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NATURAL DRUGS

INVESTIGATION INTO BIOLOGICALLY ACTIVE CONSTITUENTS OF *GEUM RIVALE* L.

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Abstract: Aerial and underground parts of *Geum rivale* (*Rosaceae*) were investigated. Tiliroside, gallic acid, ellagic acid and a sterol fraction were isolated from aerial parts of the plant. The sterol fraction was analyzed using GC-MS. Eleven phenolic acids were identified in aerial parts of the plant, and eight in underground parts, by means of RP-HPLC analysis. The quantitative determination of phenolic acids, tannins and flavonoids was also carried out.

Keywords: *Geum rivale*, phenolic acids, tannins, flavonoids, sterols

Geum rivale belongs to the subfamily *Rosoideae* of the family *Rosaceae*. Plants belonging to that subfamily often contain large amounts of polyphenolic compounds, most important of which are tannins (condensed tannins as well as gallo- and ellagitannis) and flavonoids (kaempferol and quercetin glycosides). Considerable amounts of free ellagic acid were also reported along with small quantities of free gallic acid. Plants of the *Rosaceae* family are also rich in triterpenes (ursolic acid, oleanolic acid) and fitosterols (1).

In the genus Geum the most recognized specie is *G. japonicum*, in which a large variety of triterpenes and gallotannins were identified. Some flavonoids were isolated from *G. bulgaricum*. Several species of Geum were also proved to contain some amounts of essential oil (e.g., *G. japonicum*, *G. montanum*, *G. reptans*) (2).

G. rivale is a perennial herb widely distributed in Europe, central Asia and North America in temperate regions. It is common in Poland on damp meadows, river banks and forest edges. Rhizome of the plant is used in folk medicine as astringent and anti-inflammatory agent (3, 4).

The whole plant exhibits high tannin contents (5) and more recent studies reveal that other phenolic compounds are also present (6, 7). Thirteen flavonoids have been found in aerial parts of the plant along with several phenolic acids (6, 7). In the underground parts of the plant small amounts of oil was detected (8).

The aim of this study was to investigate the constituents of petroleum ether and methanol extracts from aerial parts of *G. rivale* as well as qualitative analysis of free phenolic acids and quantitative analysis of phenolic acids, tannins and flavonoids in aerial and underground parts of the plant.

EXPERIMENTAL

Plant material

Aerial and underground parts of *G. rivale* were collected in Łódź (Piłsudski Park, Zdrowie), in May 2009. The material was identified as *G. rivale* by Prof. Jan Gudej, Department of Pharmacognosy, Medical University of Łódź, Poland. A voucher specimen was deposited in Department of Pharmacognosy, Medical University of Łódź, Poland.

Chemicals and reagents

Column chromatography was performed on MN-Kieselgel 60 (70-270 mesh), Sephadex LH-20 (Sigma-Aldrich) and polyamide (Roth; 0.05-0.16 mm).

Acetonitrile, phosphoric acid and water (HPLC solvent grade) were obtained from POCH (Gliwice,

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Poland). Other reagents used for the extraction of plant material were obtained from Chempur (Piekary Śląskie, Poland). Phenolic acid standards were purchased from Koch-Light.

Extraction

The air-dried pulverized aerial parts (1000 g) of *G. rivale* were exhaustively extracted with petroleum ether and chloroform in Soxhlet apparatus and then with boiling methanol. After evaporation of the solvents under reduced pressure, the following amounts of extracts were obtained: 25.3 g of petroleum ether extract, 12.8 g of chloroform extract and 118 g of methanol extract. The methanol extract was dissolved in hot water and left for 24 h. After that time, water-insoluble precipitate was centrifugated and water solution was successively extracted with diethyl ether, ethyl acetate and n-butanol to yield 2.2, 15.8 and 32.3 g of extracts, respectively.

Isolation

The petroleum ether extract from aerial parts of the plant was subjected to column chromatography over silica gel (50 × 650 mm) and eluted with solvents of increasing polarity (n-hexane, gradient n-hexane to benzene, benzene, gradient benzene to chloroform, chloroform, gradient chloroform to ethyl acetate, ethyl acetate, gradient ethyl acetate to methanol, methanol). The fractions eluted with n-hexane-benzene 2 :3 v/v (FD), and n-hexane-benzene 3 : 7 v/v (FE) were analyzed using GC-MS.

GC-MS analysis was performed using an Agilent 6890N gas chromatograph with Agilent 5973 mass detector. Samples were analyzed on a DB-5MS column (30 m × 0.25 mm, 0.25 µm). The oven temperature was raised from 80 to 270°C (0-60 min) at a rate of 10°C /min. The mass spectrometer was operated with the electron impact (EI) at 70 eV as ionization potential. The injector temperature was kept constant at 250°C. The detector was kept at 250°C. The carrier gas was helium at a constant flow rate of 0.6 mL/min.

The diethyl ether extract from aerial parts of *G. rivale* (2.5 g) was fractionated by column chromatography over polyamide (24 × 490 mm). The column was eluted with water-methanol gradient. Fractions eluted with water (F1), 60% aqueous methanol (F6) and 100% methanol (F8) were further purified by column chromatography over Sephadex LH-20 (20 × 410 mm) leading to isolation of compound **I** (from fraction F1), compound **II** (from fraction F6) and compound **III** from (fraction F8). The isolated compounds were identified using UV and NMR spectra.

Analysis of phenolic acids

The diethyl ether extract of aerial and underground parts of *G. rivale* was purified by extraction with sodium bicarbonate, acidification and reextraction with diethyl ether. The diethyl ether extract was evaporated under reduced pressure and the residue was dissolved in methanol and subjected to HPLC analysis. RP-HPLC analysis was carried out with a Hewlett-Packard 1100 series chromatograph equipped with a quaternary pump HP1311A, an HP1322A vacuum degasser, an HP3395 series integrator, an HP1314A variable wavelength UV/VIS detector, a manual injector 20 µL and an HPLC column LichroCART (250 × 4 mm) packed with Lichrosphere RP-18 (dp = 5 µm). The mobile phase consisted of solvent A (0.5% water solution of H_3PO_4) and solvent B (acetonitrile) with the elution profile as follows: 0-9 min: 6-10% B in A, 9-15 min: 10-13% B in A, 15-35 min: 13% B in A, 35-40 min: 13-30% B in A. The elution was carried out at room temperature at a flow rate of 1.0 mL/min, injection volume 20 µL, detection wavelength 254 nm. Identification of phenolic acids was based on their retention times, which were compared to the retention times of standards.

The quantitative analysis of phenolic acids was performed according to the method described in Polish Pharmacopoeia VI (9). All samples were analyzed in triplicates and the results were averaged. The total phenolic acid content is expressed as milligrams of caffeic acid equivalents (CAE) per 1 g of dry plant material.

Analysis of tannins

Quantitative analysis of tannins was carried out according to the method described in DAB X (10). All samples were analyzed in triplicates and the results were averaged. The total tannin content is expressed as milligrams of tannins per 1 g of dry plant material.

Analysis of flavonoids

Quantitative analysis of flavonoids was carried out according to the method described in Polish Pharmacopoeia VIII (11). All samples were analyzed in triplicates and the results were averaged. The total flavonoid content is expressed as milligrams of quercetin equivalents (QE) per 1 g of dry plant material.

RESULTS AND DISCUSSION

Isolation

Dried and powdered aerial parts of *G. rivale* were extracted successively with petroleum ether,

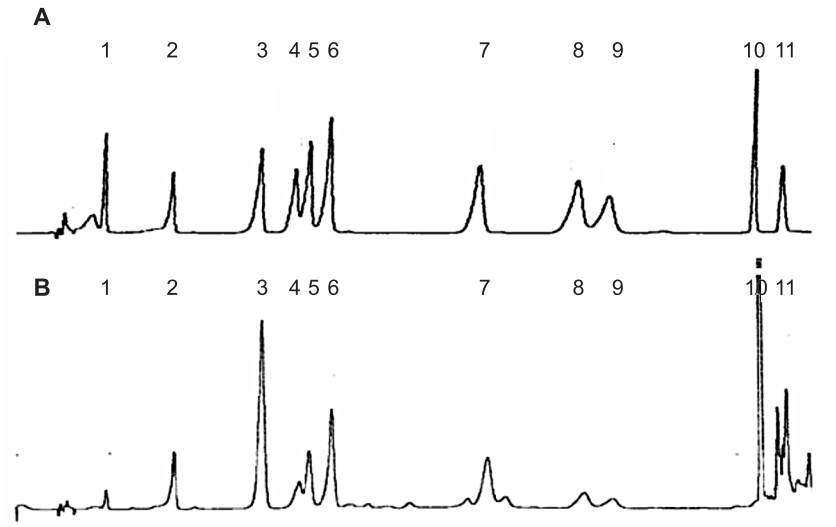

Figure 1. Sample chromatographs of RP-HPLC analysis of free phenolic acids from *G. rivale*. **A** – standards, **B** – aerial parts of *G. rivale*. Numeration of peaks according to Table 1

Table 1. The results of the RP-HPLC analysis of free phenolic acids in *G. rivale*.

No.	Phenolic acid	RP-HPLC Rt [min] ± SD	Presence in the plant material	
			Aerial parts	Underground parts
1	Gallic	5.045 ± 0.131	+	+
2	Protocatechuic	8.921 ± 0.184	++	+
3	p-Hydroxybenzoic	13.994 ± 0.154	+++	++
4	Vanillic	16.046 ± 0.187	+	-
5	Caffeic	16.648 ± 0.162	++	-
6	Syringic	17.909 ± 0.144	+	+
7	p-Coumaric	26.035 ± 0.163	++	-
8	Ferulic	32.241 ± 0.165	+	+
9	Sinapic	33.845 ± 0.173	+	+
10	Ellagic	40.903 ± 0.180	++++	++++
11	Salicylic	42.446 ± 0.169	++	+

+, ++, +++, ++++ - increasing amout of phenolic acid

Table 2. The results of quantitative determination of tannins and flavonoids in *G. rivale*.

Compounds	Content in the plant material	
	Underground parts	Aerial parts
Phenolic acids [mg CAE/1 g]	18.9	5.9
Tannins [mg/1 g]	136.1	31.4
Flavonoids [mg QE/1 g]	0.3	3.0

chloroform and methanol. The methanol extract was fractionated by extraction with diethyl ether, ethyl acetate and n-butanol. The petroleum ether extract was subjected to column chromatography using silica gel to give fractions FA and FB, that were analyzed by GC-MS. Analysis of FA showed high content of β-sitosterol (about 70% of the fraction) as well as small amounts of other sterols – campesterol

and stigmasterol. Fraction FB contained traces of α-amyrin and a mixture of long-chain aliphatic alcohols.

The diethyl ether extract was fractionated by a combination of column chromatography over polyamide and Sephadex LH-20. Three known compounds were obtained. The compounds were identified as tiliroside (**II**), gallic acid (**I**) and ellagic acid (**III**) by comparing their spectroscopic data with the data reported in the literature (12). Compounds **I** and **III** have been already isolated from *G. rivale* (6, 7). Tiliroside is a common constituent of plants from *Rosaceae* family (12-14) and its presence in *G. rivale* was also signalized (6).

HPLC analysis of phenolic acids

The qualitative analysis of phenolic acids was performed using RP-HPLC method. The results are given in Table 1. Figure 1 presents a sample chromatogram. Eleven phenolic acids were identified in aerial parts of *G. rivale*, while only eight in underground parts. Three of them (gallic acid, ellagic acid and caffeic acid) have been already isolated from *G. rivale* (6, 7). Ellagic acid was the dominant constituent of both extracts.

Quantitative analysis of phenolic acids, tannins and flavonoids

The results of the quantitative analysis of phenolic acids, tannins and flavonoids are presented in Table 2. Underground parts of *G. rivale* were proved to contain about three times more phenolic acids (18.9 mg/1 g) in comparison with aerial parts (5.9 mg/1 g). Especially high content of tannins was detected also in underground parts of the plant (about 130 mg/1 g), whereas amount of flavonoids was very low (only 0.3 mg QE/1 g). The content of tannins and flavonoids in aerial parts of the plant was about 30 mg/1 g and 3 mg QE/1 g, respectively. The previous studies suggested that the plant contains much larger amounts of tannins (even up to 29% in the rhizome) (5) but the methods used are no longer considered as reliable and might have given false high results.

CONCLUSIONS

Current study recognizes some of phenolic acids present in aerial and underground parts of *G. rivale*. Quantitative analysis of phenolic acids, tannins and flavonoids was also carried out. These groups of active compounds are considered to be mainly responsible for the biological activity of the plants belonging to the *Rosaceae* family. The result indicates that underground parts of *G. rivale* contain much larger amount of phenolic acids and tannins than aerial parts, which are in turn richer in flavonoids and in which the set of phenolic acids is more diverse. Our study and other studies that have been carried out so far, suggest that due to interesting composition, both aerial and underground parts of *G. rivale* could be used more widely in medicine.

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