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Identification and quantification of flavonoids and hydroxycinnamic acids in yellow wood anemone (*Anemone ranunculoides* L.) by UHPLC-DAD-HESI/MS analysis

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Abstract

Introduction. Yellow wood anemone (*Anemone ranunculoides* L.) is a herbaceous perennial. This plant grows in the European parts of Russia, Ciscaucasia, Siberia, Central Europe and other regions. It has many different pharmacological activities: immunomodulatory, sedative, anti-inflammatory, antitoxic, diuretic, antibacterial, antioxidant, antitumor and antirheumatic activity. These various effects are due to their biologically active compounds, which include ephemeroids, protoanemonin, saponins, tannins, resins, ascorbic acid, ranunculin, oils lipids and triterpene glycosides. As to phenolic compounds, currently there is no sufficient information on flavonoids and hydroxycinnamic acids (HCAs) profiles in different species of genus *Anemone*. Because these groups of compounds are quite specific for yellow wood anemone, their detailed study seems to be relevant.

Aim. The objective of this research is to identify and quantify flavonoids, HCAs, and their conjugates in Yellow wood anemone leaves, flowers and rhizomes with roots.

Materials and methods. The identification was carried out by using ultra-high-performance liquid chromatography with diode array and electrospray ionization-mass spectrometric detection (UHPLC–DAD–ESI-MS) with quantification of individual compounds by external calibration method.

Results and discussion. Among the 47 compounds, 30 flavonoids and 17 derivatives of HCAs were detected. Quercetin glycosides were found to be the major flavonoids in aerial parts whereas chalcone glycosides – in underground parts. The major HCA in rhizomes with roots was feruloyltartaric acid (1.18 mg/g) whereas chlorogenic acid was predominant in leaves and flowers (8.68 mg/g and 2.62 mg/g accordingly). The total content of phenolic compounds was estimated at 60 mg/g on a dry weight basis.

Conclusion. As a result of the research a detailed profile of flavonoids and HCAs acids of anemon was described. The data obtained can serve to identify this species in the standardization of medicinal plant materials and taxonomic studies.

Keywords: *Anemone ranunculoides*, flavonoids, hydroxycinnamic acids

Conflict of interest. The authors declare that they have no obvious and potential conflicts of interest related to the publication of this article.

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Идентификация и количественное определение флавоноидов и гидроксикоричных кислот ветреницы лютичной (*Anemone ranunculoides* L.) методом UHPLC-DAD-HESI/MS

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Резюме

Введение. Ветреница лютичная (*Anemone ranunculoides* L.) – многолетнее травянистое растение произрастает в европейской части России, Предкавказье, Сибири, Центральной Европе и в других регионах. Ветреница лютичная обладает разнообразной фармакологической активностью: иммуномодулирующей, седативной, противовоспалительной, антитоксической, мочегонной, антибактериальной, антиоксидантной, противоопухолевой и противоревматической. Эти многочисленные эффекты обусловлены биологически активными соединениями, входящими в состав этого эфемероида, среди которых протоанемонин, сапонины, танины, смолы, аскорбиновая кислота, ранункулин, масла, липиды и тритерпеновые гликозиды. Говоря о фенольных соединениях, следует отметить, что в настоящее время нет достаточной информации о профиле флавоноидов и гидроксикоричных кислот (ГКК) разных видов рода *Anemone*. Поскольку эти группы соединений достаточно специфичны для ветреницы лютичной, их детальное изучение представляется актуальным.

Цель. Целью данного исследования является идентификация и количественное определение флавоноидов, ГКК и их конъюгатов в листьях, цветках и корневищах с корнями ветреницы лютичной.

Материалы и методы. Идентификацию проводили методом UHPLC-DAD-HESI/MS, для количественного определения индивидуальных соединений использовался метод внешнего стандарта.

Результаты и обсуждение. Среди 47 соединений обнаружено 30 флавоноидов и 17 производных ГКК. Установлено, что в надземной части основными флавоноидами являются гликозиды кверцетина, а в подземной части – халконовые гликозиды. Основной ГКК в корневищах с корнями была ферулоилвинная кислота (1,18 мг/г), тогда как в листьях и цветках преобладала хлорогеновая кислота (8,68 мг/г и 2,62 мг/г соответственно). Общее содержание фенольных соединений составило 60 мг/г в пересчете на сухую массу сырья.

Заключение. В результате представлен подробный профиль флавоноидов и ГКК ветреницы лютичной. Полученные данные могут использоваться для идентификации этого вида при стандартизации лекарственного растительного сырья и в таксономических исследованиях.

Ключевые слова: *Anemone ranunculoides*, флавоноиды, гидроксикоричные кислоты

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Вклад авторов. А. Н. Луферов, Н. В. Бобкова, Д. О. Боков, Е. В. Сергунова, Т. Ю. Ковалёва, Т. Д. Рендюк, А. В. Стреляева, А. М. Анцышкина, Т. В. Простодушева, С. Г. Зайчикова, В. М. Баева, И. Б. Перова, К. И. Эллер, В. В. Бессонов осуществляли планирование эксперимента. Д. О. Боков, М. Н. Родин, И. Б. Перова проводили экспериментальные исследования. А. Н. Луферов, Н. В. Бобкова, Д. О. Боков, М. Н. Родин, Е. В. Сергунова, Т. Ю. Ковалёва, Т. Д. Рендюк, А. В. Стреляева, А. М. Анцышкина, Т. В. Простодушева, С. Г. Зайчикова, В. М. Баева, И. Б. Перова, К. И. Эллер, В. В. Бессонов участвовали в написании текста статьи, включая заключение и обсуждение результатов. Все авторы прочитали и согласились с опубликованной версией рукописи.

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INTRODUCTION

The genus *Anemone* L. includes more than 150 species[1], which are a fairly large taxonomic group within the subfamily, *Ranunculoideae* Arn. family *Ranunculaceae* Juss. [1–3]. Windflowers (anemones) are distributed mainly in the extratropical regions of Eurasia and North and South America [2, 4].

Yellow wood anemone (*Anemone ranunculoides* L.), also known as yellow anemone or buttercup anemone, is a herbaceous perennial 5–20 (25) cm high. *Anemone ranunculoides* belongs to the subgenus *Anemonoides*

[1] The Plant List. Available at: http://www.theplantlist.org/ Accessed: 28.09.2023.

(Mill.) Luferov, section *Sylvia* Spach, and subsection *Ranunculoides* (Starodubtsev) Luferov. Characteristic features of the subgenus *Anemonoides* are petiolate stem leaves, sessile or almost sessile nuts with short pubescence, as well as arcuate or hook-shaped stylodia. For section *Sylvia*, diagnostic features are long (rarely short) horizontal rhizomes; nuts with coarse hairs. The subsection *Ranunculoides* includes species with jointed rhizomes, consisting of thick and thin sections of different lengths. Tepals of a simple perianth, 5–7 (rarely up to 12), appressed-hairy on the underside [1–6]. Blossoms in April – May, bears fruit in the second half of May – June; it is spring ephemeroid.

The main habitats of plants of this species are broad-leaved (*Querqus*, *Acer*, *Fraxinus*, *Fagus*, *Tilia* species) and small-leaved (*Betula*, *Populus*, *Alnus incana* species) forests, as well as in mixed forests with *Picea* species, less often other conifers, among thickets of species *Corylus*, *Sambucus*, *Euonymus*, *Viburnum*, and other shrubs, on edges, glades, in parks and gardens. The plant is a European-West Siberian-Caucasian forest species in terms of its geographical distribution [3]. Many anemones contain valuable biologically active compounds that provide their healing effect in various diseases. For example, they are used as antibacterial, antioxidant, anti-inflammatory, antitoxic, diuretic, antitumor, antirheumatic, immunomodulatory, sedative agents [1, 7, 8]. *Anemone nemorosa* L. is known as a folk antitumor agent [9]. In traditional medicine, *A. ranunculoides* drugs are used for stomach pain, whooping cough, gout, paralysis, and also as a drug of enhancing the activity of the kidneys and lungs. An alcoholic tincture of *A. ranunculoides* is used externally for edema, rheumatism and radiculitis [10]. Buttercup anemone is used to produce homeopathic medicines [11]. Insufficient chemical and pharmacological knowledge of *Anemone* species limits the possibilities of their use in practical pharmacy. The use of anemones poses a certain danger due to the content of toxic anemonol, which, when dried, turns into an equally toxic anemonin.

The chemical composition of biologically active substances of the plant is not well studied. Buttercup anemone is one of the most common forest ephemeroids. Contains the toxic substance protoanemonin, saponins, tannins, resins, ascorbic acid. Fresh leaves contain the substance ranunculin, which, when dried, breaks down into protoanemonin and glucose. Protoamonin is a mitotic poison, an oily liquid with a pungent odor and taste; by the chemical structure, it belongs to lactones. It polymerizes to form anemonin – a crystalline substance, sparingly soluble in water and well – in organic solvents. All these active ingredients form the basis of the chemical composition of buttercup anemone [12]. Oils lipids with specific fatty acid composition are accumulated in seeds [13]. *A. ranunculoides* aerial part contain saponins (ranunculin). Anemonin and ranunculin, the potent anti-inflammatory and anticancer compounds, are abundant in tribes *Ranunculeae* and *Anemoneae* [1].

Another important group of biologically active compounds (BAS) in *A. ranunculoides* is triterpene glycosides. Six glycosides, anemonosides A, B, C, D, E, and F in the order of increasing polarity gave an aglycone which was identified as an oleanolic acid (by chromatography, IR spectroscopy, and a mixed melting point). Data on the composition of *A. ranunculoides* flavonoids and hydroxycinnamic acids (HCAs), as well as for other species of *Anemone* genus, are not available in the literature. Because these groups of compounds are quite specific for anemone, their detailed study seems to be relevant (figure 1).

Figure 1. ***A. ranunculoides*** **at blooming stage**

MATERIALS AND METHODS

Standards and reagents

For high-performance liquid chromatography with diode array and electrospray ionization-mass spectrometric detection (HPLC–DAD–ESI-MS) analysis Thermo Fisher Scientific Ultimate 3000 liquid chromatograph (Thermo Fisher Scientific Inc., USA) interfaced with a TSQ Endura triple quadrupole mass spectrometer (Thermo Fisher Scientific Inc., USA) was used. UHPLC system was equipped with a degasser, triple pump, and column compartments with autosampler and a PDA detector controlled by Chromeleon 7.2 Software. Samples were separated on a Waters NovaPak® C18 (150 × 4.6 mm, 4 µm; Waters Corporation, USA) maintained at 25 °C. The following gradient elution program was applied at a flow rate of 0.5 mL/min, where eluent A was 0.1 % (v/v) formic acid in water and eluent B was 0.1 % (v/v) formic acid in acetonitrile: 0–10 min 15–30 % (v/v) B, 10–20 min 30–45 % (v/v) B, 20–30 min 45–60 % (v/v) B, 30–35 min 60–70 % (v/v) B, 35–38 min 70 % (v/v) B, 38–40 min 70–15 % (v/v) B, 40-50 min 15 % (v/v) B. Injection volume was 10 mL.

Analytical wavelengths were 290, 338, 350, 370 nm; UV spectra were recorded between 190 and 700 nm. Ionization was carried out by a heated electrospray in positive (HESI/MS^+) and negative ion mode (HESI/MS^-) in the m/z range from 150 to 1000 Da. Scanning speed

was 1000 Da/sec; quadrupole resolution Q1: 0.7. Parameters of the ionization source were: capillary voltage – 3500 V (positive mode) and 2500 V (negative mode); Sheath gas (nitrogen) flow: 5.6 l/min; auxiliary gas flow: 10.1 l/min; sweep gas flow: 2.7 l/min; ion transfer tube temperature: 350 °C; evaporator temperature: 400 °C. As standard samples, commercially available individual substances were used: rutin (≥94 %, Sigma), kaempferol-3-O-glucoside (≥95 %, PhytoLab GmbH & Co. KG, Germany), apigenin-7-O-glucoside (≥97.0 %, Sigma-Aldrich, USA), isoliquiritin (≥95 %, Shanghai Tauto Biotech Co., China), caftaric acid (≥98 %, Fluka), chlorogenic acid (≥95 %, Sigma-Aldrich, USA), caffeic acid (≥98.0 %, Sigma), trans-ferulic acid (99 %, Aldrich), chicoric acid (≥95 %, Sigma), *p*-coumaric acid (≥98.0 %, Sigma).

Identification of flavonoids and HCAs derivatives based on comparison of retention times, UV- and mass-spectra with those of above-mentioned standards and literature data. Quantification of individual compounds was carried out by external standard calibration method using DAD signals: 370 nm (chalcone glycosides), 350 nm (flavonolglycosides), 338 nm (apigenin derivatives), 330 nm (caffeic, ferulic acids and their derivatives), 310 nm (*p*-coumaric acid and it's derivatives). Rutin, kaempferol-3-O-glucoside, apigenin-7-O-glucoside, isoliquiritin calibration curves were created at concentration range 0.001–0.100 mg/mL ($R^2 > 0.999$), and HCAs standards – at concentration range 0.0005–0.150 mg/mL ($R^2 > 0.999$). These calibration curves were used for quantitative estimation of similar compounds from appropriate groups mentioned above. Screening assessment makes it possible to make a preliminary quantification without assessing the detector response of each compound.

Plant material and sample preparation

Aerial parts (stems with leaves, flowers) and underground parts (rhizomes with roots) were collected in Moscow, Russia (55° 41' 23.83" N, 37° 48' 1.95" E) during the blooming stage in April 2018. The raw materials were dried for one week at 25 °C. Plant samples were authenticated in the Pharmaceutical Natural Sciences Department (Sechenov University, Russia); voucher specimens were deposited.

0.5–1.0 g of the sample (accurately weighed) was transferred to a 50 ml round bottom flask, 25 ml of 60 % aqueous methanol was added, placed in a boiling water bath under reflux for 1 h. Then the sample was transferred into a 25 ml volumetric flask, brought 60 % aqueous methanol to the mark, centrifuged at 15 000 rpm for 5 minutes.

RESULTS AND DISCUSSION

Flavonoids

Data of chromatographic detection of the main flavonoid compounds of aboveground and underground parts of *A. ranunculoides* are given in table 1 and in figures 2–6. Three flavonoid glycosides found in extracts from roots with rhizomes were identified as chalcone derivatives based on specific band I UV absorption at 365–370 nm and minor band II – at 235–255 nm (small shoulder in UV spectra) and ions with 425, 439, 601 m/z in the mass spectra. Ions with 425, 439 and 601 m/z are presumably correspond to hexoside, glucuronide and hexoside-glucuronide of chalcone aglicon with 263 m/z respectively. These compounds are found exclusively in the underground part. A richer flavonoid composition is characteristic of the *A. ranunculoides* herb (stems and leaves). The ions with 303, 287, 271 m/z along with characteristic UV spectra data found in extracts indicated that the discovered compounds belonged to derivatives of quercetin, kaempferol, and apigenin. In the herb, 9 quercetin glycosides (No. 1–5, 7, 10, 21, 22), 2 kaempferol glycosides (No. 9, 24) and one apigenin (No. 28) were found. Flowers contain 14 quercetin glycosides (No. 1–8, 10, 15, 16, 17, 21, 22), 10 kaempferol glycosides (11, 12, 13, 19, 23, 24, 25, 27, 29, 30), two apigenin glycosides (20, 28) were found to be present. Moreover, compound No. 9 (kaempferol glycoside) is present only in the herb; while 15 compounds are present in flowers that are not found in other parts of the plant (No. 6, 8, 11, 12, 13, 15, 16, 17, 19, 20, 23, 25, 27, 29, 30).

Table 1. Flavonoids composition and content in the rhizomes with roots (1), leaves (2) and flowers (3) of *A. ranunculoides*

№	Flavonoid	Rt, min (±0.1)	λ_{max}, nm (±2)	m/z		Content, mg/g		
				HESI/MS$^+$	HESI/MS$^-$	1	2	3
1	Quercetin + rhamnose + hexose + glucuronic acid isomer	12.5	255, 270, 350	787 [M + H]$^+$, 641 [M – rha + H]$^+$, 479 [M – rha – hex + H]$^+$, 303 [M – rha – hex – gluA + H]$^+$	785 [M – H]$^-$	–	0.316	0.047
2	Quercetin + rhamnose + hexose + glucuronic acid isomer	12.7	255, 270, 350	787 [M + H]$^+$, 641 [M – rha + H]$^+$, 479 [M – rha – hex + H]$^+$, 303 [M – rha – hex – gluA + H]$^+$	785 [M – H]$^-$, 639 [M – rha – H]$^-$	–	0.570	0.055
3	Quercetin + rhamnose + hexose + glucuronic acid	12.8	255, 270, 355	787 [M + H]$^+$, 641 [M – rha + H]$^+$, 479 [M – rha – hex + H]$^+$, 303 [M – rha – hex – gluA + H]$^+$	785 [M – H]$^-$, 639 [M – rha – H]$^-$	–	2.634	0.426

Continuation of table 1

№	Flavonoid	Rt, min (±0.1)	λ_{max}, nm (±2)	m/z		Content, mg/g		
				HESI/MS$^+$	HESI/MS$^-$	1	2	3
4	Quercetin + rhamnose + 2 hexoses	13.1	255, 270, 350	773 [M + H]$^+$, 627 [M – rha + H]$^+$, 611 [M – hex + H]$^+$, 465 [M – rha – hex + H]$^+$, 303 [M – rha – 2hex + H]$^+$	771 [M – H]$^-$, 625 [M – rha – H]$^-$, 609 [M – hex – H]$^-$	–	3.736	0.958
5	Quercetin + pentose + glucuronic acid + hexose	13.3	350	773 [M + H]$^+$, 641 [M – pent + H]$^+$, 465 [M – pent – gluA + H]$^+$, 303 [M – pent – gluA – hex + H]$^+$	771 [M – H]$^-$, 639 [M – pent – H]$^-$, 463 [M – rha – gluA – H]$^-$	–	0.077	0.136
6	Quercetin + 2 hexoses	13.4	350	627 [M + H]$^+$, 465 [M – 2hex + H]$^+$	625 [M – H]$^-$, 463 [M – 2hex – H]$^-$	–	–	0.113
7	Quercetin + rhamnose + hexose + pentose	13.8	255, 270, 355	743 [M + H]$^+$, 597 [M – rha + H]$^+$, 435 [M – rha – hex + H]$^+$, 303 [M – rha – hex – pent + H]$^+$	741 [M – H]$^-$, 595 [M – rha – H]$^-$	–	7.340	1.442
8	Quercetin + rhamnose + hexose + pentose isomer	14.0	255, 270, 355	743 [M + H]$^+$, 597 [M – rha + H]$^+$, 435 [M – rha – hex + H]$^+$, 303 [M – rha – hex – pent + H]$^+$	741 [M – H]$^-$, 595 [M – rha – H]$^-$	–	–	0.300
9	Kaempferol + rhamnose + 2 hexoses	14.0	265, 345	757 [M + H]$^+$, 611 [M – rha + H]$^+$, 449 [M – rha – hex + H]$^+$, 287 [M – rha – 2hex + H]$^+$	755 [M – H]$^-$, 609 [M – rha – H]$^-$	–	0.277	–
10	Quercetin + hexose + pentose	14.2	255, 270, 350	597 [M + H]$^+$, 435 [M – hex + H]$^+$, 303 [M – hex – pent + H]$^+$	595 [M – H]$^-$, 433 [M – hex – H]$^-$	–	0.495	0.260
11	Kaempferol + rhamnose + 2 hexose isomer	14.7	265, 350	757 [M + H]$^+$, 611 [M – rha + H]$^+$	755 [M – H]$^-$, 593 [M – hex – H]$^-$, 447 [M – hex – rha – H]$^-$	–	–	0.277
12	Kaempferol + 2 hexoses	14.8	265, 340	611 [M + H]$^+$, 449 [M – hex + H]$^+$, 287 [M – 2hex + H]$^+$	609 [M – H]$^-$, 447 [M – hex – H]$^-$	–	–	0.635
13	Kaempferol + 3 hexoses	15.5	345	773 [M + H]$^+$, 611 [M – hex + H]$^+$	771 [M – H]$^-$	–	–	0.263
14	Chalcone glycoside	16.1	280, 310, 370	601 [M + H]$^+$	599 [M – H]$^-$	0.055	–	–
15	Quercetin + 2 rhamnose + hexose	16.4	350	757 [M + H]$^+$, 611 [M – rha + H]$^+$, 449 [M – rha – hex + H]$^+$, 303 [M – 2rha – hex + H]$^+$	755 [M – H]$^-$, 609 [M – rha – H]$^-$	–	–	0.734
16	Quercetin + hexose + rhamnose	16.6	255, 270, 355	611 [M + H]$^+$, 449 [M – hex + H]$^+$, 303 [M – hex – rha + H]$^+$	609 [M – H]$^-$, 463 [M – rha – H]$^-$, 447 [M – hex – H]$^-$	–	–	3.314
17	Quercetin + hexose + rhamnose	16.9	255, 270, 355	611 [M + H]$^+$, 449 [M – hex + H]$^+$, 303 [M – hex – rha + H]$^+$	609 [M – H]$^-$, 447 [M – hex – H]$^-$	–	–	1.487
18	Chalcone glycoside	17.7	215, 370	425 [M + H]$^+$	423 [M – H]$^-$	0.440	–	–
19	Kaempferol + hexose + rhamnose	17.8	265, 345	595 [M + H]$^+$, 433 [M – hex + H]$^+$, 287 [M – hex – rha + H]$^+$	593 [M – H]$^-$, 447 [M – rha – H]$^-$	–	–	0.278
20	Apigenin + 2 hexoses	18.4	265, 335	595 [M + H]$^+$, 433 [M – hex + H]$^+$, 271 [M – 2hex + H]$^+$	593 [M – H]$^-$, 431 [M – hex – H]$^-$	–	–	0.414
21	Quercetin + pentose + hexose	18.8	255, 270, 355	597 [M + H]$^+$, 465 [M – pent + H]$^+$, 303 [M – pent – hex + H]$^+$	595 [M – H]$^-$, 463 [M – pent – H]$^-$	–	0.352	2.613
22	Rutin (quercetin-3-O-rutinoside)	19.9	255, 270, 355	611 [M + H]$^+$, 465 [M – rha + H]$^+$, 303 [M – rut + H]$^+$	609 [M – H]$^-$, 463 [M – rha – H]$^-$	–	0.506	0.440

End of table 1

№	Flavonoid	Rt, min (±0.1)	λ_{max}, nm (±2)	m/z		Content, mg/g		
				HESI/MS$^+$	HESI/MS$^-$	1	2	3
23	Kaempferol + 2 hexoses + rhamnose	21.2	265, 350	757 [M + H]$^+$	755 [M – H]$^-$, 593 [M – hex – H]$^-$	–	–	0.180
24	Kaempferol-glucuronide	21.5	265, 350	463 [M + H]$^+$, 287 [M – gluA + H]$^+$	461 [M – H]$^-$, 285 [M – gluA – H]$^-$	–	1.765	0.150
25	Kaempferol + rhamnose + hexose	21.7	265, 350	595 [M + H]$^+$	593 [M – H]$^-$	–	–	0.086
26	Chalcone glycoside*	22.3	220, 365	439 [M + H]$^+$	437 [M – H]$^-$	0.517	–	–
27	Astragalin (Kaempferol 3-O-glucoside)	23.2	265, 350	449 [M + H]$^+$, 287 [M – glu + H]$^+$	447 [M – H]$^-$, 285 [M – glu – H]$^-$	–	–	0.357
28	Apigenin-7-glucuronide	24.2	265, 335	447 [M + H]$^+$, 271 [M – gluA + H]$^+$	445 [M – H]$^-$, 269 [M – gluA – H]$^-$	–	0.703	0.191
29	Kaempferol-*p*-coumaroylhexoside	29.3	265, 315	595 [M + H]$^+$, 287 [M – *p*-coumhex + H]$^-$	593 [M – H]$^-$, 285 [M – *p*-coumhex – H]$^-$	–	–	1.338
30	Kaempferol-*p*-coumaroylhexoside isomer	30.2	265, 315	595 [M + H]$^+$, 287 [M – *p*-coumhex + H]$^-$	593 [M – H]$^-$, 285 [M – *p*-coumhex – H]$^-$	–	–	0.343
Total flavonoid content						**1.012**	**18.771**	**16.836**

Note. * Identification and quantification of the compound with a retention time of 22.3 min is not entirely clear; another interpretation is possible (chalcone and HCA).

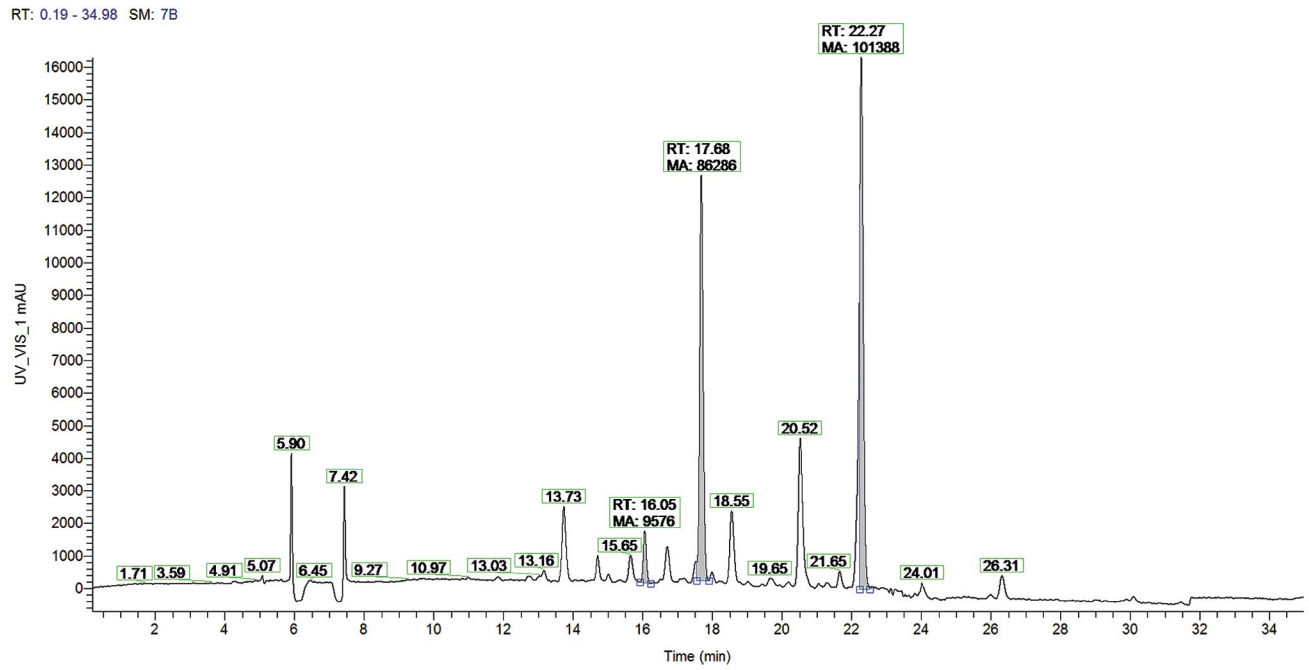

Figure 2. Chromatogram of *A. ranunculoides* rhizomes with roots extract at λ = 370 nm (chalcone glycosides)

Hydroxycinnamic acids (HCA)

The data of chromatographic detection of the main HCA of the aerial and underground parts of *A. ranunculoides* are shown in table 2 and in figures 7–8. The herb is the richest in HCAs, their content in the grass is the highest compared to other parts of the plant. 11 compounds were reliably identified (No. 10, 11, 12, 13, 15, 18, 19, 24, 25, 28, 29, 15 HCA were not identified), major ones are chlorogenic and caffeoyltartaric acids. The flowers contain almost three times less HCAs, 5 compounds were identified (No. 10, 12, 16, 19, 24, 29 HCAs were not identified), chlorogenic acid is a major one. In roots, slightly more compounds were identified than in flowers – 8 compounds (No. 6, 10, 12, 16, 17, 20, 26, 27; 23 HCAs were not identified), their content is almost on the same level as in flowers; feruloyl tartaric acid is predominant.

Table 2. **HCA derivatives composition and content in the rhizomes with roots (1), leaves (2) and flowers (3) of *A. ranunculoides***

№	Hydroxycinnamic acid derivative	Rt, min (±0.1)	λ_{max}, nm (±2)	HESI-MS$^-$	Content, mg/g		
					1	2	3
1	NI*	11.0	300sh, 330	–	0.02	–	–
2	NI	11.1	290sh, 325	–	–	0.09	0.09
3	NI	11.9	300sh, 330	–	0.02	–	–
4	NI	12.8	305sh, 330	311	0.02	–	–
5	NI	13.1	305sh, 330	–	0.04	–	–
6	*trans*-Caftaric acid	13.8	300sh, 330	311 [M – H]$^-$, 179 caffeic acid [M – H]$^-$, 149 tartaric acid [M – H]$^-$	0.32	–	–
7	NI	14.3	290sh, 325	–	–	0.06	–
8	NI	14.5	290sh, 325	–	–	0.02	–
9	NI	14.6	290sh, 320	–	–	0.05	–
10	Chlorogenic acid	15.0	296sh, 325	353 [M – H]$^-$, 191 quinic acid [M – H]$^-$	0.05	8.68	2.62
11	Cryptochlorogenic acid	15.3	296sh, 325	353, 191 quinic acid [M – H]$^-$, 179 caffeic acid [M – H]$^-$	–	0.67	–
12	*cis*-Caftaric acid (possibly)	15.6	300sh, 330	311 [M – H]$^-$, 179 caffeic acid [M – H]$^-$, 149 tartaric acid [M – H]$^-$	0.14	2.69	0.25
13	*p*-coumaric acid derivative	16.6	295sh, 315	163 *p*-coumaric acid [M – H]$^-$	–	0.12	–
14	NI	16.7	300sh, 325	–	0.21	–	–
15	Caffeoylquinic acid	17.1	296sh, 320	353 [M – H]$^-$	–	0.49	–
16	Ferulic acid derivative	17.5	300sh, 330	193	0.17	–	0.15
17	Caffeoylquinic acid	18.0	305sh, 325	353 [M – H]$^-$, 191 quinic acid [M – H]$^-$	0.05	–	–
18	*p*-coumaroylquinic acid	18.0	295sh, 315	337 [M – H]$^-$, 191 quinic acid [M – H]$^-$	–	0.41	–
19	Caffeic acid	18.3	295sh, 325	179 [M – H]$^-$	–	0.25	0.06
20	Ferulic acid derivative	18.6	300sh, 330	193	0.65	–	–
21	NI	18.8	295sh, 320	–	–	0.41	–
22	NI	19.0	296sh, 325	–	–	0.40	–
23	NI	19.7	306sh, 325	–	0.05	–	–
24	Feruloyltartaric acid	19.7	296sh, 325	325 [M – H]$^-$, 193 Ferulic acid [M – H]$^-$	–	0.62	0.25
25	*p*-coumaric acid derivative	20.3	295sh, 310	162 *p*-coumaric acid [M – H]$^-$	–	0.05	–
26	Feruloyltartaric acid isomer	20.5	302sh, 330	324 [M – H]$^-$, 193 ferulic acid [M – H]$^-$	1.18	–	–
27	Chicoric acid	22.2	302sh, 330	473 [M – H]$^-$, 311 [M – dehydrocaffeic acid]$^-$, 293 [M – caffeic acid]$^-$, 179 caffeic acid [M – H]$^-$, 149 tartaric acid [M – H]$^-$	0.34	–	–
28	*p*-coumaric acid	22.5	290sh, 310	163 [M – H]$^-$	–	0.08	–
29	*p*-coumaric acid derivative	22.6	292sh, 315	163 *p*-coumaric acid [M – H]$^-$	–	0.05	–
30	NI	23.2	308sh, 330	–	–	–	0.17
31	NI	23.4	300sh, 320	–	–	0.05	–
32	NI	23.6	296sh, 325	–	–	0.32	0.29
33	NI	24.3	307sh, 325	–	–	–	0.17
34	NI	26.0	300sh, 325	–	–	0.06	–
35	NI	26.4	306sh, 335	–	0.14	–	–
36	NI	26.5	300sh, 325	–	–	0.06	–
37	NI	28.5	300sh, 325	–	–	0.05	–
38	NI	28.9	300sh, 320	–	–	0.12	–
Total hydroxycinnamic acid derivative content					**3.39**	**15.80**	**4.06**

Note. * NI – not identified hydroxycinnamic acid derivative.

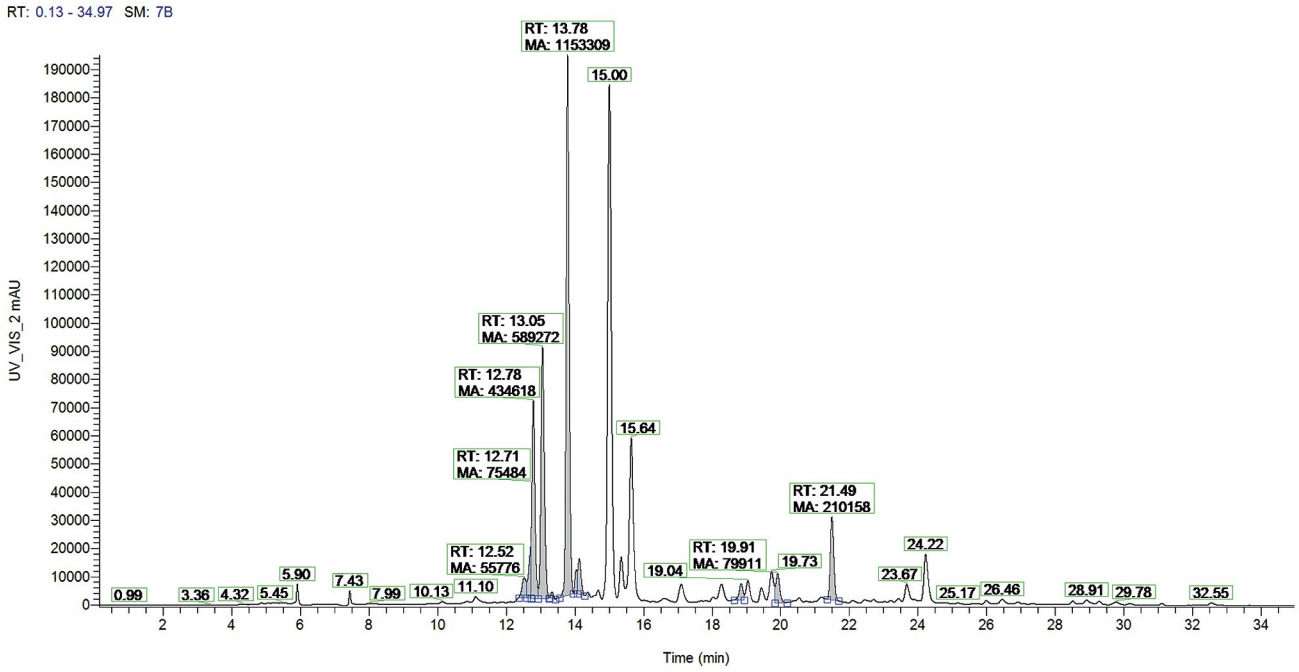

Figure 3. **Chromatogram of *A. ranunculoides* leaves extract at λ = 350 nm (flavonol glycosides).**

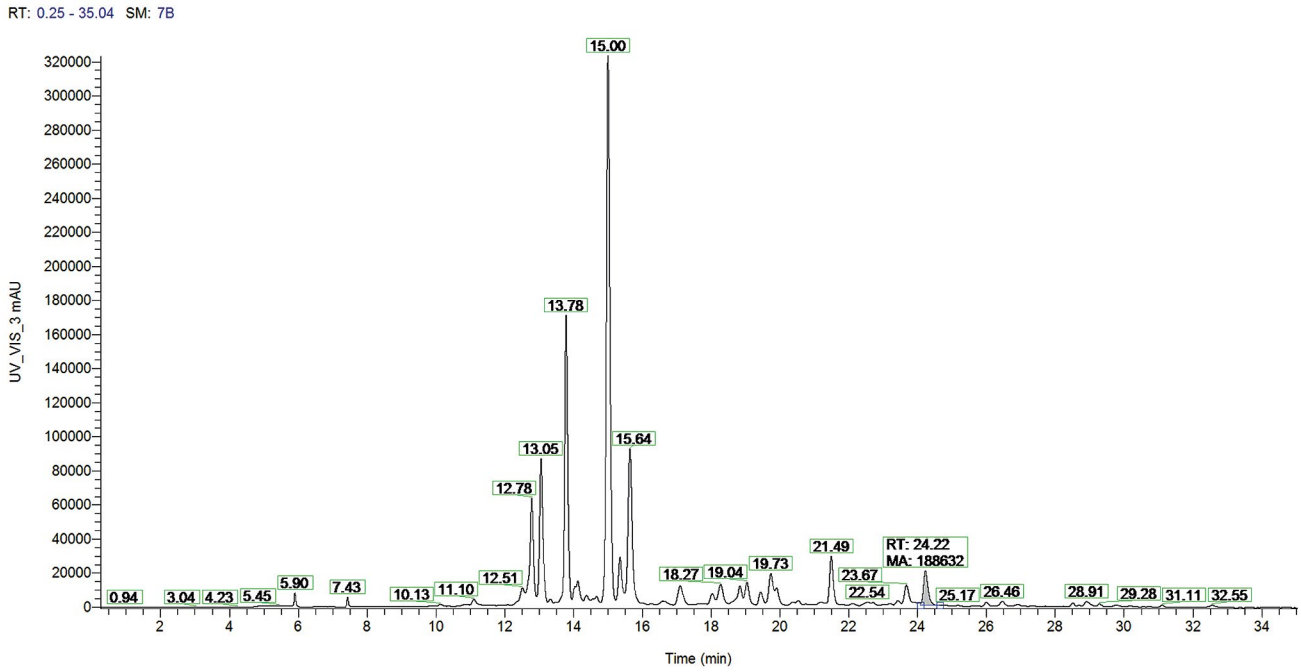

Figure 4. **Chromatogram of *A. ranunculoides* leaves extract at λ = 338 nm (apigenin-7-glucuronide).**

CONCLUSION

A methanol extracts of the aerial and underground parts of *A. ranunculoides* growing in Moscow were studied by HPLC with UV- and mass selective detection. Characteristic chromatographic profiles of flavonoids and HCAs were collected at analytical wavelengths of 215–370 and 310, 330 nm, respectively. In the group of flavonoids, major components were identified as quercetin glycosides in herb and flowers, chalcone glycosides in rhizomes with roots. In the HCA group, feruloyltartaric acid was presented in rhizomes with roots in a great amount, chlorogenic acid was predominant in the aerial part.

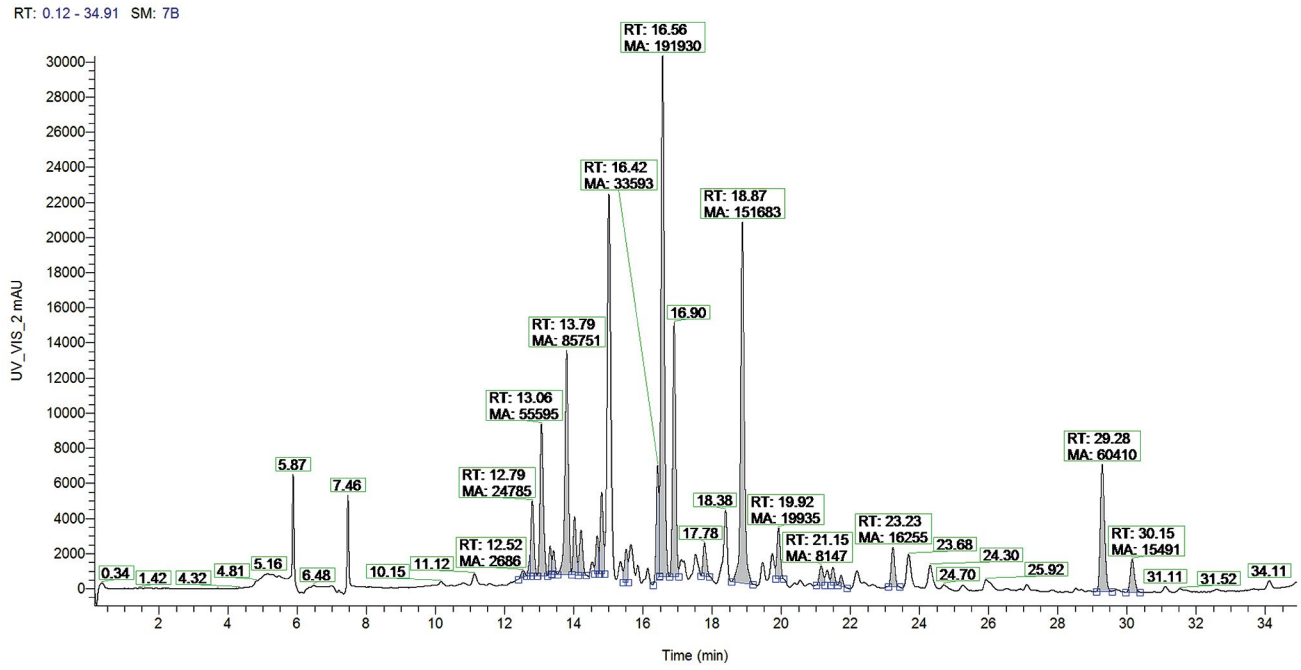

Figure 5. **Chromatogram of *A. ranunculoides* flowers extract at λ = 350 nm (flavonol glycosides).**

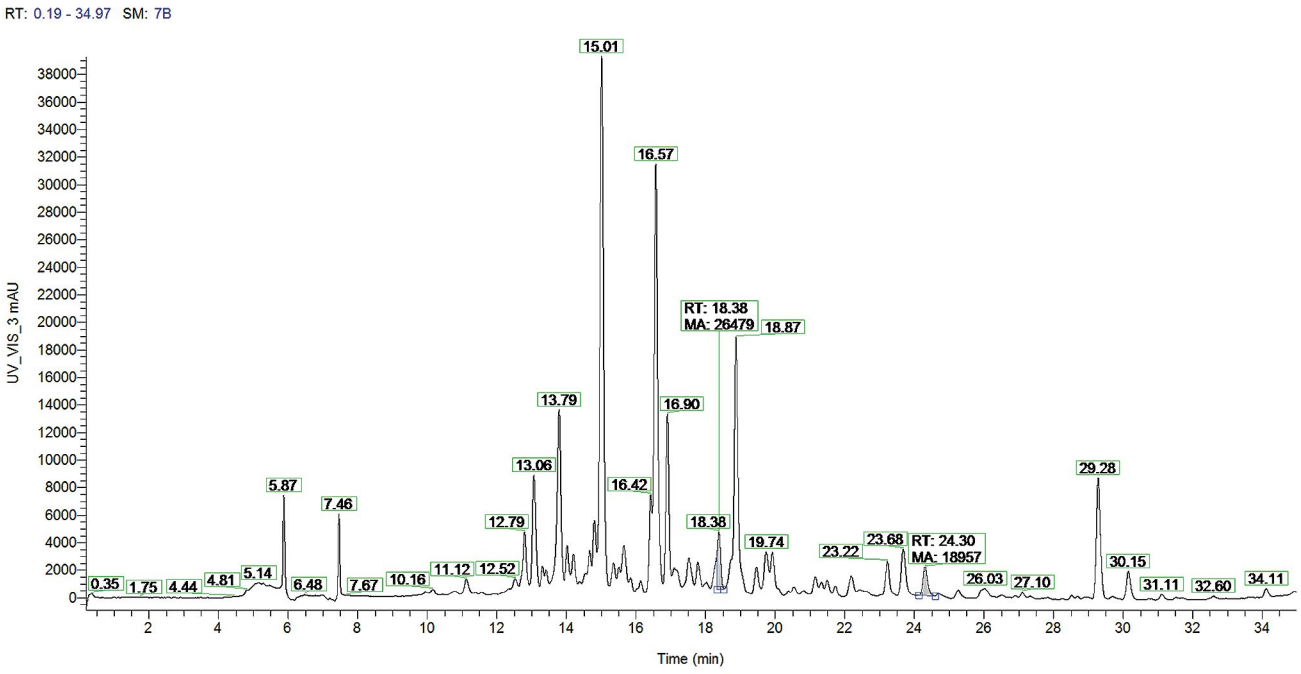

Figure 6. **Chromatogram of *A. ranunculoides* flowers extract at λ = 338 nm (apigenin derivatives).**

The identified chromatographic profiles of polyphenolic complex can be used to identify the medicinal plant . The total content of flavonoids and HCAs in the methanol extracts was approximately 60 mg/g on dry weight basis, which indicates a significant accumulation of phenolics during the flowering period of *A. ranunculoides*.

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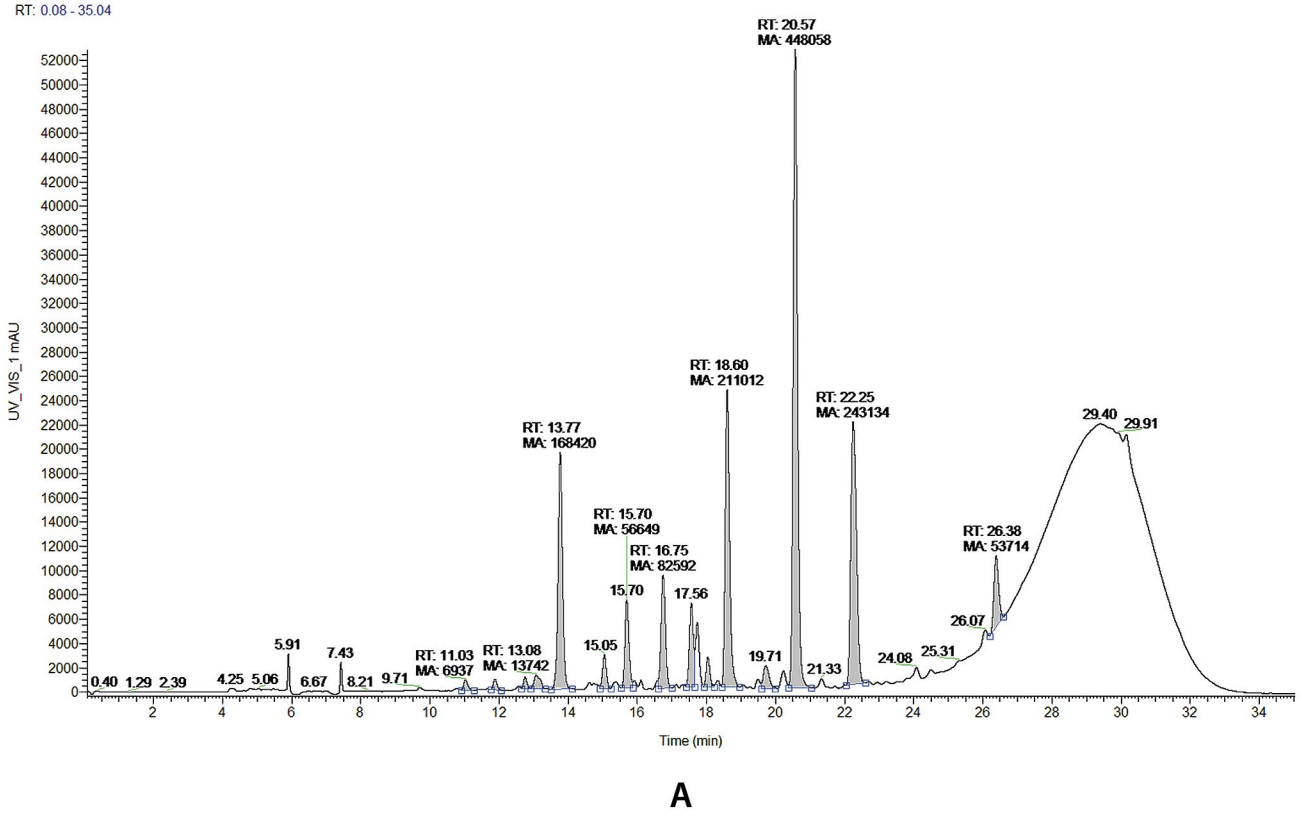

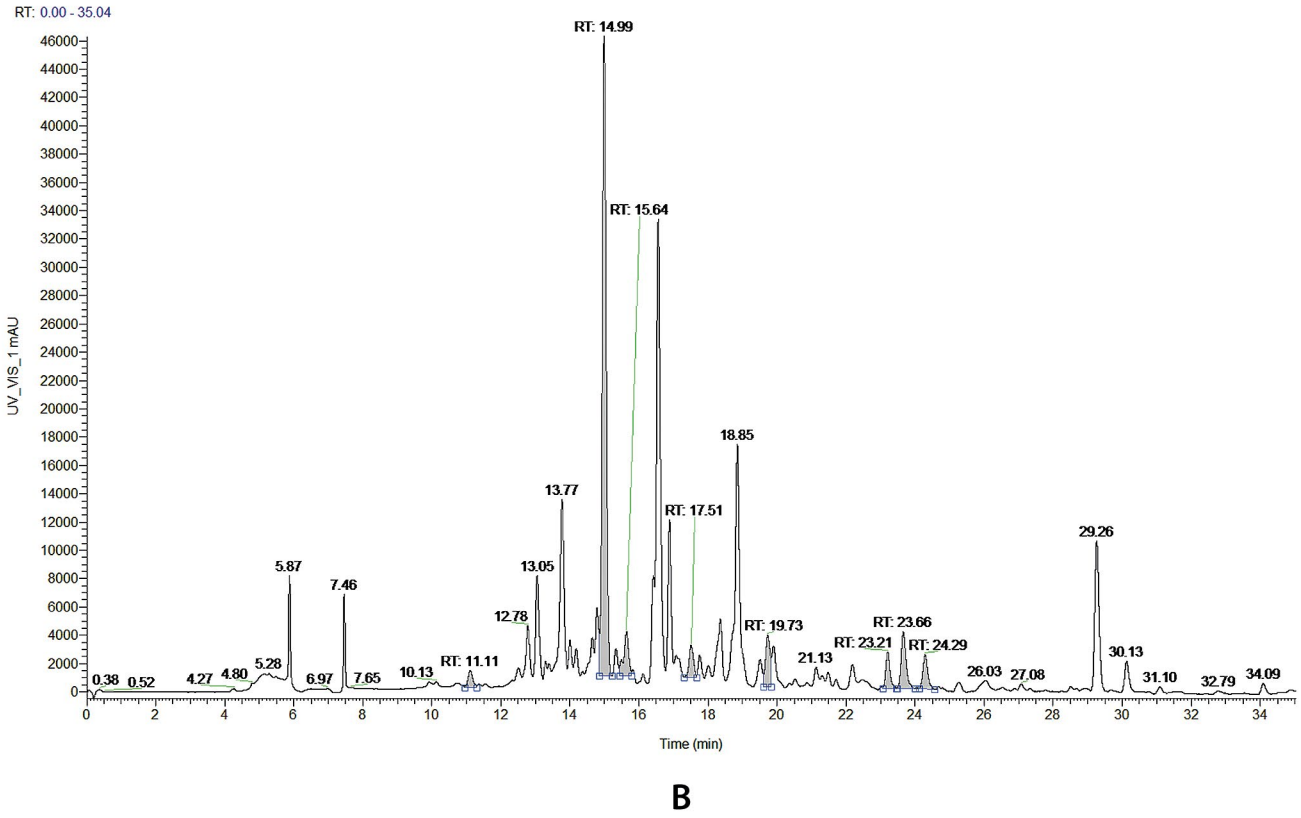

Figure 7. **Chromatogram of *A. ranunculoides* rhizomes with roots extract (A) and flowers extract (B) at λ = 330 nm (hydroxycinnamic acid derivatives)**

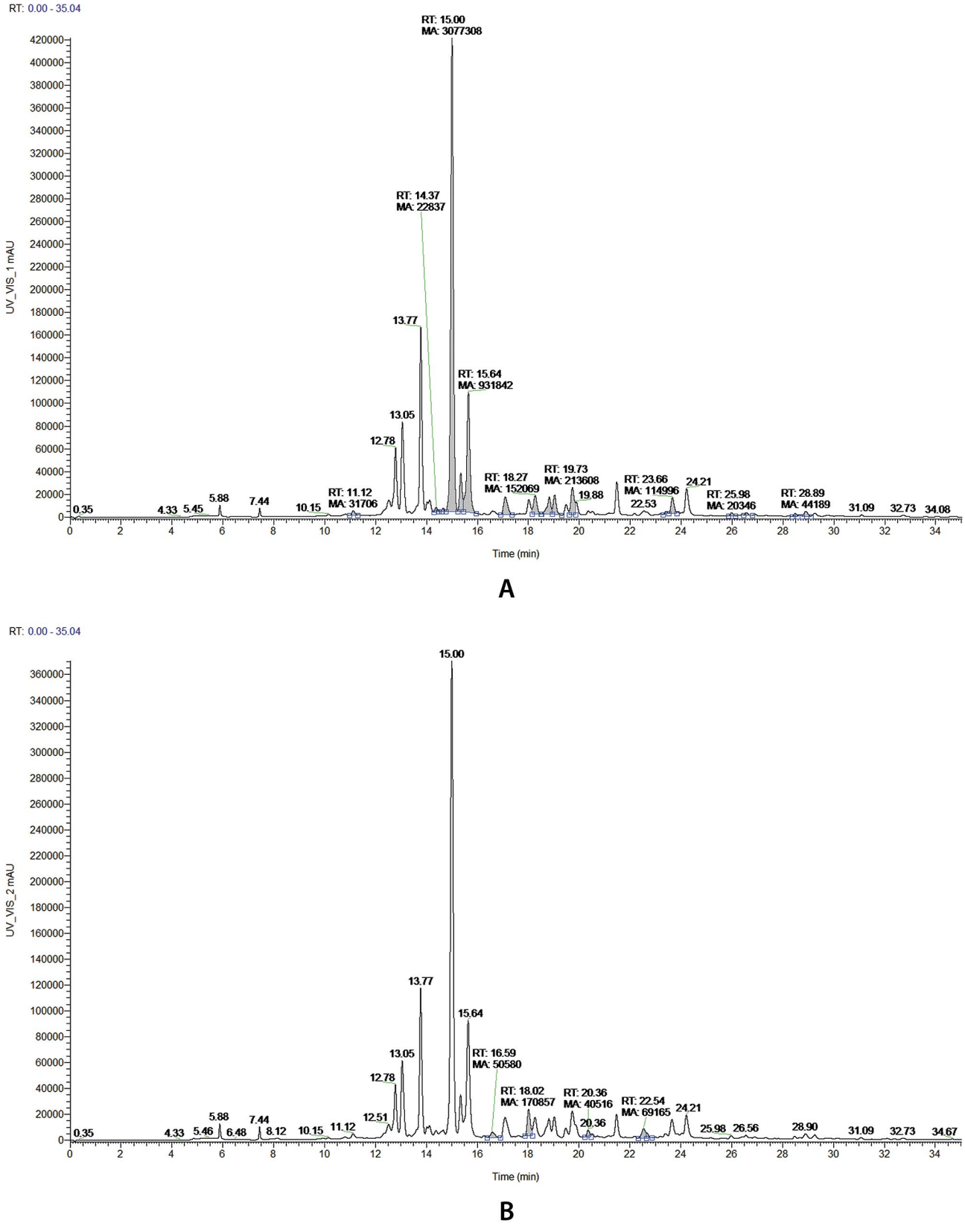

Figure 8. **Chromatogram of *A. ranunculoides* leaves extract at λ = 330 nm (A), 310 nm (B) (hydroxycinnamic acid derivatives)**

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