenin-7-*O*-glucoside (prunin) (**4**) was detected in higher concentrations in the barks of both species, which were subject to investigation, than in the case of *S. purpurea* barks (Tab. 3). The highest content of naringenin-5-*O*-glucosides (salipurposides) (41.93 mg/g) was determined in the bark of *S. purpurea* (**VIII**), which was collected from a natural habitat (Tab. 3). In this plant material, (+)-naringenin-5-*O*-glucoside (**2**) was dominant (25.30 mg/g) besides their isomer (–)-naringenin-5-*O*-glucoside (**3**) (15.87 mg/g) (Tab. 3). The concentration of naringenin-7-*O*-glucoside (**4**) was significantly lower (0.68 mg/g) (Tab. 3). Similar differences in composition of naringenin glucosides were noted in

Table 3. The content of flavanones [mg/g] in the MeOH and dried extracts of analyzed willow barks determined by HPLC.

No.	Sample	Content [mg/g]				
		2[a])	**3**[a])	**4**	**1**	Σ flavanones
I	*Salix acutifolia*	4.82 ± 0.09	3.02 ± 0.09	3.64 ± 0.07	0.11 ± 0.01	11.59 ± 0.11
II	*S. daphnoides*	20.19 ± 0.21	13.16 ± 0.17	3.27 ± 0.08	0.12 ± 0.02	36.75 ± 0.26
III	*S. daphnoides* clone 1095	6.69 ± 0.09	3.96 ± 0.11	15.82 ± 0.14	0.14 ± 0.01	26.60 ± 0.17
IV	*S. daphnoides* (Labofarm)	6.17 ± 0.14	3.63 ± 0.08	4.57 ± 0.11	0.014 ± 0.007	14.37 ± 0.12
V	*S. purpurea*	4.49 ± 0.11	2.81 ± 0.09	0.23 ± 0.03	–	7.53 ± 0.09
VI	*S. purpurea*	3.35 ± 0.08	2.15 ± 0.06	0.22 ± 0.05	–	5.72 ± 0.06
VII	*S. purpurea* clone 1132	2.63 ± 0.13	1.81 ± 0.04	0.24 ± 0.08	0.01 ± 0.005	4.69 ± 0.08
VIII	*S. purpurea* (Herbapol Wrocław)	25.30 ± 0.24	15.87 ± 0.17	0.68 ± 0.07	0.08 ± 0.01	41.93 ± 0.21
IX	Dry extract (Herbapol Wrocław)	61.78 ± 0.19	41.47 ± 0.22	–	0.82 ± 0.06	104.07 ± 0.22
X	Dry extract (Finzelberg)	4.72 ± 0.08	4.40 ± 0.13	–	0.20 ± 0.05	9.32 ± 0.12

[a]) – the content determined by external standard – naringenin-7-*O*-glucoside (**4**)

Salix daphnoides samples. The bark of violet willow, which originated from a natural habitat (**II**), contained a high concentration of salipurposides (**2** and **3**) (33.35 mg/g) aside from a relatively low content of prunin (**4**) (3.27 mg/g). On the other hand, both compounds were determined to be practically at the same level (salipurposides (**2** and **3**) – 10.65 mg/g and prunin (**4**) – 15.82 mg/g) in the bark of *S. daphnoides* clone 1095 (**III**) (Tab. 3).

There is no information about the concentration of 6”-*O*-*p*-coumaroylisosalipurposide (**6**) in *Salicis cortex*, in contrast to isosalipurposide, whose content was determined by Meier et al. (0.15–2.20%) (7).

The essential difficulty behind HPLC determination of chalcones was caused by the high values of retention times of isosalipurposide and its *p*-coumaroyl ester. To solve this problem, SPE method was developed to serve as the fractionation process for MeOH extracts obtained from the analyzed willow barks. Experiments were performed on column filled with silica gel Lichroprep RP-18. Once MeOH extract was introduced into the column, it was eluted with mixtures of ACN and H_2O in different ratios – 20 : 80 and 30 : 70 (v/v). Two fractions were collected containing isosalipurposide (**5**) and its p-coumaroyl ester (**6**). The combined fractions were analyzed using HPLC method on Discovery C18 column with gradient elution and the concentration of ACN increasing from 20% to 50% in a mixture of ACN/H_2O + H_3PO_4 (99.1 + 0.1; v/v) (t_G 15 min) (Fig. 3).

In the established SPE method, the recovery of isosalipurposide (**5**) and 6”-*O*-*p*-coumaroylisosalipurposide (**6**) was at the level of 82.51% ± 2.16 and 95,83% ± 1.74 (n = 6), respectively. A quantitative analysis was performed by means of the external standard method, using isolated chalcones as standard compounds and, additionally, a commercially available chalcone – isoliquiritigenin (**7**). It was found that the estimated concentrations of isosalipurposide (**5**), calculated on isoliquiritigenin (**7**), were lower than the concentrations determined using, as a standard, this compound itself, while the concentrations of 6”-*O*-*p*-coumaroylisosalipurposide (**6**), calculated on isoliquiritigenin (**7**), were higher (Tab. 2). The observed differences in concentrations of single compounds resulted from differences in their absorption intensity at the wavelength of 280 nm demonstrated by, both, chalcones (**5** and **6**) and isoliquiritigenin (**7**) (Fig. 4).

In purple willow barks, the contents of isosalipurposide (**5**) varied between 1.04 mg/g to 10.0 mg/g and were nearly twice lower than the concen-

trations in violet willows barks (1.85 mg/g and 17.54 mg/g, respectively) (Tab. 2). Among the purple willow barks, the highest concentration of isosalipurposide (**5**) characterized *S. purpurea* clone 1132 (**VII**) (10.0 mg/g) (Tab. 2).

On the other hand, 6"-*O*-*p*-coumaroylisosalipurposide (**6**) was present in the highest concentration in the bark of *S. daphnoides* clone 1095 (**III**) (4.47 mg/g), (Tab. 2). In contrast to other barks, this clone (**III**) was marked out by a high content of both chalcones (22.01 mg/g) (Tab. 2).

The conducted chromatographic analysis of the dry extracts from willow barks – *S. purpurea* (Herbapol Wrocław) (**IX**) and *Salix sp.* (Finzelberg) (**X**), both standardized on salicin content, confirms the differences in concentration of active compounds due to the origin and chemical composition of the starting plant material. In contrast to the extract obtained from Herbapol Wrocław (**IX**), the amounts of naringenin-5-*O*-glucosides (**2** and **3**) and isosalipurposide (**5**) in the extract of Finzelberg (**X**) were about 11 times lower (Tab. 2 and 3).

With regard to the biological properties, namely the anti-inflammatory and antiulcer activities of flavanones and chalcones present in the willow bark (9–13), the differences in content of flavonoids could have an influence on the antiphlogistic activity of extracts obtained from them.

NSAIDs, used in therapy of rheumatic diseases, stimulate the formation of stomach and duodenum ulceration by COX-1 inhibit (3). It might be possible that, due to the presence of flavanones and chalcones, willow bark extracts have a protective effect on gastric mucosa (9, 11–13).

Regarding the complex chemical composition of plant extracts, and the synergistic effect of compounds (22), which they contain, the observed differences in flavonoid concentrations can have an influence on the pharmacological effect of extracts originating from different willow barks.

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