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NATURAL DRUGS

POLYPHENOLIC COMPOUNDS IN *SCOPOLIA CAUCASICA* KOLESN. ex KREYER (*SOLANACEAE*)

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Abstract: The qualitative and quantitative determinations of coumarins, phenolic acids and flavonoids in the leaves and underground parts of *Scopolia caucasica* using paper chromatography and HPLC methods were described. From the leaves of this plant, kaempferol 3-O-(2-glucosyl)-galactoside-7-O-glucoside, kaempferol 3-O-(2-glucosyl)-galactoside and quercetin 3-O-glucoside were isolated and identified by spectroscopic methods (UV, ^{1}H- and ^{13}C-NMR).

Keywords: *Scopolia caucasica* Kolesn. ex Kreyer, leaves and underground parts; coumarins, phenolic acids, flavonoids; isolation, determination, HPLC

Scopolia caucasica Kolesn. ex Kreyer (*S. caucasica* Kolesn.) is a perennial, naturally occurring in the mountainous region of the Caucasus and South-Western Europe (1, 2). It can be successfully planted in Poland (3).

According to literature, underground parts of this plant contain tropane alkaloids – atropine and scopolamine (about 0.3-0.7%), widely used in therapy as spasmolytic agents (4-6). Moreover, in the leaves and the underground parts, the following polyphenols: scopoletin, scopolin, caffeic acid, chlorogenic acid and additionally rutoside in leaves were chromatographically identified (7).

Our initial studies revealed that extracts from the leaves and the underground parts of *S. caucasica* are much more complex in polyphenolic composition than it has been described in the literature. This paper is a continuation of this research. The study was undertaken to describe biologically active components of genus *Scopolia* Jacq. (8-12).

EXPERIMENTAL

Material and methods

The plant material (leaves and underground parts) was collected from 12-year-old plants grown in the Garden of Medicinal Plants, Department of Pharmacognosy at the Medical University of Łódź (June and October 2000). The seeds were provided by the Botanical Garden in Petersburg (Russia). All the materials were pulverized and the specimens for quantitative studies were sieved through sieves acc. to Polish Pharmacopoeia VI (sieves 0.315 and 0.074 mm). Voucher specimens were deposited at the Department of Pharmacognosy.

Paper chromatography (PC) was carried out on Whatman No. 1 with: n-BuOH/AcOH/H_2O (4:1:5, v/v/v, upper phase) (System S-1), 15% AcOH (S-2), C_6H_6/AcOH/H_2O (6:7:3, v/v/v, upper phase) (S-3), HCOONa/HCOOH/H_2O (10:1:200) (S-4), AcOEt/HCOOH/H_2O (10:2:3, upper phase) (S-5); TLC was run on silica gel 60 precoated plates (Merck) with EtOH/25% NH_4OH/H_2O (20:1:4) (S-6). Column chromatography (CC) on polyamide SC-6 (MN) and Sephadex LH-20 (Fluka). HPLC, chromatograph Hewlett-Packard 1100 series, was equipped with a quaternary pump HP1311A, vacuum degasser HP1322A, integrator HP series 3395, variable wavelength UV/VIS detector HP1314A, manual injector 20 μL (HP) and Hypersil ODS 5 μm column 125 × 4 mm (HP).

Melting points (uncorr.) were determined on a Boetius apparatus. Spectra were recorded using the following instruments: UV, Unicam SP 800 in MeOH and after addition of the usual shifts reagents, acc. to standard procedures (13); ^{1}H- and ^{13}C-NMR, Bruker MSL 300 MHz (in DMSO-d_6, TMS as internal standard).

Qualitative analysis of polyphenolic compounds by 2D-PC method

Leaves and underground parts (10 g of each) were extracted with $CHCl_3$ in a Soxhlet apparatus for 18 h. The chloroform extracts were concentrated and purified by precipitation of ballast with hot water and filtered. The aqeous filtrate was reextract-

ed with $CHCl_3$ (5 × 50 mL). The solvent was removed in vacuo from the collected extracts and the residue was dissolved in 5 mL of chloroform, creating F-1 fraction.

In the next stage, the plant materials were extracted with 70% MeOH (3 × 150 mL) at the boiling temp. of the solvent for 5 h. The obtained extracts were collected, concentrated to 100 mL and extracted by turns with: Et_2O (5 × 50 mL), as well as EtOAc and n-BuOH (5 × 50 mL and 3 × 50 mL, respectively). After concentration under the reduced pressure, the dry residues were dissolved in 5 mL of MeOH, providing the F-2 fraction and F-3 fraction, respectively.

F-1, F-2 and F-3 fractions and 0.1% methanolic solutions of standards – coumarins (8) and phenolic acids (Fluka, Merck) were spotted (0.02 and 0.04 mL) on Whatman paper. Chromatograms were developed in Chroppa chambers by two-dimensional ascending technique and observed in UV light (254 and 366 nm) before and after treatment with ammonia vapor. Next, the spots were visualized with 1% solution of $AlCl_3$ and 0.5% solution of diazosulfanilic acid in 10% Na_2CO_3 and observed in the daylight.

The results of the 2D-PC analysis of the F-1, F-2 and F-3 fractions are given in Table 1.

Extraction, isolation and identification of flavonoids

Leaves (0.3 kg) were extracted with petrol and $CHCl_3$ in Soxhlet apparatus, and later exhaustively extracted with MeOH and 70% MeOH at the boiling temp. of the solvents. Combined methanol extracts were evaporated to 300 mL, cooled, and the balast precipitates were filtered off. The remaining filtrate (aqeous solution) was extracted with Et_2O. The ethereal extracts did not contain flavonoids and were discarded. The aq. solution was extracted with EtOAc (20 × 100 mL) and with n-BuOH (5 × 100 mL). After combination, AcOEt-BuOH extract (14.2 g) was obtained. Next, the extract was submitted to CC on polyamide (eluent H_2O-MeOH with increasing gradient of MeOH). The fractions eluted with 10% aq. MeOH, after evaporation were rechromatographed on polyamide columns using mixture of C_6H_6–MeOH with MeOH gradient. Following the separation, compound **I** (380 mg) was obtained. The 30% and 60-70% MeOH fractions were independently rechromatographed on Sephadex columns (eluent MeOH) to give compounds **II** (85 mg) and **III** (11 mg), respectively.

Complete acid hydrolysis. A 10 mg sample of **I** was refluxed with 5% H_2SO_4 for 2 h, and 5-10 mg

Table 1. The occurrence of polyphenolic compounds in *Scopolia caucasica* Kolesn. ex Kreyer.

Compound	2D-PC, R_f		2D-PC, R_f		F-1 fraction		F-2 fraction		F-3 fraction	
	S-1	S-2	S-3	S-4	L (0.2g)	Up (0.2g)	L (0.2g)	Up (0.1g)	L (0.9g)	Up (0.4g)
scopoletin	0.82	0.63	0.52	0.22	+	(+)	+	+	+	–
scopolin	0.50	0.78	–	–	(+)	(+)	–	–	++	+
fabiatrin	0.31	0.83	–	–	–	–	–	–	+++	+
synapic acid	–	–	0.83	0.21, 0.52	–	–	+	–	–	–
ferulic acid	–	–	0.78	0.30, 0.65	–	–	(+)	+	–	–
vanillic acid	–	–	0.77	0.49	–	–	+	+	–	–
p-coumaric acid	–	–	0.46	0.31, 0.77	–	–	+	+	–	–
p-hydroxybenzoic acid	–	–	0.43	0.58	–	–	+	(+)	–	–
caffeic acid	0.80	0.47, 0.58	0.20	0.33, 0.73	–	–	+	+	+	(+)
chlorogenic acid	0.60	0.75, 0.80	0.10	0.61, 0.75	–	–	++	+	+++	+
flavonol glycoside	0.57	0.39	–	–	–	–	(+)	–	(+)	–
flavonol glycoside	0.43	0.68	–	–	–	–	+	–	+	–
flavonol glycoside	0.15	0.80	–	–	–	–	+	–	+++	–

Abbreviations: L – leaves, Up – underground parts; – absent, (+) trace amounts; +,++,+++ increasing amounts

Table 2. ^{13}C- and ^{1}H-NMR data for compounds **I**, **II** and trifolin (14), (δ ppm in DMSO-d_6)*.

Carbon or proton number	**I**		**II**		Trifolin	
	δ C	δ H	δ C	δ H	δ C	δ H
kaempferol						
2	155.9		155.7		156.4	
3	133.2		132.9		132.6	
4	177.7		177.5		177.5	
5	160.9		161.2		161.2	
6	99.3	6.43 d (2.1)	98.6	6.25 d (2.0)	98.9	6.05 d (2.0)
7	162.8		164.0		165.0	
8	94.5	6.80 d (2.1)	93.6	6.46 d (2.0)	93.8	6.25 d (2.0)
9	156.2		156.3		156.5	
10	105.6		103.9		103.2	
1’	120.8		120.9		120.8	
2’and 6’	131.2	8.12 d (8.9)	131.1	8.14 d (8.9)	130.6	8.01 d (8.0)
3’ and 5’	115.4	6.92 d (8.9)	115.3	6.93 d (8.9)	115.2	6.86 d (8.3)
4’	160.2		159.8		159.7	
3-galactosyl						
1’’	98.3	5.69 d (7.5)	98.0	5.72 d (7.3)	102.6	5.32 d (7.0)
2’’	80.6		80.5		71.5	
3’’	73.2		73.3		73.4	
4’’	67.7		67.8		68.3	
5’’	76.0		75.9		75.5	
6’’	60.0		60.1		60.4	
2’’-glucosyl						
1’’’	104.3	4.58 d (7.5)	104.3	4.60 d (7.5)		
2’’’	74.5		74.4			
3’’’	77.0		76.9			
4’’’	69.7		69.7			
5’’’	76.5		76.8			
6’’’	60.8		60.7			
7-glucosyl						
1’’’’	99.8	5.08 d (7.2)				
2’’’’	73.4					
3’’’’	77.1					
4’’’’	69.7					
5’’’’	77.2					
6’’’’	60.7					

* Numbers is parentheses denote coupling constans (*J*) in Hz.

samples of **II** and **III** were refluxed with 2.5% H_2SO_4 for 1 h. Aglycone was filtered off and identified by coPC (S-1) with the standard kaempferol (R_f 0.88) or quercetin (R_f 0.78). The sugars from the aqueous solution were identified by coPC (S-1) and coTLC (S-6) and visualized by spraying with aniline phthalate and heating at 105°C. Compounds **I** and **II** gave D-galactose R_fs: 0.16 (S-1), 0.34 (S-6) and D-glucose R_fs: 0.16 (S-1), 0.49 (S-6), compound **III** gave D-glucose.

Partial acid hydrolysis. A 10 mg sample of **I** was refluxed with 10 mL of mixture of 1 M trifluoroacetic acid–MeOH (1:1, v/v) for 30 min. The mixture showed (PC, S-1) unhydrolyzable glycoside (**I**), monoglycoside (**I-a**) (R_f 0.60, yellow under UV light) and sugar (**I-b**). The **I-a** and **I-b** were obtained by preparative PC (S-1). Moreover, they were refluxed with 5% H_2SO_4 for 1 h. The **I-a** hydrolyzate showed kaempferol and D-glucose, the **I-b** showed D-glucose and D-galactose, identified by comparison with standards.

KAEMPFEROL 3-*O*-(2’’-*O*-β-D-GLUCOPYRANOSYL)-β-D-GALACTOPYRANOSIDE-7-*O*-β-D-GLUCOPYRANOSIDE (**I**)

Yellow needles, m.p. 217-219°C (MeOH), 228-230°C (70% MeOH). PC: 0.16 (S-1), 0.81 (S-2), 0.26 (S-5). UV λ_{max}^{MeOH} nm: 267, 321sh, 349; MeONa 270, 300sh, 337sh, 393; $AlCl_3$ 275, 301sh,

349, 394; $AlCl_3$/HCl 275, 300sh, 346, 396; AcONA 267, 308sh, 361, 395sh; AcONa/H_3BO_3 267, 325sh, 350. For ^{1}H- and ^{13}C-NMR data see Table 2.

KAEMPFEROL 3-*O*-(2''-*O*-β-D-GLUCOPYRANOSYL)-β-D-GALACTOPYRANOSIDE (**II**)

Yellow needles, m.p. 192-195°C (MeOH), 185-187°C (aq. MeOH). PC: 0.42 (S-1), 0.68 (S-2), 0.70 (S-5). UV λ_{max}^{MeOH} nm: 268, 300sh, 320sh, 348; MeONa 274, 326, 398; $AlCl_3$ 274, 305, 348, 395; $AlCl_3$/HCl 275, 305, 346, 395; AcONa 275, 307, 384; NaOAc/H_3BO_3 267, 300sh, 353. For ^{1}H- and ^{13}C-NMR data see Table 2.

QUERCETIN 3-*O*-β-D-GLUCOPYRANOSIDE, ISOQUERCITRIN (**III**)

Yellow powder, m.p. 212-216°C (MeOH). PC: 0.58 (S-1), 0.40 (S-2), 0.62 (S-5). UV λ_{max}^{MeOH} nm: 255, 268sh, 303sh, 355; MeONA 269, 325, 408; $AlCl_3$ 272, 303sh, 335sh, 436; $AlCl_3$/HCl 269, 300sh, 365, 398; AcONa 270, 323sh, 398; AcONa/H_3BO_3 267, 320sh, 379.

Quantitative analysis of the main polyphenolic compounds by RP-HPLC method

Solvents, standards, HPLC procedure

All solvents were of analytical reagent grade for HPLC (Merck, Germany). Coumarins-scopolin and fabiatrin isolated from the underground parts of *Scopolia lurida* (8), kaempferol 3-*O*-(2''-*O*-glucosyl)-galactoside-7-*O*-glucoside isolated from the leaves of *Scopolia caucasica* and chlorogenic acid (Fluka, Germany) were used as the standards. The calibration curves were established for each compound.

Solutions of standards were prepared by dissolving 1-2 mg of compounds in 10 mL of MeOH, in volumetric flasks. By successive dilutions, eight different solutions of compounds were prepared and used for the estimation of calibration curves. Correlation coefficients have not been lower than 0.9961 for all examined compounds.

The mobile phase consisted of solvent A (MeOH), solvent B (0.7% tetrahydrofuran) and solvent C (0.5% AcOH) with the elution profile as follows: 0-11 min: 5-16.4% A in B+C (linear gradient), 11-19 min: 16.4% A in B+C (isocratic).

The flow rate was 1.1 mL/min., the injection volume 20 μL (three times) and detection at 270 nm.

Sample preparation

A 2.0 g of raw material was thoroughly extracted with chloroform in a Soxhlet apparatus. After drying, the material was extracted with MeOH (5 × 100 mL) at the boiling temp. of the solvent for 2.5 h. The combined methanolic extracts were evaporated under vacuum. The residue of methanolic extracts from underground parts was dissolved in 25 mL of MeOH, however, methanolic extracts from the leaves were dissolved in 50 mL of methanol. Next, the obtained extracts from the leaves were diluted with MeOH in parts 1:9 (means flavonoid and chlorogenic acid) and 2:8 (means scopolin and fabiatrin). A part of the obtained solutions, without and after dilution were filtered through a syringe filter PTFE 13 mm and dosed successively to the optimized HPLC system. Quantitative determination of the compounds was made on the basis of linear dependence between their peak areas and the concentations. For each of the studied plant materials, three extracts were made and each prepared solution was injected three times into the optimized HPLC system. The results of determination of the polyphenolic compounds are presented in Table 3.

RESULTS AND CONCLUSIONS

This work is a continuation of our study on polyphenolic compounds in species of genus *Scopolia* Jacq. (8, 9, 11, 12).

In the leaves and underground parts of *S. caucasica*, the presence of coumarins, phenolic acids and flavonoid glycosides in the leaves were detected by means of 2D-PC method. Scopoletin in a very small quantity and scopolin (scopoletin 7-*O*-β-D-glucopyranoside) in a larger quantity were found in chloroform extract as well as in diethyl ether fraction and ethyl acetate-butanol fraction of methanolic extract. In the last fraction, fabiatrin (scopoletin 7-*O*-(6'-*O*-β-D-xylopyranosyl)-β-D-glucopyranoside) was additionally identified as predominant component. Investigation of the diethyl ether fraction of

Table 3. The contents (%) of main polyphenolic compounds in *Scopolia caucasica* Kolesn. ex Kreyer.

Compound	t_R(min)	Leaves	Underground parts
scopolin	12.45	0.260	0.110
fabiatrin	13.80	0.821	0.108
chlorogenic acid	14.97	3.980	0.198
kaempferol 3-(2''-glucosyl)-galactoside-7-glucoside	15.96	1.647	absent

methanolic extract on the presence of phenolic acids, allowed identification of seven compounds in the leaves and six compounds in the underground parts of this plant. Chlorogenic acid was a predominant constituent followed by other acids: p-coumaric, caffeic, ferulic, p-hydroxybenzoic, vanillic and additionally synapic acid in the leaves. The presence of three flavonoid glycosides was detected in the leaves of *S. caucasica*. There were no flavonoids in the underground parts of this plant.

Three flavonoid glycosides **I – III** were isolated from the ethyl acetate–butanol fraction of metanolic extracts from the leaves of *S. caucasica*, by preparative column chromatography. Compound **I** after complete acid hydrolysis gave kaempferol, D-glucose and D-galactose. The UV analysis revealed that aglycone possesses two blocked OH groups in positions C-3 and C-7. Partial hydrolysis gave two products: kaempferol 7-*O*-glucoside and disaccharide–glucosylgalactose or galactosylglucose at C-3 aglycone. The spectral analysis by ^{1}H- and ^{13}C-NMR methods confirmed that the disaccharide rest is substituted at C-3, moreover, it showed that the glycoside bonds are in β-configuration, and internal sugar is galactopyranose substituted at C-2'' by glucopyranose molecule. Thus, compound **I** was identified as kaempferol 3-*O*-(2''-*O*-β-D-glucopyranosyl)-β-D-galactopyranoside-7-*O*-β-D-glucopyranoside. Literature data show that the compound of such structure has been earlier isolated from the flowers of some species of *Nicotiana* genus (15), as well from the leaves of *Scopolia carniolica* and *S. sinensis* (11).

The physico-chemical properties of compounds **II** and **III**, as well as the products of their hydrolysis indicate that they are identical with kaempferol 3-*O*-(2''-*O*-β-D-glucopyranosyl)-β-D-galactopyranoside and quercetin 3-*O*-β-D-glucopyranoside, respectively (11, 14, 16, 17). Compounds **I** and **II**, relatively rare in nature, and compound **III** were isolated from the leaves of *S. caucasica* for the first time. However, the presence of rutin in the leaves of this taxon reported in ref. (7) could not be detected.

We have determined the content of the main polyphenolic components in the raw materials by the RP-HPLC method. The conducted determinations show a high concentration of the studied polyphenols in this specie. The leaves are characterized by significant contents of chlorogenic acid (ca. 4%), kaempferol 3-(2''-glucosyl)-galactoside-7-glucoside (ca. 1.6%) and coumarins (ca. 1.1%). Moreover, in the group of analyzed coumarins, the amount of fabiatrin was larger (ca. 0.8%) than scopolin (ca. 0.3%). The smaller amount of all studied polyphenolic compounds was seen in the underground parts in comparison with the leaves. Thus, in the lower parts, the content of coumarins is five times lower ca. 0.2%. Moreover, fabiatrin and scopolin occur in similar quantities, about 0.1%. Also chlorogenic acid is present in considerably smaller amount ca. 0.2%.

The existence of kaempferol triglycoside has not been affirmed in the underground parts. Thus, it confirms earlier results of the qualitative analysis.

A comparison of the obtained results with earlier studies conducted for *S. carniolica* Jacq., *S. lurida* Dun. and *S. sinensis* Hemsl. has led to conclusion that qualitative and quantitative composition of polyphenols in leaves and underground parts of *S. caucasica* Kolesn. ex Kreyer is the most similar to the species *S. carniolica* Jacq. (12). In particular, the similarity concerns the contents of chlorogenic acid and the flavonoid glycoside in both raw materials of these species. Therefore, the drawn conclusion can confirm legitimacy of Sandina's theory, which classifies both species *S. carniolica* Jacq. and *S. caucasica* Kolesn. ex Kreyer into the same *Scopolia* Jacq. genus. Moreover, according to this theory, *S. lurida* Dun. (*Whitleya stramonifolia* D. Don ex Sweet) was included in *Whitleya* D. Don ex Sweet genus, while *S. sinensis* Hemsl. (*Atropanthe sinensis* (Hemsl.) Pasch.) in *Atropanthe* Pasch. genus (2).

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