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PHYTOCHEMICAL STUDY ON SOME POLYPHENOLS OF *Geranium pyrenaicum*

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In order to continue our previous studies concerning Geranium pyrenaicum *Burm. (Geraniaceae), we have performed spectrophotometric determinations and a HPLC study of some polyphenols. We have analyzed the dried* Geranii pyrenaici herba *(harvested from Cluj-Napoca, district of Cluj, Romania). We have established the content in flavonoids (0.316%), phenolic acids (0.099%), tannins (5.295%), and anthocyanins (12.030 mg/100 g vegetal product). We have identified and measured by HPLC the following compounds: hyperoside (21.61 μg/100 mg), ellagic acid (1810.44 μg/100 mg), isoquercitrine (11.197 μg/100 mg), and caftaric acid (76.83 μg/100 mg). We have also analyzed by HPLC a hydrolyzed sample of the same drug in which we have identified and measured: ellagic acid (4139.33 μg/100 mg), quercetol (29.65 μg/100 mg), kaempherol (41.48 μg/100 mg), and caftaric acid (20.721 μg/100 mg).*

Key words: *Geranium pyrenaicum* Burm., polyphenol, HPLC, spectrophotometric methods.

Species of *Geranium* have been used for a long time in folk medicine for different actions, including wound healing, diarrhea, ulcer healing, and antibacterial properties [1–3]. These actions are due to the various contents of polyphenolic compounds. Studies have been conducted by researchers from different countries all over the world in order to study these compounds [4–9].

We have initiated a comparative phytochemical study of some *Geranium* species from Romania using methods based on spectrophotometry, identity reactions, and HPLC [10–12]. We continue our work by analyzing some polyphenols from *Geranium pyrenaicum* Burm., using spectrophotometric techniques and an original HPLC method developed in 2003 by a group of young researchers from the University of Medicine and Pharmacy of Cluj-Napoca [13]. This method is of particular interest when the amounts of polyphenols in the plant are too small to allow investigation by TLC methods. It also allows measuring the amount for every identified active principle, while the quantitative spectrophotometric methods that we have used before do not allow measuring each particular polyphenolic compound.

The results of the quantitative spectrophotometric determinations are: flavonoids (0.316%), phenolic compounds (0.099%), tannins (5.295%), anthocyanins (12.030 mg/100g dried product).

From these quantitative determinations, the tannins seem to be the main group of active principles, in accordance with previous data cited in the international literature.

We have identified and measured by HPLC six polyphenolic compounds in *Geranii pyrenaici herba,* working in the conditions described before: hyperoside, ellagic acid, isoquercitrine, caftaric acid, quercetol, and kaempherol. Good linearity of the calibration curve for a five-point plot was established in the 0.5–50 μg/mL range for all identified compounds. We present the concentrations (μg polyphenolic compound/100 mg dried herba) for these six compounds (Table 1). We also present the HPLC chromatogram for *Geranii pyrenaici herba* samples, before hydrolysis (Fig. 1) and after hydrolysis (Fig. 2).

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TABLE 1. Concentration of Polyphenolic Compounds (μg Polyphenolic Compound/100 mg Dried Herba)

Polyphenolic compound	Concentration, μg/100 mg	
	BH	AH
Caftaric acid	76.83	20.72
Hyperoside	21.61	-
Ellagic acid	1810.44	4139.33
Isoquercitrine	11.19	-
Quercetol	-	29.65
Kaempherol	-	41.48

BH: before hydrolysis; AH: after hydrolysis.

TABLE 2. Retention Times for All Standards

Polyphenolic compound	Retention time 330 nm	Polyphenolic compound	Retention time 330 nm
Caftaric acid	3.27	Ellagic acid	19.90
Gentisic acid	3.76	Isoquercitrine	20.27
Caffeic acid	6.10	Rutoside	20.78
Chlorogenic acid	6.80	Quercitrine	23.64
p-Coumaric acid	9.49	Quercetol	27.57
Ferulic acid	12.80	Patuletine	29.39
Sinapic acid	15.01	Luteoline	29.93
Cichoric acid	15.83	Kaempherol	32.50
Hyperoside	19.32	Apigenine	33.95

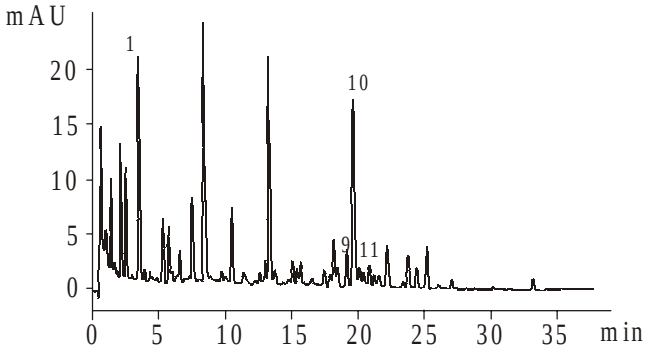

Fig. 1

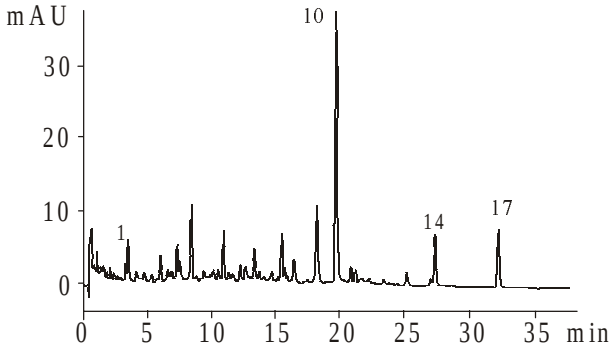

Fig. 2

Fig. 1. HPLC chromatogram of *Geranii pyrenaici herba* before hydrolysis: *1* – caftaric acid, *9* – hyperoside, *10* – ellagic acid, *11* – isoquercitrine.

Fig. 2. HPLC chromatogram of *Geranii pyrenaici herba* after hydrolysis: *1* – caftaric acid, *10* – ellagic acid, *14* – quercetol, *17* – kaempherol.

EXPERIMENTAL

We have analyzed the air-dried drug *Geranii pyrenaici herba* (harvested from Cluj-Napoca, district of Cluj, Romania).

Spectrophotometric determinations:

The quantitative analysis of tannins was made using the method described in the Romanian Pharmacopoeia Xth Edition [14].

The quantitative analysis of flavonoids and phenolic acids was made using the methods described in the Romanian Pharmacopoeia IXth Edition for the drug *Cynarae folium* [15].

The quantitative determination of anthocyanins was made using the technique described by Markakis [16].

HPLC determinations:

Apparatus and Chromatographic Conditions. We used an Agilent 1100 HPLC Series (Agilent technologies, USA) equipped with a degasser G1322A, a quaternary gradient pump G1311A, an autosampler G1313A, a column oven G1316A, and a Zorbax SB-C18 reversed-phase analytical column 100 mm × 3.0 mm i.d., 3.5 μm particle (Agilent technologies, USA) and we operated at 48°C. The mobile phase was a binary gradient: methanol and buffer solution. The buffer solution was prepared by dissolving potassium dihydrogen phosphate (40 mM) in water and the pH was adjusted to 2.3 with 85% orthophosphoric acid. The gradient began with a linear gradient at 5% methanol and 42% methanol over the first 35 min, followed by isocratic elution with 42% methanol over the next 3 min. The flow rate was 1 mL/min and data were collected at 330 nm. The injection volume was 10 mL.

Sample Preparation. A 200 mg air-dried portion of powdered herba was placed in a 10 mL centrifuge tube; 2 mL water and 2 mL ethanol were added in the centrifuge tube. In order to study the flavonoid aglycones that can be obtained by hydrolysis we prepared a second sample containing 200 mg air-dried powdered herba, 2 mL hydrochloric acid 2 M, and 2 mL methanol, all placed in a 10 mL centrifuge tube. In both samples we added 200 mL ascorbic acid 10% solution (Sicomed Bucharest, Romania) as antioxidant. The mixtures were heated at 80°C for 30 min on a water bath, then they were ultrasonicated for 15 min and finally heated again for another 30 min at 80°C on a water bath. After extraction the mixtures were centrifuged at 4000 rpm and the remaining solids were extracted two times with additional 5 mL buffer solution using the same procedure. The combined extracts were diluted with buffer solution in a 25 mL volumetric flask and filtered through a 0.45 μm filter before injection.

Detection. The compounds were UV-detected at 330 nm. All compounds were identified by external standard addition and comparison of their retention times with those of the standards under the same chromatographic conditions. Quantitative determinations were performed using the external standard method.

Standards. We present the retention times for all used standards at 330 nm (Table 2) because the HPLC for the standards is no longer shown.

We have confirmed by HPLC the fact that tannins are the main group of active principles (from six identified compounds, the ellagic acid has the highest concentration) and we have demonstrated indirectly the presence of ellagic tannins (the amount of ellagic acid increases after hydrolysis).

We have also identified and measured two flavonoids (hyperoside, isoquercitrine) and one phenylpropanoic compound (caftaric acid).

The flavonoidic aglycones (quercetol and kaempherol) are present as part of the molecules of the flavonoids (they were not identified as free compounds).

Our results represent the basic data for future pharmacological determinations based on phytochemistry.

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