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Investigation of phenolic compounds about ground organs of cultivated species genus *Primula* L.

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Abstract

The phytochemical research of leaves and flowers cultivated species of the genus *Primula* L. (drumstick primrose - *Primula denticulate* Smith, Julia's primrose - *Primula juliae* Kusn., rock primrose - *Primula saxatilis* Kom.) have been conducted. The highest quantitative content of the flavonoids was observed in flowers of drumstick primrose – 1,68%, flowers of Julia's primrose – 1,3%; and leaves of drumstick primrose – 0,97%; coumarins – in leaves of primrose rock – 0,045%, flowers of drumstick primrose - 0,042% and flowers of Julia's primrose – 0,04%. In the flowers and leaves of three cultivated species of the genus *Primula* L. by high performance liquid chromatography was identified aglycones: flavonols - quercetin and kaempferol; flavons – luteolin and apigenin; glycosides of flavonols - rutin, hyperoside, isoquercitrin and simple coumarins: coumarin, umbelliferone and scopoletin.

Keywords: Drumstick primrose, Julia's primrose, rock primrose, flavonoids, coumarins, leaves, flowers, high performance liquid chromatography

1. Introduction

Phenolic compounds of plant origin attracted the attention of many researchers. This is caused by the lack of toxicity, a small amount of side effects and a wide range of pharmacological action, which in turn causes the relevance of their application in herbal medicine. Particularly great interest are phenolic compounds such as flavonoids and coumarins^[1].

Flavonoids - derivatives of phenolic compounds are found in many plants. It is known that flavonoids exhibit high biological activity due to the presence in the molecule of active phenolic hydroxyl and carbonyl groups, which in the course of various biochemical modifications are involved in a number of physiological processes and discover a wide range of pharmacological activity. The most significant pharmacological action of flavonoids is to regulate condition of capillaries, particularly they increase their permeability at an atherosclerosis and thereby help to reduce and normalize blood pressure. They are characterized by diuretic, spasmolytic, choleric, sedative, estrogen and P-vitamin action; they expand the capillaries, reduce blood pressure, tone up heart muscle, dilates coronary vessels, reduce blood coagulation^[1-3, 4, 6].

Coumarins - it lactones cis-o-hydroxycinnamic (coumarin) acid. Natural coumarins depending on the structure show diverse phytotherapeutic effect on the human body, photodynamic, spasmolytic, P-vitamin, antimicrobial, anticancer, anticoagulant, anthelmintic and anti-allergic activity.

Search of medicinal plant raw material containing phenolic compounds is a leader among topical issues of pharmacy. Therefore, during the comprehensive pharmacognostic research of cultivated species of the genus *Primula* L. rational is definition of qualitative composition and quantitative content of flavonoids and coumarins in leaves and flowers of drumstick primrose, primrose Julia's and rock primrose. Plants of the genus *Primula* L. has long been used in folk medicine: infusion of leaves as an analgesic for rheumatism, as a diuretic in diseases of the kidneys and the bladder; infusion of flowers as a diaphoretic and expectorant used for fever, bronchitis, as anti-inflammatory - when an inflammation of the gums, as general strengthening - for migraines, nervousness, insomnia, tachycardia^[5, 6]. Drumstick primrose, Julia's primrose and rock primrose are widely used as flower-decorative plants and were introduced at the M.M. Gryshko National botanical garden National Academy of Sciences of Ukraine. Considering the lack amounts of information and the lack of research of these types of plants is actual their pharmacognostic comprehensive research with the future prospects of their application as officinal medicinal plant raw material.

The purpose of this work was to analyze the qualitative composition and quantitative content of flavonoids and coumarins in above-ground organs of three cultivated species of the genus *Primula* L. - drumstick primrose, Julia's primrose and rock primrose.

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2. Materials and methods.

2.1 The research objects: as a prospective medicinal raw material for investigation content of phenolic compounds was selected leaves and flowers of cultivated species of the genus *Primula* L. (drumstick primrose - *Primula denticulate* Smith, Julia's primrose - *Primula juliae* Kusn., rock primrose - *Primula saxatilis* Kom.). Plant raw materials harvested at the scientific and research section of the Department of flower-ornamental plants M.M. Gryshko National botanical garden National Academy of Sciences of Ukraine. The leaves and flowers were harvested during flowering, drumstick primrose and Julia's primrose - April-May, primrose rock - May-June 2015.

Chromatographic separation was performed on high-performance liquid chromatograph (Agilent 1200 3 D LC System Technologies, USA) with photometric diode-array detector UV-Vis G1315C equipped with a flow degasser G1322A, autosampler G1329A, column thermostat G1316A, in complex with PC software Agilent Chem Station (G2215 BA).

Conditions of chromatography: column Supelco-Discovery C18 size 250 x 4,6 mm with sorbent - silica grains with a diameter of 5 mm, eluants: (A) 0.005 mol / m³ phosphoric acid (Fluka), (B) acetonitrile. Gradient elution mode (Tab. 1). The rate of mobile phase: 0,8 ml / min (flavonoids), 0,7 ml / min (coumarins), column thermostat temperature of 25 °C. The total duration of analysis 60 minutes^[9].

Table 1: Parameters of gradient elation

Time, min	Flavonoids									
	0	30	33	38	40	41	48	49	60	
Eluant B, %	12	25	25	30	40	80	80	12	12	

2.2 Sample preparation: Sample preparation was carried out as follows: grinded raw material carefully weighed by the mass 1,00 grams ((accurately sample) and placed into a flat-bottomed flask on volume 50 ml - (flavonoids and coumarins), extracted with 50 ml of 60% methanol in boiling water bath reflux condenser under mixing for 30 minutes (flavonoids and coumarins). After the sample was treated with ultrasound for 10 minutes, filtered, quantitatively transferred to a flask of 100 ml and enhanced the volume up to mark by 60% methanol solution. Before chromatography was filtered through a disposable application filter with pore diameter of 0,45 microns^[8-9].

Identification of phenolic compounds was carried out by comparison of their RT values and UV spectra with standards data^[9].

The calculation of the concentration was performed by the calibration method (dependence of chromatographic peak areas on mass concentration of appropriate flavonoids and coumarins in a solution prepared standard sample)^[7-9].

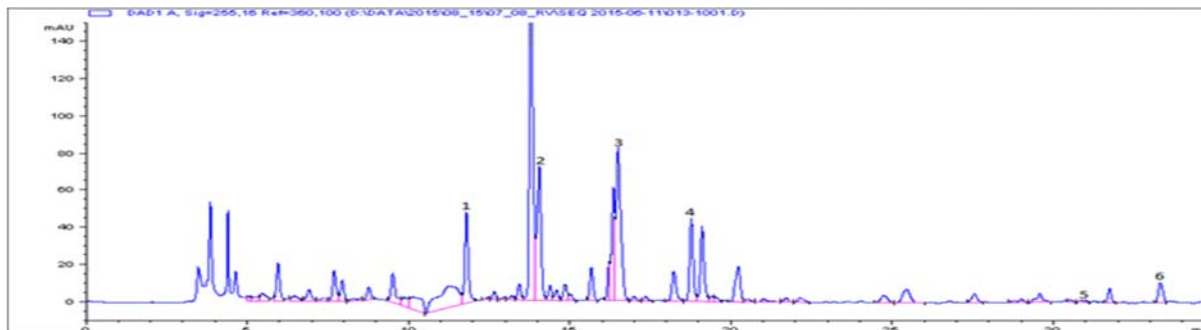
3. Results and Discussion

The investigated extracts from flowers and leaves of three cultivated species drumstick primrose, Julia's primrose and primrose rock observed a positive result of cyanidin reaction that indicates about content of flavonoids. Also positive result during the qualitative reactions to the content of coumarins such as slow hydrolyzation under the action of diluted alkali to form yellow solution of salts of coumarin acid.

By HPLC method of analysis in the studied samples of plant material it was identified and defined quantitative content of aglycones: flavonols - quercetin and kaempferol; flavons - luteolin and apigenin; glycosides of flavonols - rutin, hyperoside, isoquercitrin and simple coumarins: coumarin, umbelliferone and scopoletin. (Tab. 2).

Table 2: Qualitative composition and quantitative content of phenolic compounds in the leaves and flowers of drumstick primrose (*Primula denticulata* Smith), Julia's primrose (*Primula juliae* Kusn.), rock primrose (*Primula saxatilis* Kom.) (by HPLC method)

Phenolic compounds, %	Leaves			Flowers		
	<i>P. denticulata</i> Smith	<i>P. juliae</i> Kusn.	<i>P. saxatilis</i> Kom.	<i>P. denticulata</i> Smith	<i>P. juliae</i> Kusn.	<i>P. saxatilis</i> Kom.
Flavonoids						
Hyperoside	0,02	0,12	0,08	0,07	0,09	0,20
Rutin	0,39	0,24	0,04	0,42	0,31	0,13
Isoquercetin	0,44	0,09	0,05	0,61	0,33	0,14
Luteolin	0,11	-	0,01	0,25	0,15	0,03
Quercetin	-	0,09	-	-	0,040	0,013
Kaempferol	0,01	-	0,02	0,01	0,02	0,007
Apigenin	0,009	0,32	0,17	0,32	0,37	0,25
Coumarins						
Coumarin	0,01	0,02	0,03	0,04	0,03	-
Scopoletin	0,003	-	0,007	0,002	0,009	0,01
Umbelliferone	-	-	0,008	-	-	0,02



Chromatograms of flavonoids and coumarins of investigated species raw materials by the HPLC are presented in Figures 1 - 6.

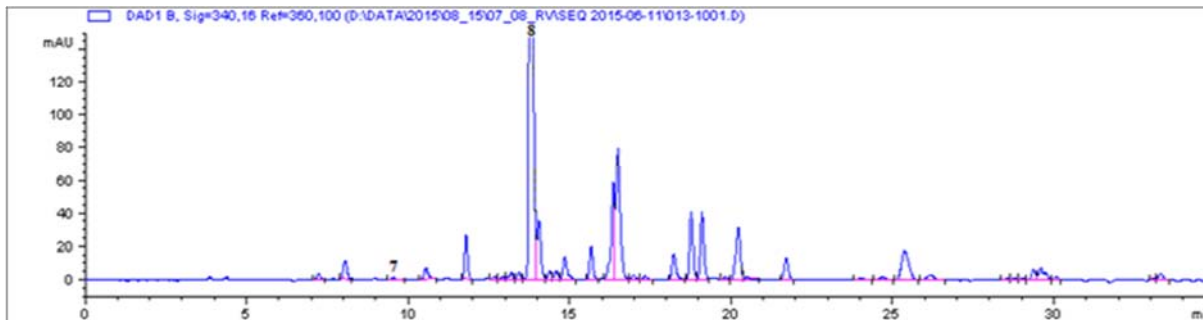


Fig 1: Chromatogram of flavonoids and coumarins flowers of *Primula denticulata* Smith ($\lambda = 255 \text{ nm}$, $\lambda = 340 \text{ nm}$): 1 – hyperoside, 2 – rutin, 3 – isoquercetin, 4 – luteolin, 5 – kaempferol, 6 – coumarin, 7 – scopoletin, 8 – apigenin.

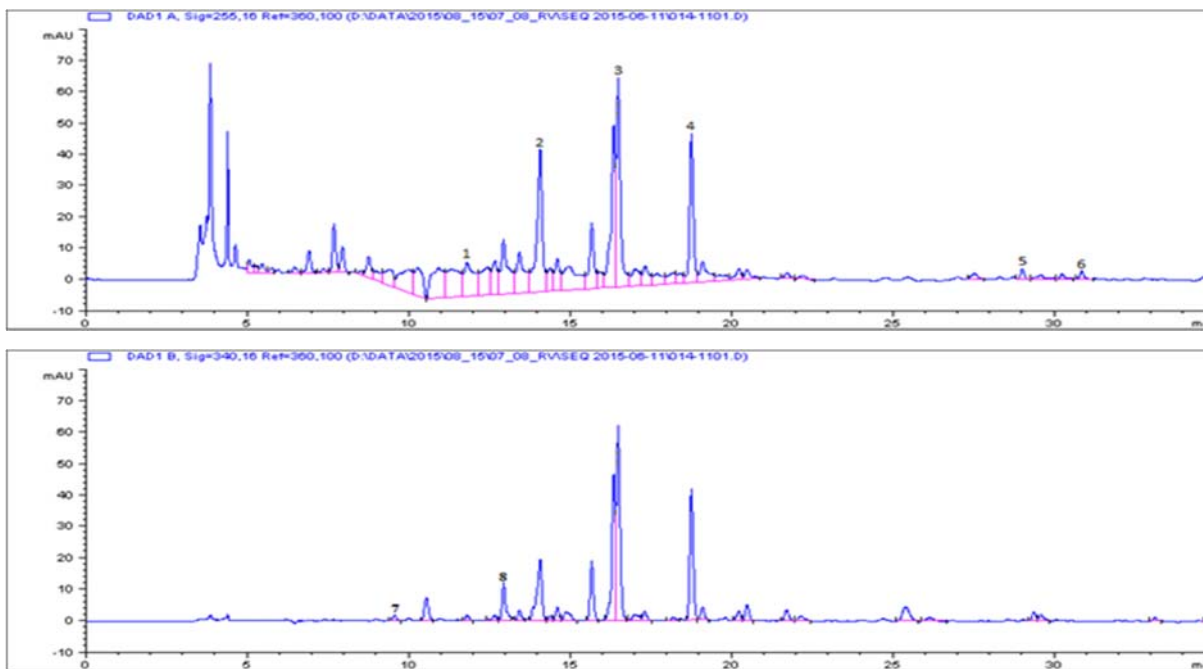


Fig 2: Chromatogram of flavonoids and coumarins leaves of *Primula denticulata* Smith ($\lambda = 255 \text{ nm}$, $\lambda = 340 \text{ nm}$): 1 – hyperoside, 2 – rutin, 3 – isoquercetin, 4 – luteolin, 5 – coumarin, 6 – kaempferol, 7 – scopoletin, 8 – apigenin.

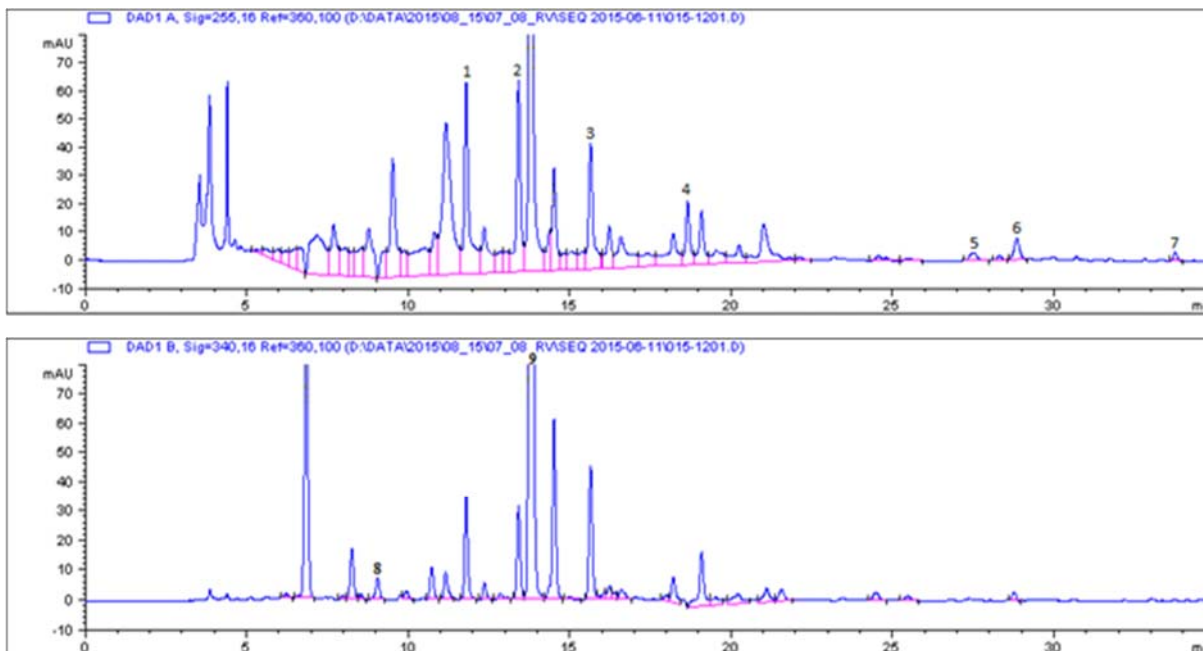


Fig 3: Chromatogram of flavonoids and coumarins flowers of *Primula Juliae* Kusn. ($\lambda = 255 \text{ nm}$, $\lambda = 340 \text{ nm}$): 1 – hyperoside, 2 – rutin, 3 – isoquercetin, 4 – luteolin, 5 – coumarin, 6 – quercetin, 7 – kaempferol, 8 – scopoletin, 9 – apigenin

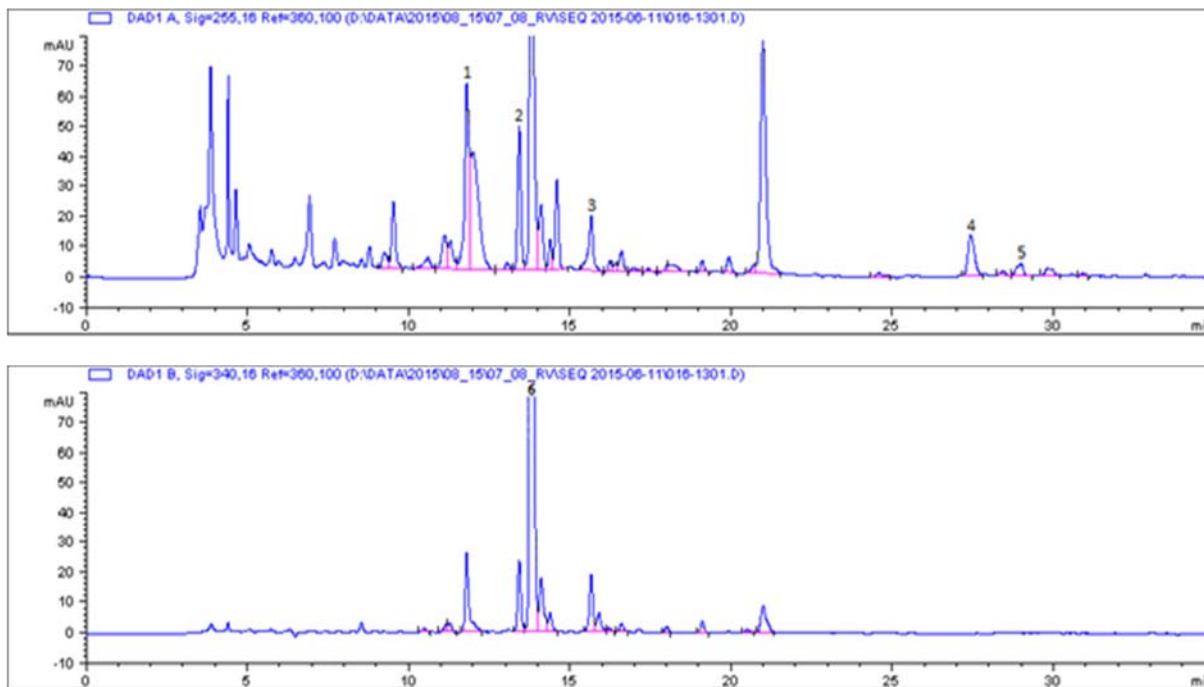


Fig 4: Chromatogram of flavonoids and coumarins leaves of *Primula juliae* Kusun. ($\lambda = 255 \text{ nm}$, $\lambda = 340 \text{ nm}$): 1 – hyperoside, 2 – rutin, 3 – isoquercetin, 4 – quercetin, 5 – coumarin, 6 – apigenin.

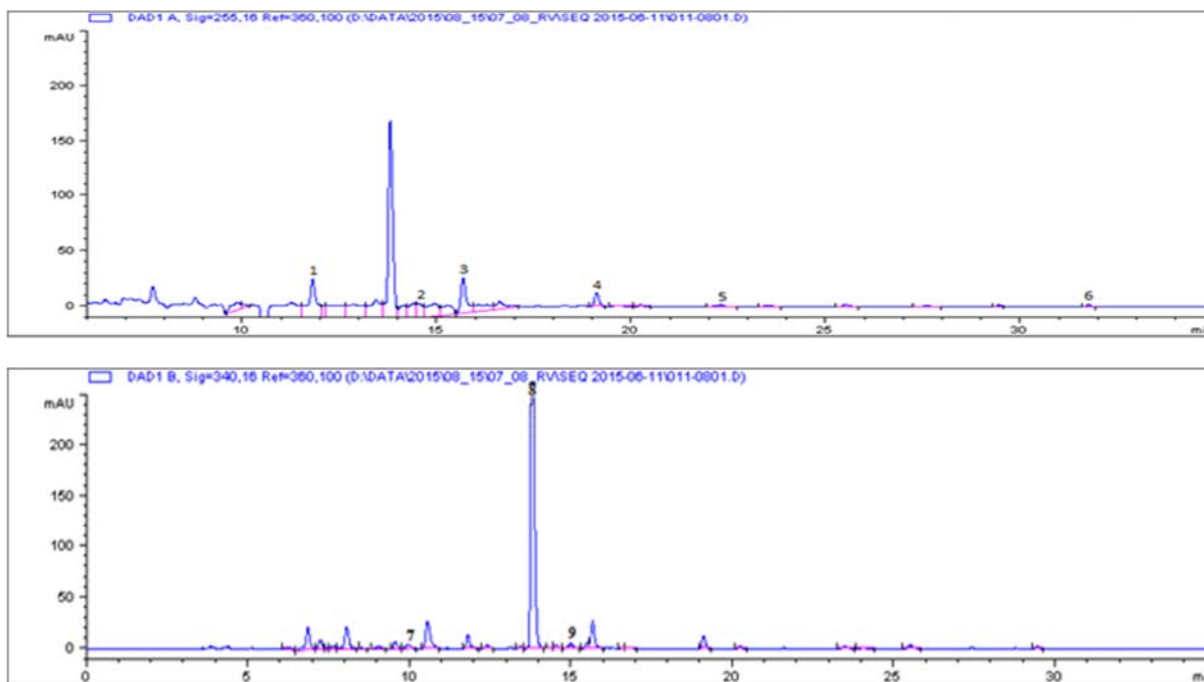
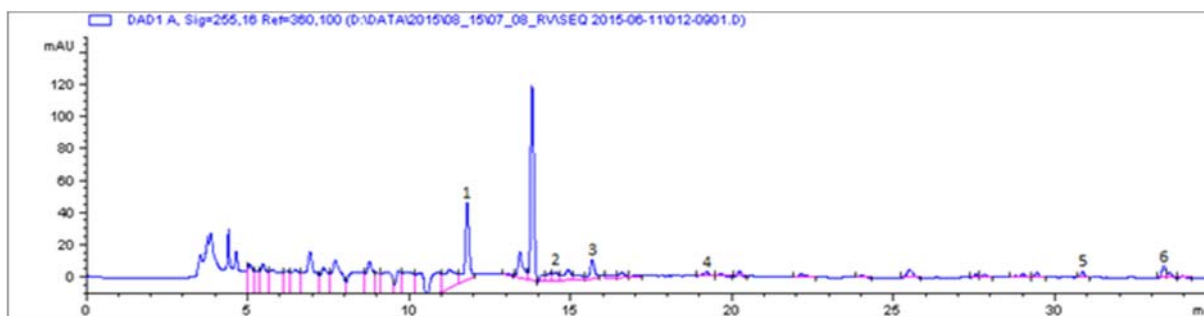


Fig 5: Chromatogram of flavonoids and coumarins flowers of *Primula saxatilis* Kom. ($\lambda = 255 \text{ nm}$, $\lambda = 340 \text{ nm}$): 1 – hyperoside, 2 – rutin, 3 – isoquercetin, 4 – luteolin, 5 – quercetin, 6 – kaempferol, 7 – scopoletin, 8 – apigenin, 9 – umbelliferone.



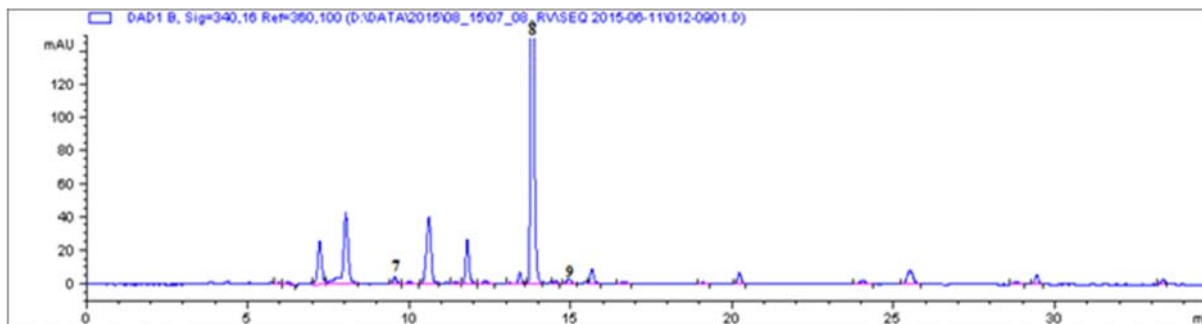


Fig 6: Chromatogram of flavonoids and coumarins leaves of *Primula saxatilis* Kom. ($\lambda = 255$ nm, $\lambda = 340$ nm): 1 – hyperoside, 2 – rutin, 3 – isoquercetin, 4 – luteolin, 5 – kaempferol, 6 – coumarin, 7 – scopoletin, 8 – apigenin, 9 – umbelliferone

Results of HPLC analysis showed that leaves of drumstick primrose, rock primrose and flowers of drumstick primrose do not contain quercetin; leaves of Julia's primrose – kaempferol and luteolin. All investigated samples contain coumarins except flowers of rock primrose; leaves of Julia's primrose do not contain scopoletin. Umbelliferone contained only leaves and flowers of rock primrose. So, experimentally approved that investigated species of genus *Primula* L. different by the quality composition and quantity content of flavonoids and coumarins.

4. Conclusions

- At the first time analyzed flavonoids and coumarins in leaves and flowers drumstick primrose (*Primula denticulate* Smith), Julia's primrose (*Primula Juliae* Kusn.), rock primrose (*Primula saxatilis* Kom.) by high performance liquid chromatography. Established that species of genus *Primula* L. different by the quality composition and quantity content of investigated biologically active substances.
- By HPLC analysis in flowers and leaves of drumstick primrose (*Primula denticulate* Smith), Julia's primrose (*Primula Juliae* Kusn.), rock primrose (*Primula saxatilis* Kom.) identified and established quantitative content of aglycones: flavonols - quercetin and kaempferol; flavons – luteolin and apigenin; glycosides of flavonols - rutin, hyperoside, isoquercitrin and simple coumarins: coumarin, umbelliferone and scopoletin.
- Established that in the largest quantities leaves of drumstick primrose contain rutin and isoquercetin – 0,39% and 0,44% respectively; leaves of Julia's primrose – apigenin 0,32%; flowers of drumstick primrose rutin, isoquercetin and apigenin - 0,42%, 0,61 and 0,32% respectively; flowers of Julia's primrose-isoquercetin and apigenin - 0,33% and 0,37% respectively.

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