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## PHENOLIC COMPOUND COMPOSITION OF HERB OF PULSATILLA PRATENSIS (L.) MILL

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**Abstract.** The manuscript presents the results of the determination of phenolic compounds in the herb of meadow pasqueflower. Determination of phenolic compounds has been performed by the high performance liquid chromatography (HPLC). Identification of separated substances was performed by the comparison of the retention time of main peaks in the chromatogram of meadow pasqueflower herb with the retention time of standard substances and by reference of UV-spectra with spectra of standard substances like quercetin, hyperoside, luteolin, rutin, apigenin-7-glucoside, caffeic and ferulic acid, umbelliferone and scopoletin.

9 phenolic compounds were identified in the meadow pasqueflower herb using HPLC. Such flavanoids as rutin, hyperoside, apigenin-7-glucoside, luteolin and quercetin were identified and quantified in the herb of meadow pasqueflower. Hyperoside (0.02437 %) and luteolin (0.01568 %) are found in higher quantity. Content of hydroxycinnamic acids, such as caffeic and ferulic acids, is 0.00257 % and 0.00228 %, respectively. Coumarins like umbelliferone (0.00770 %) and scopoletin (0.00037 %) were also found.

**Keywords:** meadow pasqueflower, phenolic compounds, herb, high performance liquid chromatography.

**Introduction.** Meadow pasqueflower (*Pulsatilla pratensis* (L.) Mill. s.l. (incl. *P. bohémica* (Skalický) Tzvelev = *P. pratensis* (L.) Mill. subsp. *bohémica* Skalický; *P. nigricans* auct. non Stöerck, nom. illeg.; *P. ucranica* (Ugr.) Wissjul.) is a creeping stem grass of Ranunculaceae family. This plant grows on the territory of the Balkans, in the Middle and Eastern Europe. Meadow pasqueflower is native of forest, forest-steppe and steppe zone of Ukraine except western-most regions and Crimea [3, 5].

Populations of this plant are numerous but nowadays their quantity decreases. Slope terracing at the forest-growing, devastation of meadow steppes, pasturage, gathering the flowers for bunches, grubbing up and trampling down the grass belong to the main reasons of population change [5, 6].

Protective measures have been implemented in order to protect population of this plant, particularly, meadow pasqueflower is protected in National park “Podilski Tovtry”, “Svyati Hory”, in wildlife management areas: Kanevskiy, Ukrainian steppe, Luhansk, Black Sea biosphere reserve, and in the regional landscape parks: «Mejirechenskiy» and «Granitno-stepove-pobujjya». Culling flowers, burning of dry grass, breach of growth conditions are restricted. It has to be said that discussed plant is cultivated in botanical gardens: Donetsk botanical garden of National Academy of Sciences of Ukraine, Krivoy Rog botanical garden of National Academy of Sciences of Ukraine, botanical gardens of Dnipropetrovsk and Odesa universities, in arboretum of biosphere reserve “Askania-Nova” [5].

Meadow pasqueflower contains anemonin, ranunkulin, protoanemonic, ether oil, tannin, vitamin C, organic acids, flavonoids, traces of alkaloids, tanning substances (near 4.5 %). Seeds contain fatty oil (17.4 %) [2, 6].

Phenolic compounds show high biological activity and they are active metabolites of cellular metabolism. These compounds play an important role in different physiological processes. Medicinal preparations based on the phenolic compounds are widely used as antimicrobial, antiinflammatory, haemostatis, cholagogue, diuretic, hypotensive, tonic agents.

This plant has hypotensive, sedative, hypnotic, antispasmodic, antimicrobial, anesthetic activity, it decreases heart rate and stimulates breath. In scientific medicine meadow pasqueflower is

used at the treatment of hypertensive disease (stages 1 and 2), glaucoma and at the heartbeating, it is also used as sedative and hypnotic agent at the mental illnesses. In traditional medicine it is often applied as depressant at the increased nervous fever and associated headache, faintness, anhypnosis, hysterical chorea, convulsions, dysmenorrhoeas. Meadow pasqueflower is indicated for the treatment of chin cough, bronchial allergy, bronchitis, bilious headache as antispasmodic drug [2]. This plant is widely used in homeopathy. Fresh plants gathered at the time of flowering are the herbal raw for homeopathic preparations named Pulsatilla [4].

Plant antianxiety drugs suppress excitative processes of CNS. Like bromides herbal medicinal products affect the cerebral cortex and also the subcortical centers of brain. Effect of herbal medicinal products that are aqueous or alcohol extracts from crude medicines is due to the combination of active substances. Esters, ethereal oils, organic acids and alkaloids are of great importance. Sedative herbal medicinal products have also antispasmodic activity on smooth muscle organs [4].

It is known that tea or tincture from meadow pasqueflower herb is used as sedative or anaesthetic in gynecology [5].

Pulsatilla-based homeopathic medicine prepared by potentiometry is also known [6]. Actaea racemosa and Magnesium phosphoricum are prepared by the homeopathic method using the same dilution of medicinal components.

Detailed photochemical study of meadow pasqueflower as starting materials of herbal origin is actual in view of common usage of plants in traditional and official medicine and presence of herbal medicinal products on Ukrainian pharmaceutical market.

That's why *the purpose of our work* was investigation of phenolic compounds of meadow pasqueflower herb.

**Experimental section.** Determination of phenolic compounds of meadow pasqueflower herb was performed by the high performance liquid chromatography (HPLC).

The chromatographic separation was performed on liquid chromatograph Shimadzu HPLC-system, ser.20, equipped with photodiode array detector. Chromatographic conditions are the following:

1. 250 mm x 4.6 mm Phenomenex Luna C18(2) column, 5 $\mu$ m;
  2. column temperature is 35 °C;
  3. detection wavelength is 330 nm (for hydroxycinnamic acids, glycosides, flavonoids), 350 nm (for aglycons of flavonoids), 280 nm (for tanning substances);
  4. flow rate of mobile phase is 1 mL/min;
  5. sample injection is 5  $\mu$ l ;
- Mobile phase: Eluent A: 0.1 % trifluoroacetic acid solution in water;  
Eluent B: 0.1 % trifluoroacetic acid solution in acetonitrile.

Table 1.

Run time (min)	Eluent A, %	Eluent B, %
0–5	95	5
5–35	95 → 75	5 → 25
35–40	75	25
40–60	75 → 50	25 → 50
60–65	50 → 20	50 → 80
65–70	20	80
70–85	95	5

Aforementioned chromatographic conditions make possible the determination of biologically active substances in the meadow pasqueflower herb.

Calculations were done using the following formula, % (on the dried basis):

$$X, \% = \frac{A_{pr} \times m_{st} \times V_{pr} \times P \times 100}{A_{st} \times V_{st} \times m_{pr} \times 100} \quad (1)$$

$A_{pr}$  – peak area of the substance in the chromatogram of test solution;

$A_{st}$  – peak area of the substance in the chromatogram of reference solution;

$m_{st}$  – mass of reference standard substance in the reference solution, mg;

$m_t$  – mass of preparation, mg;  
 $V_{pr}$  – dilution of test solution, mL;  
 $V_{st}$  - dilution of reference solution, mL;  
 $P$  – potency of standard substance, %

**Results and discussions.** Identification of separated substances was performed by the comparison of the retention time of main peaks in the chromatogram of meadow pasqueflower herb with the retention time of standard substances and by reference of UV-spectra with spectra of standard substances like quercetin, hyperoside, luteolin, rutin, apigenin-7-glucoside, caffeic and ferulic acid, umbelliferone and scopoletin.

9 phenolic compounds were identified in the meadow pasqueflower herb using HPLC (Table 1). Chromatogram of the meadow pasqueflower herb is given on the Fig. 1.

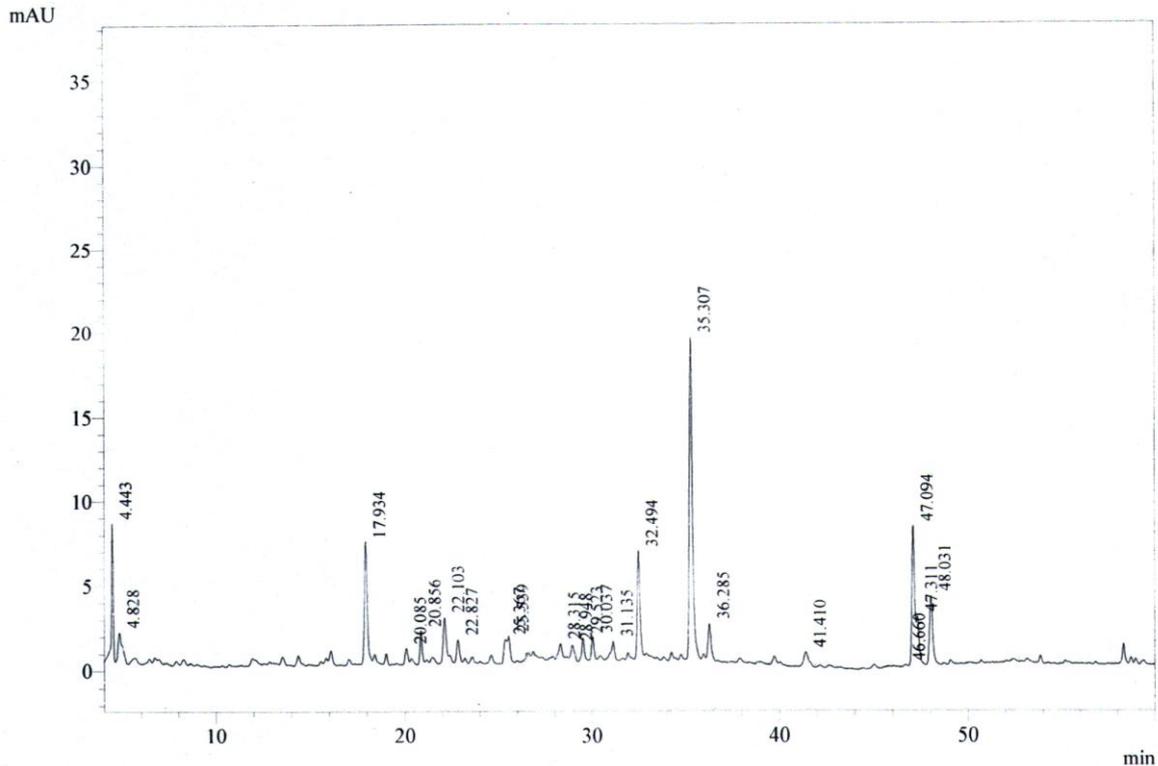


Fig. 1. Chromatogram of the meadow pasqueflower herb

Table 1. Quantitative results of phenolic compound composition of the meadow pasqueflower herb

№	Retention time, min	Peak area,			Nme of substance	Content, %
		330 nm	280 nm	350 nm		
1.	22.1	67037	25264	29658	Caffeic acid	0.00257
2.	30.44	18396			Umbelliferon	0.00770
3.	31.02	8266			Scopoletin	0.00037
4.	31.13	10610	15792	16363	Rutin	0.00136
5.	31.63	11653			Ferulic acid	0.00228
6.	32.49	3807	62690	85435	Hyperosid	0.02437
7.	36.28	786449	22840	51751	Apigenin-7-glucoside	0.10909
8.	47.09	158470	86525	35068	Luteolin	0.01568
9.	47.3	39648	29385	10980	Quercetin	0.00326

Such flavanoids as rutin, hyperoside, apigenin-7-glucoside, luteolin and quercetin were identified and quantified in the herb of meadow pasqueflower. Hyperoside (0.02437 %) and luteolin (0.01568 %) are found in higher quantity. Content of hydroxycinnamic acids, such as caffeic and ferulic acids, is 0.00257 % and 0.00228 %, respectively. Coumarins like umbelliferone (0.00770 %) and scopoletin (0.00037 %) were also found.

**Conclusions.** Thus, it may be concluded that 9 phenolic compounds, 5 flavanoids, hydroxycinnamic acids and 2 coumarins were identified using high performance liquid chromatography.

Obtained experimental findings give additional information about the chemical composition of herbal raw material such as meadow pasqueflower and it can be used in further complex pharmacological investigations.

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