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STUDY OF ORGANIC ACIDS PROFILE OF GENUS *PERSICARIA* MILL SPECIES

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The genus *Persicaria* Mill. species belonging to the buckwheat family (*Polygonaceae* Juss.) and widespread in Russia, are weeds. The chemical composition's main components of the genus *Persicaria* Mill. species**,** are flavonoid glycosides (rutin, avicularin, hyperoside, etc.). The data concerning a comparative study of the organic acids in the herb representatives of genus *Persicaria* Mill., have not been detected in the available literature.

The aim of the research is a comparative study of the organic acids qualitative and quantitative composition in the genus *Persicaria* Mill. species growing in the Voronezh region.

Materials and methods. The objects of the study were dried herb samples of the genus *Persicaria* Mill. species. All the species were harvested in the Voronezh region during the blooming period. The quantitative content of ascorbic acid and the amount of organic acids in terms of malic (hydroxy-succinic) acid was carried out according to the titrimetric methods of the Russian Federation State Pharmacopeia, the XIVth ed. The study of the qualitative composition of the organic acids profile and their quantitative content assessment in the studied objects, the herbs, was carried out by the method of capillary electrophoresis ("Kapel", St. Petersburg, Russia).

Results. With the help of pharmacopoeial titrimetric methods, it was established that the highest content of the organic acids total amount is characteristic of the *Persicaria maculosa* Mill. herb (5,60%), the lowest one – of the *Persicaria tomentósa* (Schrank) E. P. Bicknell herb (4.03%). *Persicaria maculosa* S. F. Gray and *Persicaria hydropiper* (L.) Delarbre are the richest in ascorbic acid (0.17% and 0.15%, respectively). Using the method of capillary electrophoresis, the composition of the total amount of the studied organic acids has been established. It is represented by oxalic, formic, citric, malic, wine, propionic, lactic, benzoic and other acids.

Conclusion. The study of the organic acids of the genus *Persicaria* Mill. species has been carried out. It has been established that in the studied species, the organic acids total amount in terms of malic acid and the amount of ascorbic acid are similar. By the method of capillary electrophoresis, a complete composition of organic acids has been studied, and the quantitative content of each component has been established. In all the studied *Persicaria* Mill. species, the predominance of oxalic, formic and malic acids has been revealed.

Keywords: *genus Persicaria*; *Persicaria*; medicinal herbal raw materials; organic acids; capillary electrophoresis; titrimetry

Abbreviations: AsA – ascorbic acid; SP – State Pharmacopoeia; OAs – organic acids; GPM – General Pharmacopoeia Monograph; PM – Pharmacopoeial Monograph; BASs – biologically active substances.

ИЗУЧЕНИЕ ПРОФИЛЯ ОРГАНИЧЕСКИХ КИСЛОТ ВИДОВ РОДА ГОРЕЦ (*PERSICARIA* MILL.)

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Виды рода горец (*Persicaria* Mill. (L.), относящиеся к семейству гречишных (*Polygonaceae* Juss.), широко распространены на территории России, являются сорняками. Основными компонентами химического состава травы видов рода горец являются флавоноидные гликозиды (рутин, авикулярин, гиперозид и др.). Данных, касающихся сравнительного изучения органических кислот в траве представителей рода горец, в доступной литературе не обнаружено.

Цель. Сравнительное изучение качественного и количественного состава органических кислот видов рода горец (*Persicaria* Mill. (L.), произрастающих в Воронежской области.

Материалы и методы. Объектами исследования служили высушенные образцы травы видов рода горец. Все виды были заготовлены в Воронежской области во время цветения. Количественное содержание аскорбиновой кислоты и суммы органических кислот в пересчете на яблочную (гидроксибутандовую) кислоту проводили согласно титриметрических методик, рекомендованных Государственной Фармакопеей Российской Федерации XIV изд. Изучение качественного состава профиля органических кислот и оценку их количественного содержания в траве изучаемых объектов проводили методом капиллярного электрофореза (Капель, СПб, Россия).

Результаты. С помощью фармакопейных титриметрических методик выявлено, что наибольшее содержание суммы органических кислот характерно для травы горца почечуйного (5,60%), наименьшее для травы горца войлочного (4,03%). Аскорбиновой кислотой наиболее богаты горцы почечуйный и перечный (0,17% и 0,15% соответственно). При использовании метода капиллярного электрофореза был установлен состав суммы органических кислот изучаемых растений, представленный щавелевой, муравьиной, лимонной, яблочной, янтарной, пропионовой, молочной, бензойной и другими кислотами.

Заключение. Проведено исследование органических кислот видов рода горец. Установлено, что содержание суммы органических кислот в пересчете на яблочную кислоту и количество аскорбиновой кислоты в изучаемых видах сходно. Методом капиллярного электрофореза изучен полный состав органических кислот и установлено количественное содержание каждого компонента. Выявлено преобладание щавелевой, муравьиной и яблочной кислот во всех изучаемых видах рода горец.

Ключевые слова: род горец; *Persicaria*; лекарственное растительное сырье; органические кислоты; капиллярный электрофорез; титриметрия

Список сокращений: АсК – Аскорбиновая кислота; ГФ – Государственная Фармакопея; ОК – органические кислоты; ОФС – общая фармакопейная статья; ФС – фармакопейная статья; БАВ – биологически активные вещества.

INTRODUCTION

Organic acids (OAs) of plants are active metabolites [1]; they are involved in the synthesis of amino acids [2] and detoxification of heavy metals in plants [2, 3]. Such OAs as ascorbic acid (AsA or vitamin C), citric, malic acids are important for normal functioning of a human body [4–6], take part in metabolic processes, regulate the activity of the digestive system, have bactericidal [1] and antioxidant effects, promote the reduction the inflammatory response, accelerate tissue regeneration [7–10], activate cellular respiration and protein synthesis [4, 5].

Such a wide range of pharmacological activities explains the interest of Russian and foreign authors in the study of the qualitative and the quantitative content of OAs in plants [1, 14–16]. However, despite the significant list of positive OAs effects, there are also negative ones. Therefore, the scientists DO Bokov, et al. [17] notify that a high content of oxalic acid in the human diet can lead to the development of urolithiasis caused by metabolic disorders (the most important factor is a violation of the acid-base balance). Oxalic acid, which enters the urine, forms compounds with calcium ions. This fact leads to the formation of oxalate crystals.

The genus *Persicaria* Mill. species belonging to the buckwheat family (*Polygonaceae* Juss.), are promising study objects. According to the latest data from the international information base "The Plant List"[1], *genus Persicaria* includes about 66 species that are widespread in both hemispheres. Numerous works are devoted to the study of the taxonomy and quantitative composition of the genus *Persicaria* Mill. species (S. Hassannejad, et al: GI Vysochina; FA Vagabova, et al.) [18–20], and some of them touched on the issues of species chemosystematics, developed on the study of the flavonoid composition. Based on the research data, the genus *Persicaria* Mill. species were divided into 4 ranges: (*Persicariaeformes* Kom., *Hydropiperiformes* Kom., *Lapathiiformes* Worosh., *Amphibiae* Kom. However, given the peculiarities of the genus *Persicaria* Mill. species, which, growing in the same territory, can interbreed with each other and form various polymorphic forms, there are still disagreements among scientists. These disagreements regard the self-dependence of some species (for example, in the Lapathiiformes range, the probability of singling out *Persicaria tomentósa* (Schrank) into a separate species is doubtful.

In the officinal medicine, *Persicaria maculosa* S.F. Gray and *Persicaria hydropiper* (L.) Delarbre included in the State Pharmacopoeia of the Russian Federation of the XIVth ed.[2], are certified and recommended for use as hemostatic agents. Other representatives of the genus are considered impurity plants and cannot be harvested as medicinal plant materials. However, as a result of assessing their pharmacological activity, anti-inflamma-

[1] The Plant List. Available from: http://www.theplantlist.org.

[2] State Pharmacopoeia of the Russian Federation, 14th ed. 4 volumes. Available from: http://femb.ru/femb/pharmacopea.php.

tory, antioxidant and membrane-protective effects have been established [21]. The main components of the genus *Persicaria* Mill. species herb chemical composition are believed flavonoid glycosides (rutin, avicularin, hyperoside, glycosides of kaempferol, quercetin, etc.) [18, 19, 22–24], tannins, phylloquinone [25], calcium salts [26]. The data concerning a comparative study of the organic acids in the herb representatives of genus *Persicaria* Mill., have not been detected in the available literature.

THE AIM of the research is a comparative study of the organic acids qualitative and quantitative composition in the genus *Persicaria* Mill. species growing in the Voronezh region.

The experimental part of this work is aimed at solving two problems. The first task is aimed at assessing the quantitative content of the total amount of organic acids in terms of malic acid, as well as AsA in the genus *Persicaria* plants using generally available pharmacopoeial methods (titrimetry). The second task is devoted to a detailed study of the qualitative composition and the quantitative content of organic acids using a modern method of analysis (capillary electrophoresis).

MATERIALS AND METHODS

Raw materials

The objects of the study were the dried herb samples of *Persicaria maculosa* S.F. Gray, *Persicaria lapathifólia* (L.) Delarbre harvested self-dependently in the village of Uglyanets (30 km north-eastward of Voronezh, the territory of the Voronezh region; *Persicaria tomentósa* (Schrank) E.P. Bicknell), collected in the Kozo-Polyansky Botanical Garden, within the city of Voronezh; *Persicaria hydropiper* (L.) Delarbre), *Persicaria minor* (Huds.) Opiz, growing in the village of Rybachy (within the city of Voronezh); two forms of *Polygonum amphibium* – terrestrial (*Persicaria amphibia var. terréstris* (Leyss.) Munshi & Javeid) and aquatic (*Persicaria amphibia* (L.) Delarbre), harvested in the coastal zone of the Voronezh River (70 km north-eastward of Voronezh). The studied species were harvested annually from the same habitats during 2016–2018. The objects were subjected to air-shadow drying. The identification of the studied species was carried out using herbarium specimens and guides to plants of the Botany and Mycology Department, Voronezh State University.

Microscopic research

The research of microscopic characteristics of the species under study was carried out according to General Pharmacopoeia Monograph.1.5.3.0003.15 "Microscopic and microchemical research techniques of medicinal plants and herbal medicinal products" (the Russian Federation State Pharmacopeia, the XIVth ed.[3]) on the "Biomed 6" microscope at ×100 magnification. Visualization of diagnostic characteristics was carried out using a Levenhuk C310 NG digital video camera (China).

Quantitation

The content of AsA and the amount of organic OAs in terms of malic acid was carried out according to the titrimetric methods represented in the Russian Federation State Pharmacopeia, the XIVth ed. (General Pharmacopoeia Monographs "Rosehip *(Rosa canina)* fruits" and "Rowan-tree *(Sorbus aucuparia)* fruits"[4].

The analysis of the quantitative content of individual organic acids was carried out by capillary electrophoresis (Kapel, Russia). The separation conditions were represented by phosphate buffer. Capillary was: L_{eff} / L_{tot} = 40/50 cm, ID = 50 μm. The sample injection was 300 mbar s. The voltage was −17 kV. The temperature was +20°C. The detection was indirect, 190 nm[5] [5, 27].

Reagents

The reagents of chemically pure and analytically pure grades (JSC "Vekton", St. Petersburg, Russia) were used. The calculation of all quantitative characteristics was carried out in terms of absolutely dry plant materials.

RESULTS AND DISCUSSION

In the studied genus *Persicaria* Mill. species, at the first stage of the research by pharmacopoeial titrimetric methods, the content of AsA and the total amount of OAs in terms of malic acid were determined. The results are shown in Table 1.

It has been found out that among the species under study, *Persicaria maculosa* S.F. Gray and *Persicaria hydropiper* (L.) Delarbre contain a greater amount of AsA (0.17±0.01 and 0.15±0.01%, respectively). The lowest AsA content is typical for the herb of *Persicaria tomentósa* (Schrank) E.P. Bicknell and *Persicaria amphibian var. terréstris* (Leyss.) (0.07±0.006 and 0.08±0.005%, respectively). According to the WHO[6] and taking into account the data on the content of AsA in the studied plants, a daily consumption of AsA is 60–80 mg/day (0.06–0.08 g/day). These plants can serve as additional sources of this compound, which must be taken into account when obtaining medicinal herbal preparations based on them.

[3] Ibid.
[4] Ibid.
[5] Komarova NV, Kamentsev YaS. Prakticheskoe rukovodstvo po ispol'zovaniyu sistem kapillyarnogo elektroforeza Kapel' [A practical guide to the use of capillary electrophoresis systems Kapel']. St. Petersburg: Veda, 2006. – 213 p. Russian
[6] MSD Reference Professional Edition. Available from: https://www.msdmanuals.com/ru-ru/профессиональный/multimedia/table/v2089460_ru.

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Table 1 – Content of ascorbic acid and the amount of OAs in terms of malic acid (P>95%, n = 7)

Characteristic value	Range of *Persicariae formes*	Range of *Lapathiiformes*		Range of *Hydropiperiformes*		Range of *Amphibiae*	
	Persicaria maculosa S.F. Gray	*Persicaria tomentósa* (Schrank)	*Persicaria lapathifólia* (L.)	*Persicaria hydropiper* (L.)	*Persicaria mínor* (Huds.) Opiz	*Persicaria amphibia var. terréstris* (Leyss.)	*Persicaria amphibia* (L.)
Ascorbic acid, %	0.170±0.010	0.070±0.006	0.110±0.007	0.150±0.010	0.100±0.010	0.080±0.005	0.110±0.010
Amount of organic acids in terms of malic acid,%	5.60±0.20	4.03±0.12	5.47±0.30	5.16±0.20	4.47±0.16	5.28±0.18	4.73±0.11

Table 2 – Contents of organic acids in genus *Persicaria* Mill. species (P>95%, n = 3)

Object under study / Organic acids,%	Range of *Persicariae-formes*	Range of *Lapathiiformes*		Range of *Hydropiperiformes*		Range of *Amphibiae*	
	Persicaria maculosa S.F. Gray	*Persicaria lapathifólia* (L.) Delarbre	*Persicaria tomentósa* (Schrank) E.P. Bicknell	*Persicaria hydropiper* (L.) Delarbre	*Persicaria mínor* (Huds.) Opiz	(*Persicaria amphibia var. terréstris* (Leyss.)	*Persicaria amphibia* (L.) Delarbre
oxalic	3.36±0.06	0.35±0.02	1.70±0.03	7.36±0.14	2.13±0.04	1.19±0.02	0.48±0.004
formic	< 0.15	2.84±0.03	< 0.15	4.71±0.09	4.47±0.08	6.69±0.12	< 0.15
fumaric	< 0.005	0.014±0.002	0.023±0.0001	0.017±0.0001	< 0.005	< 0.005	0.008±0.0002
amber	< 0.05	< 0.05	< 0.15	< 0.05	< 0.05	0.067±0.001	< 0.05
malic	0.130±0.003	0.044±0.001	0.062±0.0001	0.055±0.0001	0.073±0.0002	0.28±0.005	0.66±0.01
citric	0.07±0.001	0.28±0.005	0.20±0.004	0.25±0.005	0.12±0.002	0.20±0.002	0.72±0.01
propionic	<0.15	0.22±0.004	0.16±0.002	0.17±0.002	0.03±0.0001	0.16±0.003	< 0.15
lactic	< 0.12	< 0.12	< 0.12	< 0.12	0.29±0.006	< 0.12	< 0.12
benzoic	0.006±0.0001	0.03±0.0001	< 0.005	0.02±0.0005	0.007±0.0001	0.008±0.0001	< 0.005
sorbic	< 0.025	0.12±0.002	< 0.025	< 0.025	0.04±0.0008	< 0.025	< 0.025
wine	< 0.005	0.50±0.005	0.46±0.003	0.76±0.007	0.50±0.004	2.15±0.043	1.79±0.035
acetic	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Total amount	3.56±0.07	4.47±0.09	2.61±0.05	13.42±0.27	7.66±0.15	10.77±0.21	3.66±0.08

Table 3 – Determination of the dependence of oxalic acid content on frequency and size of calcium oxalate druses

Object under study / Parameter under study	Range of *Persicariae-formes*	Range of *Lapathiiformes*		Range of *Hydropiperiformes*		Range of *Amphibiae*	
	Persicaria maculosa S.F. Gray	*Persicaria lapathifólia* (L.)	*Persicaria tomentósa* (Schrank)	*Persicaria hydropiper* (L.) Delarbre	*Persicaria mínor* (Huds.) Opiz	*Persicaria amphibia var. terréstris* (Leyss.)	*Persicaria amphibia* (L.) Delarbre
Oxalic acid content,%	3.36±0.07	0.35±0.08	1.70±0.03	7.36±0.15	2.13±0.04	1.19±0.02	0.48±0.004
Frequency of occurrence, pieces (1 mm^2)	70±20	120±45	200±30	130±25	150±20	140±30	–
Druses diameter, µm	11.6–41.9	11.5–34.9	49.0–81.5	9.3–23.3	11.5–69.9	11.6–34.9	–

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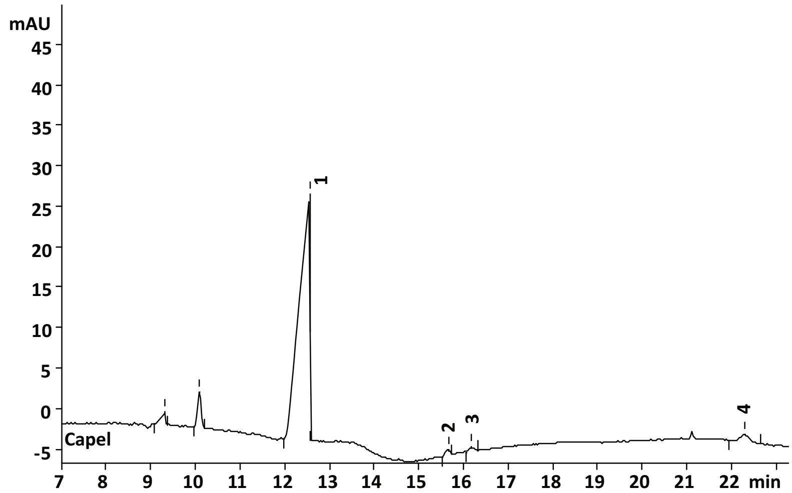

Figure 1 – Electropherogram of organic acids of *Persicaria maculosa* (S.F. Gray) herb

Note: 1 – oxalic acid; 2 – malic acid; 3 – citric acid, 4 – benzoic acid.

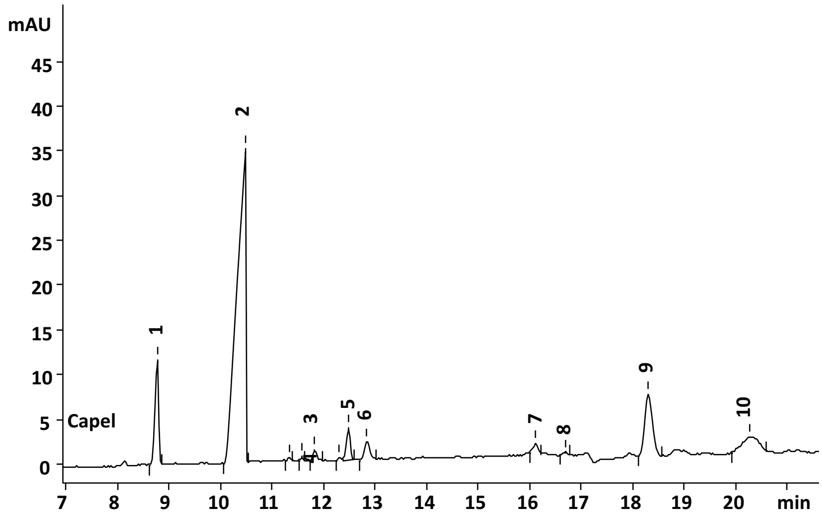

Figure 2 – Electropherogram of organic acids of *Persicaria lapathifólia* (L.) herb

Note: 1 – oxalic, 2 – formic, 3 – fumaric, 4 – malic, 5 – wine, 6 – citric, 7 – propionic, 8 – lactic, 9 – benzoic, 10 – sorbic.

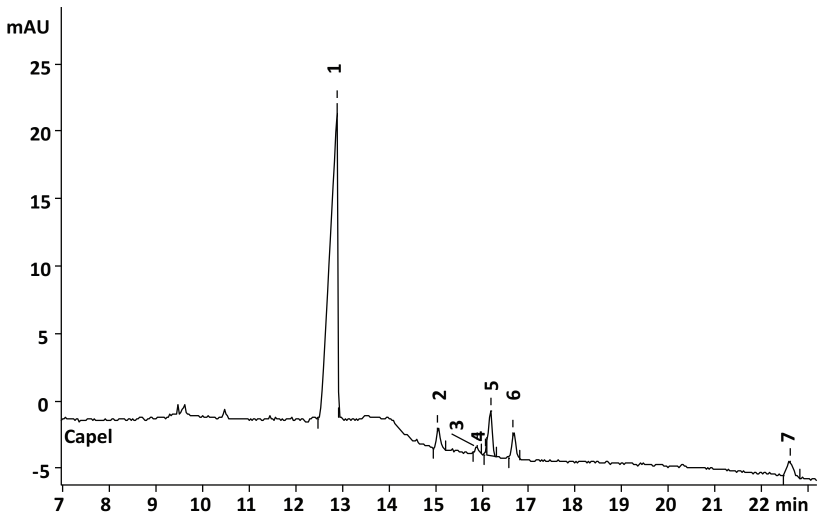

Figure 3 – Electropherogram of organic acids of *Persicaria tomentósa* (Schrank) herb

Note: 1 – oxalic, 2 – fumaric, 3 – amber, 4 – malic, 5 – wine, 6 – citric, 7 – benzoic.

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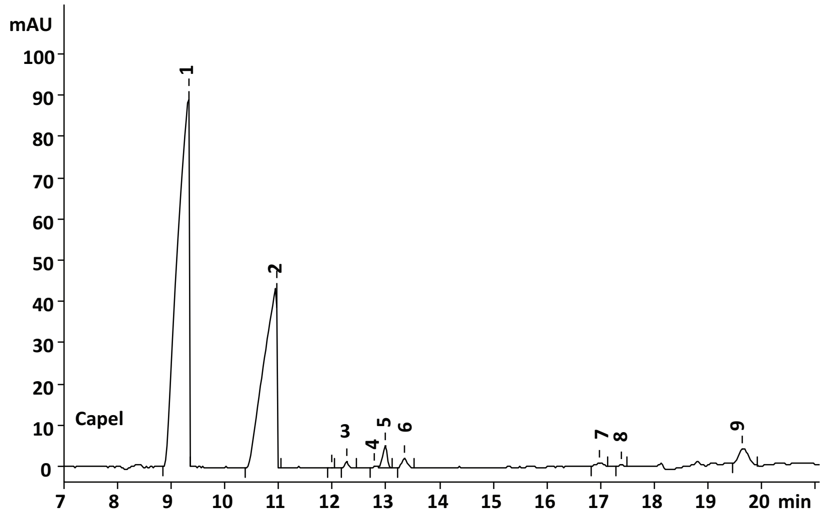

Figure 4 – Electropherogram of organic acids of *Persicaria hydropiper* (L.) herb

Note: 1 – oxalic, 2 – formic, 3 – fumaric, 4 – malic, 5 – wine, 6 – citron, 7 – propionic, 8 – lactic, 9 – benzoic.

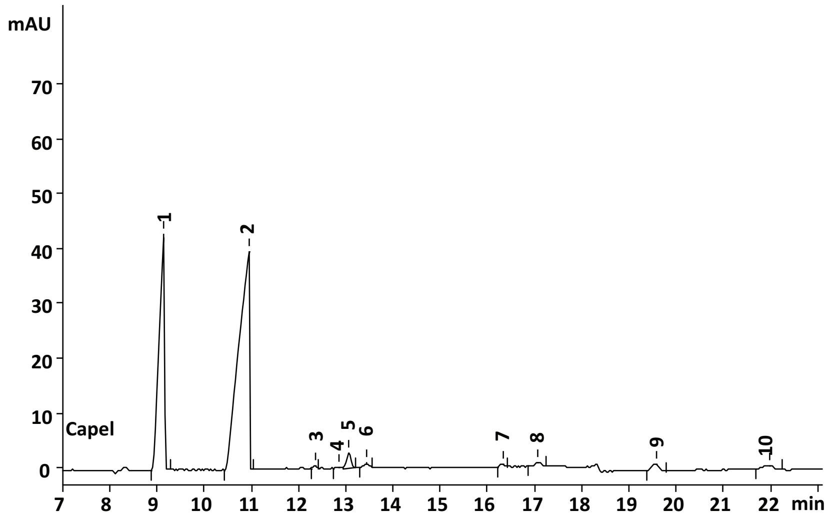

Figure 5 – Electropherogram of organic acids of *Persicaria mínor* (Huds.) herb

Note: 1 – oxalic, 2 – formic, 3 – fumaric, 4 – malic, 5 – wine, 6 – citric, 7 – propionic, 8 – lactic, 9 – benzoic, 10 – sorbic.

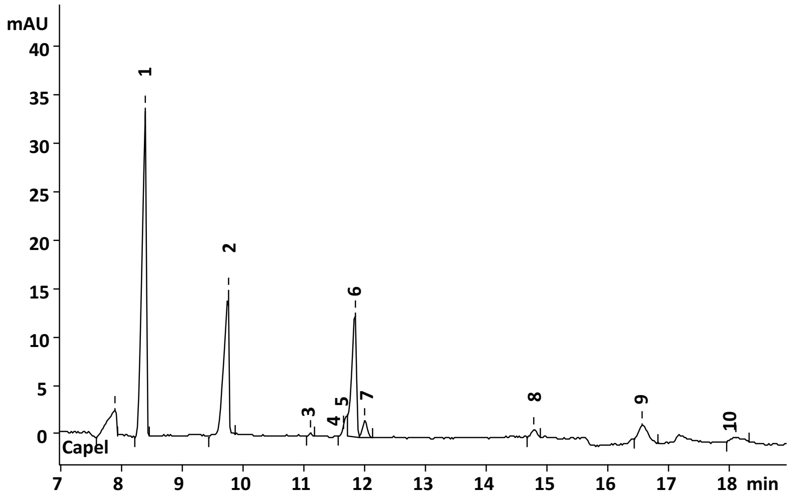

Figure 6 – Electropherogram of organic acids of *Persicaria amphibian var. terréstris* (Leyss.) herb

Note: 1 – oxalic, 2 – formic, 3 – fumaric, 4 – amber, 5 – malic, 6 – wine, 7 – citric, 8 – propionic, 9 – benzoic, 10 – sorbic.

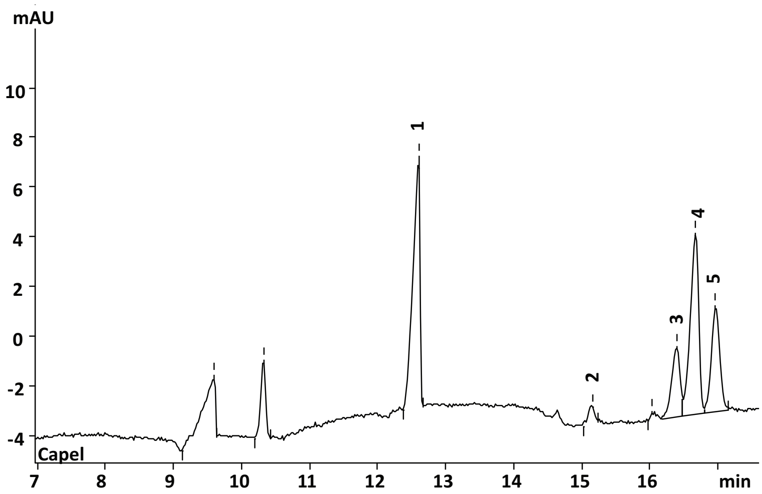

Figure 7 – Electropherogram of organic acids of the *Persicaria amphibia* (L.) herb
Note: 1 – oxalic, 2 – fumaric, 3 – malic, 4 – wine, 5 – citric.

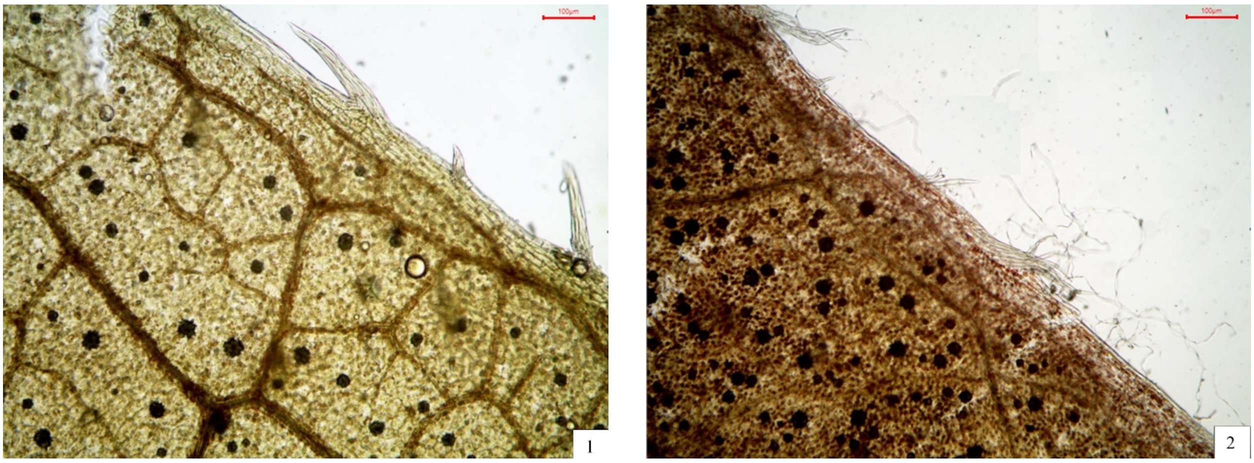

Figure 8 – Druses of calcium oxalate on micropreparations of *Persicaria maculosa* S.F. Gray (1) and *Persicaria tomentósa* (Schrank) (2) leaves

Analyzing the total amount of OAs in terms of malic acid in the studied genus *Persicaria* Mill. species, it was found out that their greater content is observed in the herb of *Persicaria maculosa* S.F. Gray and *Persicaria lapathifólia* (L.) Delarbre. In the herb of *Persicaria hydropiper* (L.) Delarbre, this indicator is 13% higher (5.16±0.20%) than in *Persicaria mínor* (Huds.) Opiz (4.47±0.16%), which is the main impurity in harvesting *Persicaria hydropiper* (L.) raw materials. The difference in the quantitative content of the both as the amount of OAs and the content of AsA is observed within two forms of one species of *Persicaria amphibia* (L.) *Delarbre.* In *Persicaria amphibia var. terréstris* (Leyss.), the amount of AsA is 27% less (0.08±0.005%), and the amount of OAs is 10% more (5, 28±0.18) than in *Persicaria amphibia* (L.) *Delarbre* (4.73±0.11).

The study of OAs and AsA in plant raw materials by pharmacopoeial methods has a number of disadvantages, in particular, malic acid, for which it is recommended to recalculate the content of the OAs amount, is not always present in the raw material in a prevailing amount. It can be in a free form and in the form of potassium and calcium salts. The pharmacopoeial method does not make it possible to establish the qualitative composition of OAs present in plant raw materials both in a free form and in the form of salts [5, 14, 28].

One of the most often used methods in the OAs analysis of plants, is capillary electrophoresis, which combines simplicity, rapidity, reliability and a low resource consumption compared to chromatographic methods of analysis [1, 5, 28–32]. The next stage of the work was the study of the complete composition of OAs (both in a free form and in the form of salts) by the method of capillary electrophoresis. Herewith, oxalic, formic, fumaric, amber, malic, citric, propionic, lactic, benzoic, sorbic, wine and acetic acids were identified in the studied *Persicaria* species, and their quantitative contents have been determined. The obtained data are shown in Table 2, the electrophoregrams are presented in Figures 1–7.

The analysis of the data obtained in the course of the study showed that despite the close relationship of the genus *Persicaria* Mill (L.) species, there is a heterogeneity in the qualitative and quantitative composition of the OAs of the plants under study. Evaluating the picture of the OAs profiles of the species under study and in accordance with their division into ranges, one can see striking differences between the *Persicariaeformes* range and others. In particular, four OAs (oxalic, malic, citric, benzoic) were reliably identified in the *Persicaria maculosa* S.F. Gray herb. Within the *Lapathiiformes* range, the differences in the composition of OAs between closely related species should be notified. In the *Persicaria lapathifólia* (L.) herb there are 9 acids (oxalic, formic, fumaric, malic, citric, propionic, benzoic, sorbic, wine), in the *Persicaria tomentósa* (Schrank) herb there are 6 acids (oxalic, fumaric, malic, citric, propionic, wine). The same situation is observed in the species of the Hydropiperiformes range: in the *Persicaria hydropiper* (L.) Delarbre herb, there are 8 acids (oxalic, formic, fumaric, malic, citric, propionic, benzoic, wine), in the *Persicaria mínor* (Huds.) Opiz herb, there are 9 acids (oxalic, formic, malic, citric, propionic, lactic, benzoic, sorbic, wine). Within the range of *Amphibiae*, in the *Persicaria amphibia var. terréstris* (Leyss.) herb, there are 8 acids (oxalic, formic, amber, malic, citric, propionic, benzoic, wine), in the *Persicaria amphibian* (L.) herb, there are 5 acids (oxalic, fumaric, malic, citric, wine). Such a difference in the patterns of the OAs profile observed in the two forms of the same species, is explained by the influence of the adaptive mechanism and a plant habitat on the synthesis of biologically active substances (BASs). Analyzing the data in Table 2, it is possible to notify the similarity in the qualitative composition of the OAs in the *Persicaria hydropiper* (L.) and *Persicaria amphibia var. terréstris* (Leyss.) herbs, which makes it possible to assume the genetic relationship between these species.

The total amount of OAs, determined by the method of capillary electrophoresis, is typical for the *Persicaria hydropiper* (L.) herb (13.42%), *Persicaria amphibia var. terréstris* (Leyss (10.77%) and *Persicaria mínor* (Huds.) Opiz (7.66%).

Oxalic acid is the main OA in the composition of the *Persicaria maculosa* S.F. Gray, *Persicaria hydropiper* (L.) Delarbre, *Persicaria tomentósa* (Schrank) herbs. In the *Persicaria maculosa* S. F. Gray herb, the content of oxalic acid was 94% of the total amount of OAs; in the *Persicaria tomentósa* (Schrank) herb it was 65%, in the *Persicaria hydropiper* (L.) herb it was 55%. One of the forms in which oxalic acid can be found in plants, is crystalline inclusions. One of the features of the buckwheat family representatives, i.e. genus *Persicaria*, is the presence of rather large and numerous druses of calcium oxalate, which are found in great numbers in idioblasts in the mesophyll of leaves, along the conductive bundles of the stem, near the base of corolla petals. In addition to determining the qualitative composition and the quantitative content of OAs in the studied species, the presence of a relationship between the quantitative content of oxalic acid, the frequency of occurrence and the size of calcium oxalate druses, revealed as a result of microscopic analysis of the studied objects, was analyzed. Fig. 8 shows, as an example, a picture of the microscopic structure of *Persicaria maculosa* S. F. Gray (1) and *Persicaria tomentósa* (Schrank) E.P. Bicknell (2) leaves, where the presence of a large amount of calcium oxalate druses on the *Persicaria tomentósa* (Schrank) E. P. Bicknell leaf is clearly visible.

Table 3 shows the results of calculating the frequency of calcium oxalate druses occurrence and determining their diameter using a Levenchuk eyepiece micrometer (China). The highest content of oxalic acid is characteristic of *Persicaria hydropiper* (L.), while the diameter of the druses, in comparison with the rest of the objects, is the smallest (9.3–23.3 microns) with an average frequency of occurrence (130±25 pieces/mm^2). The greatest number of large-diameter druses is observed in *Persicaria tomentósa* (Schrank) (200±30 pieces/mm^2), however, the content of oxalic acid in the raw material is low (1.7±0.03%). With the use of a microscopic method of analysis, calcium oxalate druses were not found out in *Persicaria amphibia* (L.), and the amount of oxalic acid, established by capillary electrophoresis, was not high (0.48±0.004%). The results obtained show that no relationship was found out between the content of oxalic acid, the frequency of occurrence and the size of calcium oxalate druses. In the studied objects, oxalic acid is found mainly in a free form, and only a small part of it – in the form of calcium salts and other compounds.

A prevailing amount of formic acid is characteristic of *Persicaria lapathifólia* (L.), *Persicaria mínor* (Huds.) Opiz, and *Persicaria amphibia var. terréstris* (Leyss.). Among the studied species, a higher content of formic acid (6.69%) is characteristic of *Persicaria amphibia var. terréstris* (Leyss.), which is 62% of the total OAs. In *Persicaria hydropiper* (L.) and *Persicaria mínor* (Huds.) Opiz, the content of formic acid is similar (4.71±0.09 and 4.47±0.08%, respectively), which is 35 and 58% of the total OAs in the plants. A smaller amount is observed in *Persicaria lapathifólia* (L.) (2.84%), however, the percentage of the total OAs is quite high and amounted to 63%. The presence of formic and oxalic acids in such high quantities explains the appearance of a not-critical irritation when plant sap comes into contact with the skin surface, which must be taken into account when harvesting raw materials.

Citric and malic acids are found in greater quantities in *Persicaria amphibia* (L.) (0.72% and 0.66%, respectively), while the content of malic acid is 50%, and citric acid

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is 70% higher than in *Persicaria amphibia var. terréstris* (Leyss.) of this species (0,28% and 0.2%, respectively). It should be notified that citric acid is unevenly distributed within the limits allocated to the genus *Persicaria* Mill. species. The content of citric acid in the *Persicaria mínor* (Huds.) Opiz herb is 50% less (0.12%) than in the *Persicaria hydropiper* (L.) herb (0.25%); it is about the same in the *Persicaria lapathifólia* (L.) herb (0.28%) and *Persicaria tomentósa* (Schrank) (0.2%)), which is 65% higher than in *Persicaria maculosa* S.F. Gray (0.07%). Malic acid plays an important role in the metabolic activity of cells and contributes to the production of ATP by the body, supports the immune system, and is a chelator of toxic metals. The pharmaceutical industry produces a number of preparations containing malic acid belonging to the group of metabolites, rehydrating agents (Sterofundin isotonic), plasma substitutes (Ionehes), antiseptics (Acerbin)[7].

A small amount of amber acid was reliably found in *Persicaria amphibia var. terréstris* (Leyss.) (0.067%) and, presumably, is a marker component for this species, since in other species its content is less than the detection limit of the device. Amber acid is an important endogenous intracellular metabolite of the Krebs cycle, which performs a universal energy-synthesizing function in the cells of the body. On the basis of amber and ascorbic acid, potentiating the action of each other, the pharmaceutical industry produces the Yantavit dietary supplement, which has general tonic, angioprotective, metabolic, antihypoxic, antioxidant properties[8].

Propionic acid (0.22%) in larger quantities is characteristic of oxalate *Persicaria lapathifólia* (L.), and lactic acid is characteristic of *Persicaria mínor* (Huds.) Opiz (0.29%).

The amount of wine acid in the *Persicaria amphibia var. terréstris* (Leyss.) herb is almost twice higher (2.15%) than in *Persicaria amphibia* (L.) (1.79%): in other plants, the amount of wine acid is low. Within the ranges of *Persicaria lapathifólia* (L.) and *Persicaria tomentósa* (Schrank), its content is similar. *Persicaria maculosa* S.F. Gray contains wine acid in the amount less than the detection limit of the device, which can be a feature of the plant and also act as an additional chemotaxonomic feature of the raw material.

Such OAs as fumaric, benzoic, sorbic, are present in the plant raw materials of *genus Persicaria* in insignificant quantities. The content of acetic acid is next to nothing, below the maximum capability of the device, which may be due to a partial loss of the substance as a result of a sample preparation (acetic acid and some others belong to the class of volatile OAs).

Thus, the carried out study made it possible to establish the qualitative composition and quantitative content of OAs in the herbs of the genus *Persicaria* Mill (L.) species and to reveal the prospects of using this group of plants as additional sources of compounds important for the vital activity of the organism.

CONCLUSION

For the first time, a comparative study of OAs in the genus *Persicaria* herbs has been carried out. With the help of pharmacopoeial methods, in the species under study, the quantitative content of the amount of OAs in terms of malic and AsA has been established: *Persicaria maculosa* S. F. Gray and *Persicaria lapathifólia* (L.) are the closest in the quantitative content of these compounds.

By the method of capillary electrophoresis, the complete composition of OAs has been studied, and their quantitative content has been established. Despite a close relationship between the herbs of the genus *Persicaria* Mill. species, a heterogeneity in the qualitative and quantitative composition of the OAs of the studied plants has been revealed. The predominance of oxalic, formic and malic acids in all the studied genus *Persicaria* Mill. species has been shown. The greatest amount of organic acids is characteristic of *Persicaria hydropiper* (L.) and *Persicaria amphibia var. terréstris* (Leyss.). It has been revealed that a characteristic feature of a number of *Amphibiae* is a higher content of malic and wine acids than in the other studied species. Amber acid acts as an identification compound of *Persicaria amphibia var. terréstris* (Leyss.). Due to the presence of a large amount of formic acid in the *Persicaria hydropiper* (L.) herb, *Persicaria mínor* (Huds.) and *Persicaria amphibia var. terréstris* (Leyss.), it is recommended to use personal protective equipment when working with these objects in order to avoid skin irritation. On the basis of the study, a possible genetic relationship between *Persicaria hydropiper* (L.) and *Persicaria amphibia var. terréstris* (Leyss.) was presupposed. During the experiment, no relationship was established between the content of oxalic acid, the frequency of occurrence and the size of calcium oxalate druses. In the studied objects, oxalic acid is found mainly in a free form, and only a small part of it – in the form of calcium salts and other compounds.

The carried out research has shown that the studied genus *Persicaria* Mill. species are promising sources of OAs. The data obtained can be used in the pharmaceutical analysis when carrying out the standardization of plant materials. The information on the qualitative composition and quantitative content of the individual components of the OAs profile in the species under study will make it possible to adjust the consumption rates of herbal medicinal products based on them.

[7] Register of medicines of Russia: reference book of medicines. Available from: https://www.rlsnet.ru.
[8] Ibid.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Anna S. Chistyakova – collecting literature data, experiment conducting, interpreting the results obtained, preparing the draft manuscript; Alevtina A. Gudkova – research planning, harvesting and drying plant materials, experiment conducting, processing the results obtained, preparing the manuscript, participating in the development of the concept and research design; Alexey I. Slivkin — manuscript publishing approval, critical review of intellectual content; Elena E. Chupandina – implementation of the experimental part of the work, discussion of the results.

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