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INVESTIGATION OF PHENOLIC COMPOUNDS OF PRIMULA VERIS L.

[1]L. G. Shostak, [1]S. M. Marchyshyn, [1]S. S. Kozachok, [2]R. V. Karbovska

[1]I. Horbachevsky Ternopil State Medical University
[2]SE «Ukrmetrteststandard»

Summary

It was established the presence and determined the quantity content of individual phenolic compounds in rhizomes with roots, leaves, flowers of Primula veris L. such as: apigenin, rosmarinic, p-coumaric, ferulic, ellagic, gallic acids, scopoletin, gallocatechin, epigallocatechin, catechin, epicatechin, catechin gallate, epicatechin gallate. The highest amount of hydroxycinnamic acids identified in the leaves and the constituents of tannins – rhizomes with roots.

Keywords: rhizomes with roots, leaves, flowers of Primula veris L., hydroxycinnamic acids, constituents of tannins, coumarins, HPLC.

Introduction. Primrose (*Primula veris* L.) – a perennial herb from Primulaceae family. The rhizomes with roots, flowers, leaves, arrows of primrose applied for treatment purposes. Primrose is widely used in official and folk medicine. Medicines from the rhizome with roots of Primula have a weak diuretic, diaphoretic and expectorant action, increase the secretory activity of mucous membranes of the upper respiratory tract and bronchus, increase the activity of ciliated epithelium and accelerate the elimination of phlegm from the respiratory tract. Medicines of the plant are used for coughs, catarrhal gastritis, diseases of the upper respiratory tract, chronic tracheitis, bronchitis and bronchopneumonia, nervous system diseases, and as a sedative tool for insomnia.

Primrose is a component of the complex herbal remedies with expectorant and mucolytic action: flowers with cups present in "Sinupret", Bionorica; roots – in "Bronchipret" Bionorica; root extract "Bronhikum elixir", A Nattermann & Cie Gmbh Germany.

Fresh leaves used as a vitamin tool for preparation of teas and concentrates of vitamin C, which applied for the treating of avitaminosis, as well as lethargy, loss of appetite, and gum disease [1].

Preliminary studies have shown that primrose contains polysaccharides, tannins, phenolic compounds (flavonoids), triterpene saponins, essential oils, organic acids, including ascorbic [2].

The aim of the work is a qualitative and quantitative determination of the phenolic compounds (hydroxycinnamic acids – HCA, coumarins – C, flavonoids – FL, constituents of tannins – T) by HPLC method in the rhizomes with roots, leaves and flowers of *Primula veris* L.

Materials and methods. Primrose was harvested in the territory of Ternopil region (Ukraine) in the appropriate vegetation's periods, leaves – early flowering, flowers – during flowering, rhizome with roots – on autumn, when the air part dies. The collected specimens were identified at the department of botany at National University of Pharmacy.

Identification and quantity content of phenolic compounds of medicinal plant materials (MPM) was performed by chromatograph (Agilent 1200 3 D LC System Technologies, USA) with diode array detector (G1315C) in complex with PC software Agilent ChemStation. Separation of the compounds was conducted on the column Discovery C_{18}, 250 x 4,6 mm (Supelco, № 505129) with the precolomn of 20 mm 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 5-20 μm, flow rate – 0.7 ml/min [3] and 0.5 ml/min [4].

For the separation of phenolic compounds it was applied the following parameters of the chromatographic analysis: gradient elution, mobile phase – bidistilled water acidulated with 0,005 N phosphoric acid solution ("A") and acetonitrile ("B") – analysis of HCA, FL, C; 0,1 % trifluoroacetic acid solution, 5 % acetonitrile solution (A) and acetonitrile 0,1 % trifluoroacetic acid solution (B) – analysis of T. Scan time was 0,6 seconds, the detection range – 190-400 nm, the wavelength of ultraviolet spectra detection – 320 (ferulic, *p*-coumaric acids) and 330 nm (HCA, C, FL) and 280 and 255 nm (T). Total analysis time was 50 and 40 minutes [3, 4].

Sample preparation grinded sample material, carefully selected about 1,00-2,00 g (exact sample) and placed into a flat-bottomed flask on 100 cm^3, extracted with 50 cm^3 of 60 % methanol solution and the flask joined to the reflux condenser for 30 minutes, heated on a magnetic mixer at 70 ^{0}C. After the sample was treated with ultrasound for 10 minutes at a frequency of 45 kHz at 70 °C. The obtained extracts was cooled and quantitatively transferred into the volumetric flask on 100 cm^3, enhanced the volume up to mark by 60% methanol solution [3]. The constituents of tannins of the investigated objects extracted with 50 cm^3 of bidistilled hot water. The flask was placed on a magnetic stirrer at 80 ° C for 30 minutes. Cooled and quantitatively transferred into the volumetric flask on 100 cm^3. Enhanced the volume up to mark by bidistilled water [4]. The obtained solutions were carefully mixed, filtered through a membrane filter with a pore size of 0.45 μm and placed into a container for chromatography.

Phenolic compounds were identified with reference compounds (retention times and UV spectra). The calculation of the concentration was performed by the calibration characteristic (dependence of chromatographic peak areas on mass concentration of standard sample).

Results of investigation and discussion. As a results of HPLC-analysis, it was identified HCA – rosmarinic, *p*-coumaric, ferulic acids, flavone – apigenin, C – scopoletin, constituents of condenced tannins – gallocatechin, epigallocatechin, catechin, epicatechin, catechin gallate, epicatechin gallate and constituents of hydrolysable tannins – ellagic, gallic acids in the investigated objects (fig. 1-7).

It was established that all studied objects of primrose contain rosmarinic and *p*-coumaric acids. In the highest amount they were identified in leaves 1108 mg/kg and 205 mg/kg, respectively. The leaves and flowers contain ferulic acid: 1202 mg/kg and 93 mg/kg, respectively. In the leaves and flowers of primrose also it was identified and determined the

quantitative content of hydroxy-, methoxycoumarin – scopoletin: 179 and 65 mg/kg, respectively, and flavones – apigenin, in leaves – 2853 mg/kg, flowers – 471 mg/kg (table. 1).

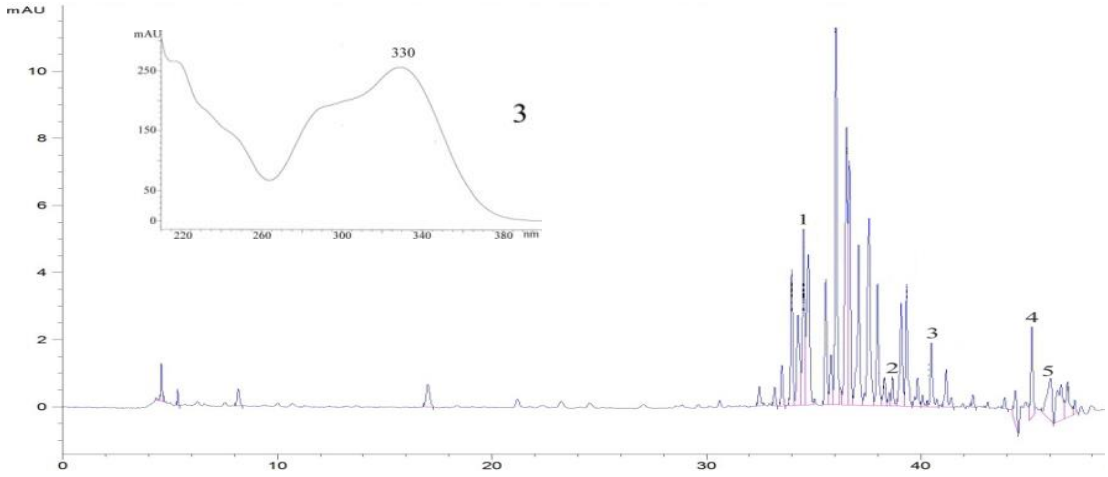

Fig. 1. The chromatogram of primrose flowers at λ = 330 nm: 1 – apigenin; 2 – *p*-coumaric acid; 3 – rosmarinic acid; 4 – ferulic acid; 5 – scopoletin.

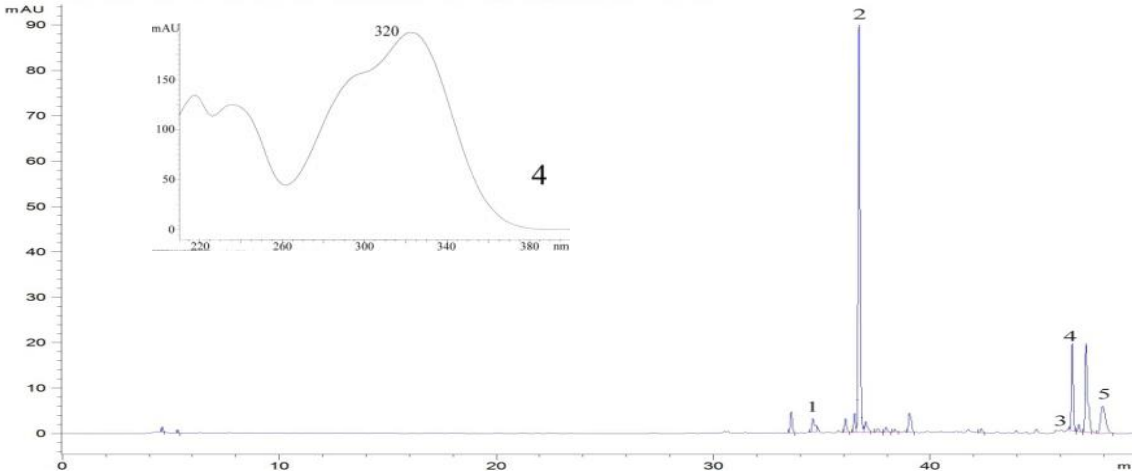

Fig. 2. The chromatogram of primrose leaves at λ= 330 nm: 1 – *p*-coumaric acid; 2 – apigenin; 3 – scopoletin; 4 – ferulic acid; 5 – rosmarinic acid.

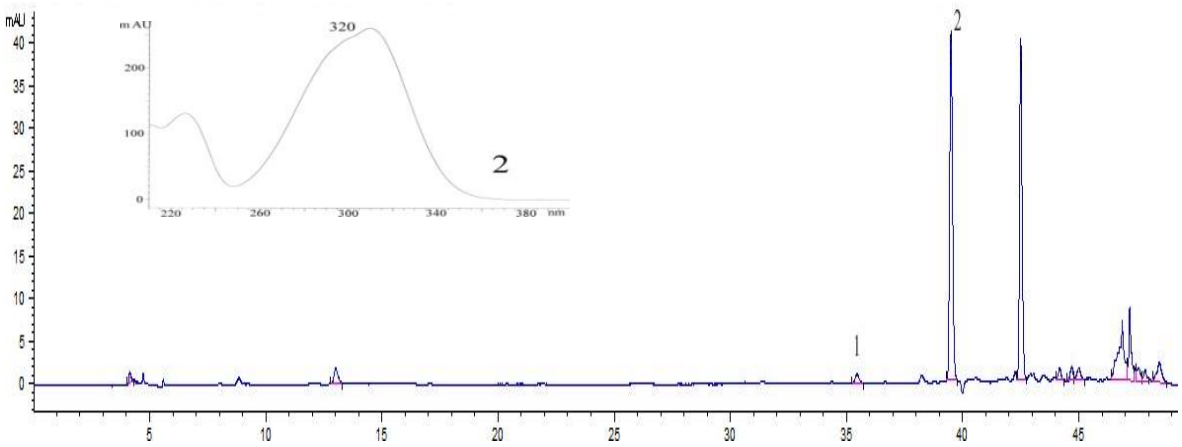

Fig 3. The chromatogram of primrose rhizomes with roots at λ= 330 nm: 1 – *p*-coumaric acid; 2 – rosmarinic acid.

Table 1

The quantitative content of phenolic compounds (mg/kg) in the investigated objects

Name	Rhizomes with roots	Leaves	Flowers
Apigenin	—	2853	471
Rosmarinic acid	26	1108	115
p-coumaric acid	18	*205*	*89*
Ferulic acid	—	1202	93
Scopoletin	—	179	65

As a results it was established, that tannins of the underground organs of primrose represented by free tannins – gallic and ellagic acids and by the constituents of condensed tannins – gallocatechin, epigallocatechin, catechin, epicatechin, epicatechin gallate. In the leaves it was observed the presence of gallocatechin, epigallocatechin, catechin, catechin, epicatechin gallate, in the flowers – catechin, epicatechin, catechin gallate. The highest amount of T it was found in primrose rhizomes with roots, among them epigallocatechin - 11226 mg/kg and catechin - 1433 mg/kg) dominated (table. 2).

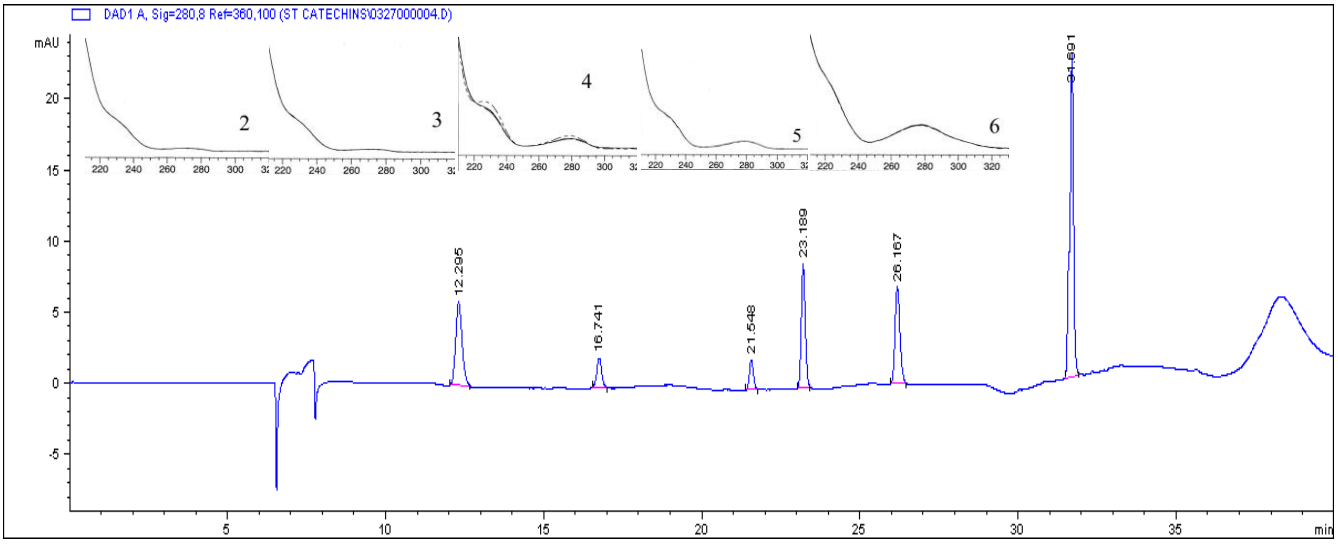

Fig. 4. The chromatograms of the mixer of gallic acid and catechins: 1 – gallic acid; 2 - gallocatechin; 3 - epigallocatechin; 4 – catechin; 5 – epicatechin; 6 – epicatechin gallate.

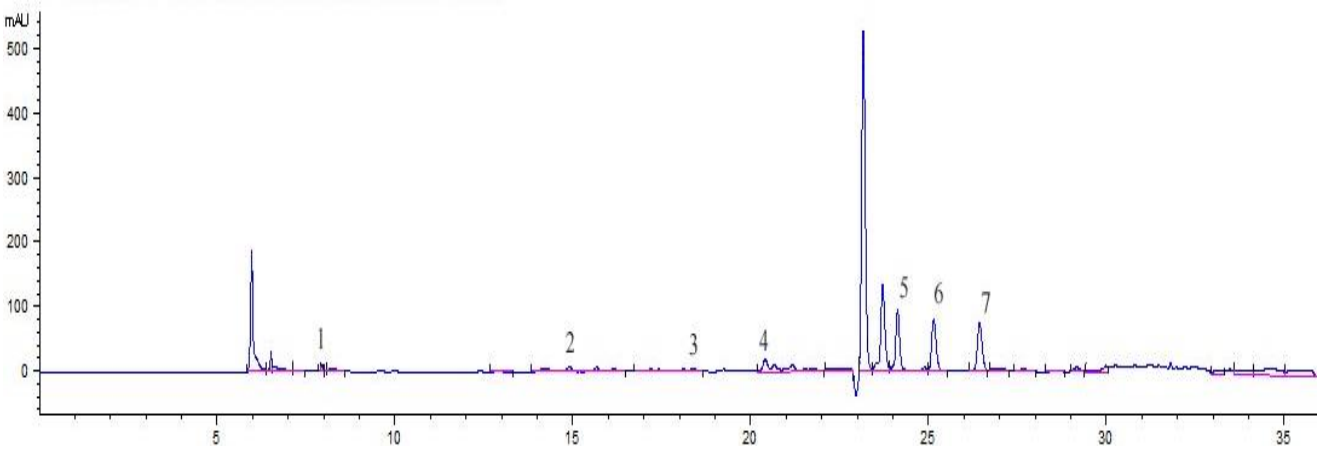

Fig. 5. The chromatogram of primrose rhizomes with roots at λ= 280 nm: 1 – ellagic acid; 2 – gallic acid; 3 – gallocatechin; 4 – epigallocatechin; 5 – catechin; 6 – epicatechin; 7 – epicatechin gallate.

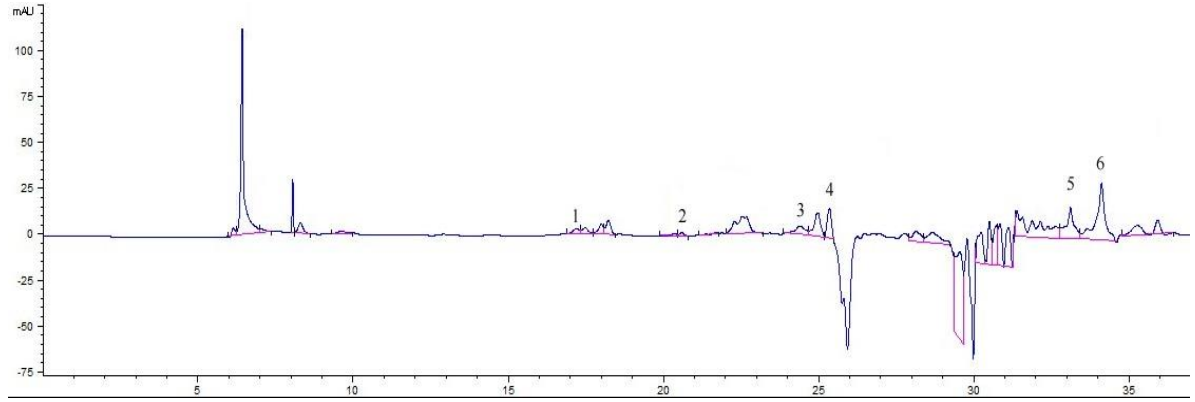

Fig. 6. The chromatogram of primrose leaves at λ= 280 nm: 1 – gallocatechin; 2 – epigallocatechin; 3 – catechin; 4 – epicatechin; 5 – catechin gallate; 6– epicatechin gallate.

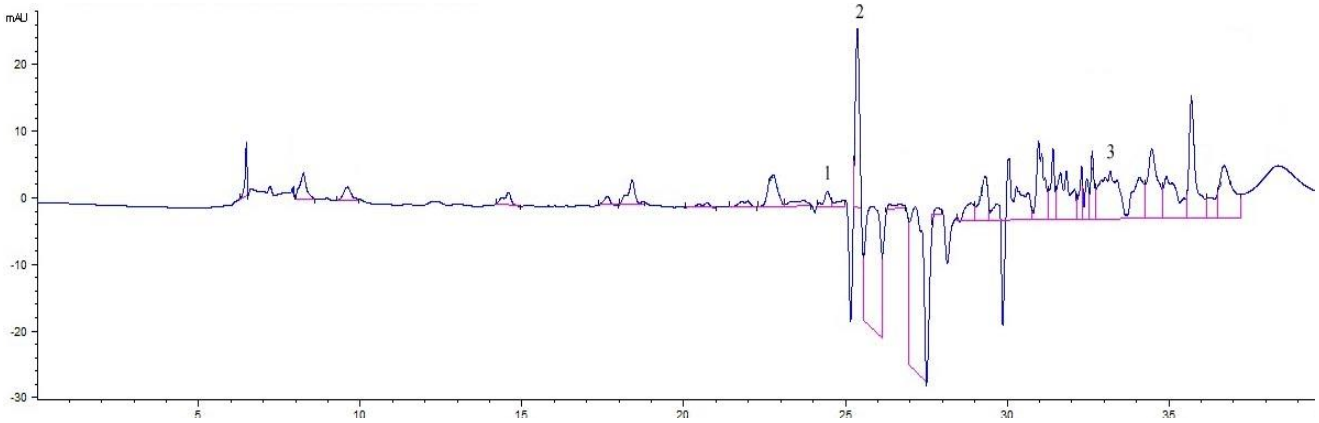

Fig. 7. The chromatogram of primrose flowers at λ = 280 nm: 1 – catechin; 2 – epicatechin; 3 – catechin gallate.

Table 2

The quantitative content of constituents of tannins (mg/kg) in the investigated objects

Name	Rhizomes with roots	Leaves	Flowers
Ellagic acid	18	–	–
Gallic acid	164	–	–
Gallocatechin	2080	334	–
Epigallocatechin	11226	685	–
Catechin	1433	403	234
Epicatechin	123	543	1613
Catechin gallate	–	279	786
Epicatechin gallate	43	739	–

A significant content of tannins in primrose determines its anti-inflammatory, antimicrobial, astringent, antioxidant activity. It is known that tannins, due to the large number of hydroxyl and carboxyl groups in their structure and high molecular weight (500-3000) can form stable complexes with proteins and macromolecules. This mechanism is the basis of their pharmacological detoxification activity. Investigated that tannins reveal a direct effect on the cell membrane, enzyme proteins and nucleic acids; improve the exchange of epinephrine, ascorbic acid, acetylcholine, affecting on the critical systems of neurohumoral and neuroendocrine regulation [5].

Conclusions. As a results of HPLC-analysis, it was established the chromatographic profile of phenolic compounds in the rhizomes with roots, leaves and flowers of primrose. Identified and determined the quantitative content of phenolic compounds in all parts of medicinal plant. The highest quantity of apigenin, scopoletin and hydroxycinnamic acids presence in the leaves, the constituents of tannins – the rhizomes with roots, which gives the reason to recommend primrose as the promising medicinal plant material for the creating of new drugs on its base with anti-inflammatory, antimicrobial, antioxidant activity.

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