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Investigation of phenolic compounds of *Antennaria dioica* (L.) Gaertn. Herb

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Abstract

It was revealed and defined the quantitative content of individual phenolic compounds in the herb of cat's foot (*Antennaria dioica* (L.) Gaertn.) by the method of high performance liquid chromatography (HPLC). It was detected 6 hydroxycinnamic acids (chlorogenic, rosmarinic, *p*-coumaric, ferulic, caffeic and isomer of chlorogenic acid), 6 flavonoids (quercetin, isoquercetin, apigenin, luteolin, rutin, hyperoside) and 2 coumarins (umbelliferone and coumarin).

The spectrophotometric method determined the quantitative content of phenolic compounds, flavonoids and hydroxycinnamic acids in the herb of cat's foot (*Antennaria dioica* (L.) Gaertn.). It was detected that hydroxycinnamic acids dominated in quantity.

Keywords: phenolic compounds, flavonoids, hydroxycinnamic acids, coumarins, cat's foot (*Antennaria dioica* (L.) Gaertn.), spectrophotometric method, high performance liquid chromatography method (HPLC)

1. Introduction

Antennaria dioica Gaertn. (Asteraceae) is a dioecious, perennial plant that grows in wastelands, dry grasslands and sandy or stony places [1]. It is scattered almost throughout the whole Ukraine; cultivated.

Nowadays the herb of *Antennaria dioica* Gaertn. is used only in folk medicine. It is recommended as a wound healing, haemostatic and cholagogue, especially used to cure hemopoiesis; intestinal, haemorrhoidal, uterine bleeding; hernia; bloody diarrhoea and pertussis. According to the choleric effect *Antennaria dioica* (L.) Gaertn. is not inferior to the *Helichrysum arenarium* (L.) DG. The herb is also used to cure the diseases of throat, lung tuberculosis and hypertension and as a sedative. Externally it is used to cure children's eczema, abscesses, skin tuberculosis; herb's powder is used to cure the wounds [2].

The analysis of literature sources showed that the herb of cat's foot (*Antennaria dioica* (L.) Gaertn.) contains tannins, resins, saponins, alkaloids, essential oils, ascorbic acid, vitamin K and phytosterol [3]. The content of its phenolic compounds was studied insufficiently. Therefore, the purpose of our research was to establish a qualitative composition and determine the quantitative content of phenolic substances in the herb of cat's foot (*Antennaria dioica* (L.) Gaertn.).

2. Materials and methods

The object of investigation was the herb of cat's foot (*Antennaria dioica* (L.) Gaertn.). It was harvested on the territory of Vyzhnytsya district, Chernivtsi region, Ukraine during the flowering period in 2015.

The qualitative composition and the quantitative content of phenolic compounds in the herb of cat's foot (*Antennaria dioica* (L.) Gaertn.) were investigated by the method of HPLC. Chromatographic separation was performed by the chromatograph Agilent 1200 3 D LC System Technologies (USA) with diode array detector G1315C, on the column Supelco Discovery C₁₈ HPLC with a grain size of 5 µm at the column thermostat temperature 25 °C. Injection of the samples was carried out by autosampler, volume of samples was 10 µl, flow rate — 0.7 ml/min, eluent working pressure — 10 000–12 000 kPa [4-5].

Solvent gradient grade acetonitrile and orthophosphate acid, for HPLC, > 99.9% (Sigma-Aldrich) were used to prepare the mobile phase. Bidistilled water was obtained on Simplicity SIMSV00 Water Purification System Millipore (Merck KGaA, Darmstadt, Germany). Solvent gradient grade methanol, for HPLC, > 99.9 % (Sigma-Aldrich) was used for the extraction of

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hydroxycinnamic acids. Standard samples are chlorogenic, caffeic, *p*-coumaric, ferulic, rosmarinic acids; apigenine, rutin, hyperoside, quercetin, isoquercetin (quercetin-3-D glucoside), kaempferol, coumarin, scopoletin and umbelliferone produced by "Sigma Chemical Co Aldrich"

Sample preparation: 1 g of plant material (accurately mass), extracted with 50 ml of 60% methanol solution for 15 minutes in a water bath with a reflux condenser while stirring. Then filtered, quantitatively transferred to a volumetric flask of 100 ml capacity, the volume of the solution was adjusted to the mark with 60% methanol. The obtained solution was filtered through a membrane filter with a pore size of 0.45 μm .

The following conditions were applied to separate phenolic compounds: gradient elution with a mixture of bidistilled water, acidified with orthophosphate acid to pH = 2.85 (A), and acetonitrile (B): 0 minutes 5% "B", 8 minutes 8% "B", 15 minutes 10% "B", 30 minutes 20% "B", 40 minutes 40% "B", 41–42 minutes 75% "B", 43–50 minutes 5%, with the detection length 320, 330 nm (hydroxycinnamic acid) and 0

minutes 12% "B", 30 minutes 25% "B", 33 minutes 25% "B", 38 minutes 30% "B", 40 minutes 40% "B", 41 minutes 80% "B", 49 min 12% with the detection length of 255, 340 nm (flavonoids). Total analysis time was 50 minutes [6-7].

Quantitative determination of phenolic compounds in the herb of cat's foot (*Antennaria dioica* (L.) Gaertn.) is based on the spectrophotometric method. The determination of hydroxycinnamic acids content was carried out recalculating on chlorogenic acid at wavelengths of 327 nm, of phenolic compounds content recalculating on gallic acid at 270 nm, and of flavonoids content recalculating on rutin at 415 nm on the spectrometer Lambda 25 Perkin Elmer (USA) [6].

3. Results and Discussion

The HPLC method identified and established the quantitative content of flavonoids, hydroxycinnamic acids and coumarins in the herb of *Antennaria dioica* (L).

HPLC chromatograms of phenolic compounds of *Antennaria dioica* (L) raw materials are shown in Figures 1–2.

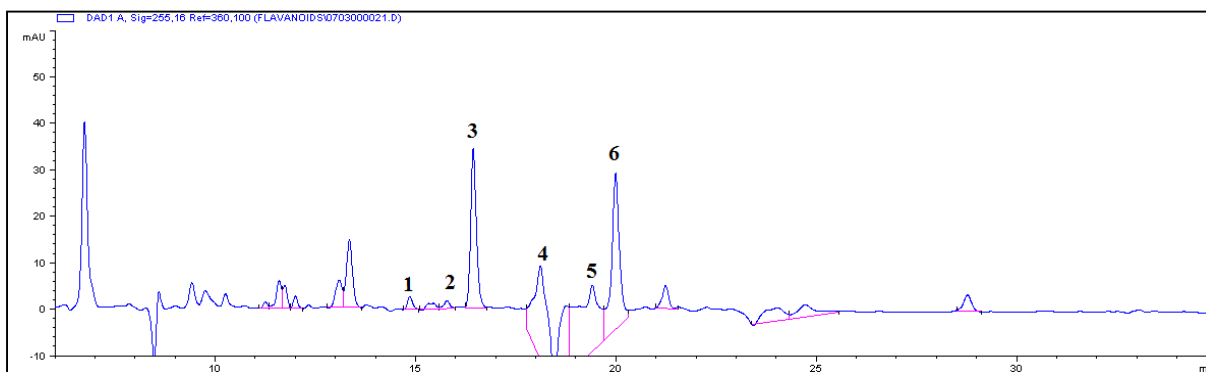


Fig 1: HPLC chromatogram of phenolic compounds of *Antennaria dioica* (L.) herb at $\lambda = 255$ nm: 1 — cumarine, 2 — quercetine, 3 — luteoline, 4 — hyperozid, 5 — rutin, 6 — quercetin-3-D-glycoside

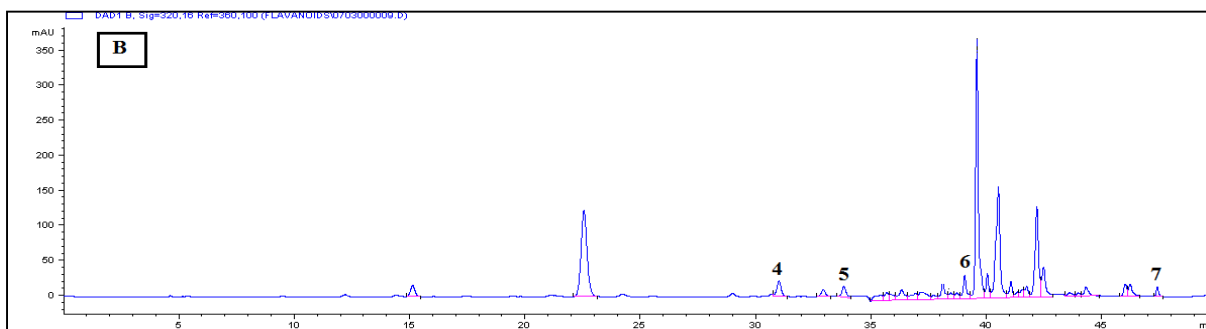
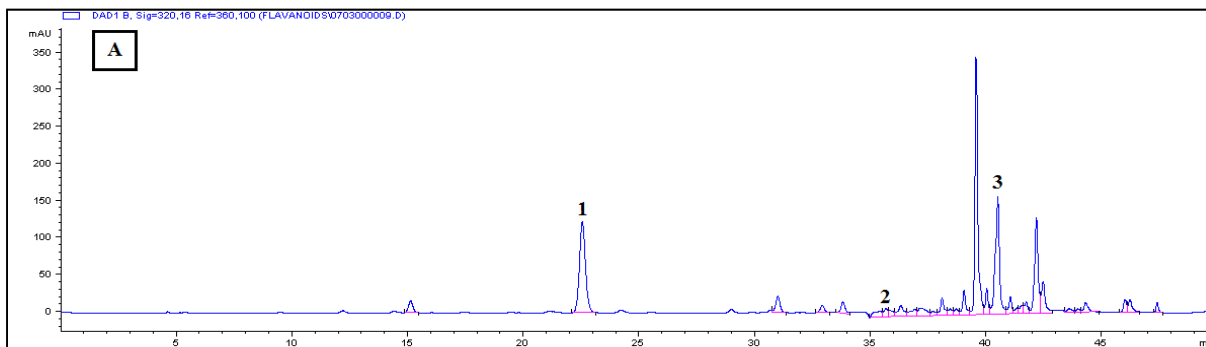


Fig 2: HPLC chromatograms of phenolic compounds of *Antennaria dioica* (L.) herb: A) at $\lambda = 330$ nm: 1 — chlorogenic acid, 2 — apigenin, 3 — rosmarinic acid; B) at $\lambda = 320$ nm: 4 — caffeic acid, 5 — *p*-coumaric acid, 6 — ferulic acid, 7 — umbelliferone.

In the investigated species of *Antennaria dioica* (L.), the following compounds of flavonoid nature were identified: quercetin-3-D-glycoside, luteolin, rutin, hyperozid, quercetin, apigenin; hydroxycinnamic acids: rozmarinic, chlorogenic and its isomer, ferulic, caffeic, *p*-coumaric; coumarins: umbelliferone and coumarin (Table 1).

Table 1: Quantitative amount of phenolic compounds in the *Antennaria dioica* (L.) herb

Phenolic Compounds	RT, min.	Quantitative amount, %
Hydroxycinnamic acids		
Chlorogenic acid	22.538	0.79
Isomer of chlorogenic acid	39.570	1.14
Rozmarinic acid	40.499	0.94
<i>p</i> -coumaric acid	33.808	0.03
Caffeic acid	30.991	0.06
Ferulic acid	39.041	0.07
Flavonoids		
Quercetin-3-D glycoside	19.982	0.16
Apigenin	35.664	0.03
Rutin	19.403	0.05
Hyperozid	18.105	0.03
Luteolin	16.428	0.12
Quercetin	15.772	0.01
Coumarins		
Umbelliferone	47.383	0.04
Coumarin	14.844	0.01

The results of researches showed that among the hydroxycinnamic acids in the investigated object rozmarinic (0.94%), chlorogenic (0.79%) and isomer of chlorogenic acid (1.14%) are dominated; among the flavonoids — quercetin-3-D-glycoside (0.16%) and luteolin (0.12%).

While determining the quantitative amount of phenolic compounds by the spectrophotometric method, it was found out that the hydroxycinnamic acids content recalculating on chlorogenic acid was (7.99 ± 0.11) %; phenolic compounds content recalculating on gallic acid was ($6.86 \pm 0,01$) %; flavonoids content recalculating on rutine (2.09 ± 0.001) % (Fig. 3).

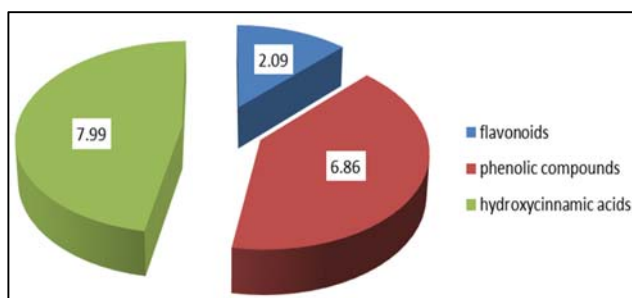


Fig 3: Quantitative amount of phenolic compounds in the herb of *Antennaria dioica* (L.)

4. Conclusions

1. It was revealed and defined the quantitative content of individual phenolic compounds in the herb of cat's foot (*Antennaria dioica* (L.) Gaertn.) by the HPLC method: hydroxycinnamic acids (rozmarinic, caffeic, ferulic, *p*-coumaric, chlorogenic and its isomer), flavonoids (quercetin-3-D-glycoside, luteolin, rutin, hyperoside, quercetin and apigenin) and cumarins (umbelliferone and coumarin).
2. The spectrophotometric method determined the

quantitative content of phenolic compounds, flavonoids and hydroxycinnamic acids in the herb of cat's foot (*Antennaria dioica* (L.) Gaertn.). It was detected that hydroxycinnamic acids dominated in quantity — (7.99 ± 0.11)%.

3. Taking into account the high content of phenolic compounds in the herb of cat's foot (*Antennaria dioica* (L.) Gaertn.), it can be considered perspective for further pharmacological studies and for development of new drugs.

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