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ORIGINAL ARTICLE

Levels of phenolic compounds in leaves of *Eranthis sibirica*, *E. stellata*, and *E. tanhoensis* (Ranunculaceae)

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Levels of phenolic compounds in leaves of plants *Eranthis sibirica* DC., *E. stellata* Maxim. and *E. tanhoensis* Erst were studied. In aqueous ethanol extracts from leaves of these *Eranthis* L. plants, 24 phenolic compounds were identified by high-performance liquid chromatography. These included chlorogenic, gentisic, caffeic, and salicylic acids, quercetin, kaempferol and hyperoside flavonols, and orientin and vitexin flavones. It was revealed that among the three species, the levels of chlorogenic (0.34–0.96 mg/g), caffeic (0.29–0.32 mg/g), and salicylic (0.25 mg/g) acids are higher in leaves of *E. sibirica*, whereas kaempferol concentration (0.42 mg/g) is higher in *E. tanhoensis* leaves. Leaves of *E. stellata* contain more orientin (1.19–4.99 mg/g) and quercetin (0.12–0.20 mg/g) as compared to the other two species. Vitexin (1.84–3.63 mg/g) was found in leaves of *E. sibirica* only. Species-specific ratios of levels of major phenolic compounds were identified. The total concentration of phenolcarboxylic acids in *E. sibirica* leaves exceeds almost 2-fold that of flavonols. On the contrary, in *E. stellata* and *E. tanhoensis* leaves, the total concentration of flavonols is higher or almost equal to the concentration of phenolcarboxylic acids.
Key words: *Eranthis sibirica*, *Eranthis stellata*, *Eranthis tanhoensis*, flavonoid, phenolcarboxylic acid, high-performance liquid chromatography, HPLC.

Introduction

Phenolic compounds are among the most common representatives of secondary metabolites in plant tissues. They play a vital role in the structural integrity of plants, protection from UV radiation, in reproduction, and internal regulation of the physiology and signaling of plant cells (Zaprometov, 1993, Falcone Ferreyra et al., 2012). Due to their high biological activity, plant polyphenols are successfully used in various industries, as well as in medicine and pharmacology, as substances with antioxidant, neuroregulatory, capillary-strengthening, immunomodulatory, anticancer, and other effects (Weinreb et al., 2008; Kim et al., 2011; Bespalov et al., 2017; Alamgir, 2018). In addition, phenolic compounds are widely employed in chemotaxonomic and phylogenetic studies owing to their widespread occurrence among plants, structural diversity, and chemical stability (Vysochina, 2004; Braunberger et al., 2015). In plants of the family Ranunculaceae Juss., phenolic compounds hold promise as chemotaxonomic markers (Hao, 2018; Erst et al., 2020).

Three species of the genus *Eranthis* L. grow on the territory of Asian Russia: *E. sibirica* DC., *E. stellata* Maxim., and *E. tanhoensis* Erst (Erst et al., 2020). The three taxa belong to section *Shibateranthis* (Nakai) Tamura. Its species have white flowers and differ from the typical yellow-flowered section *Eranthis* L. *Eranthis sibirica* and *E. stellata* in the pubescence of peduncles, stalks, and fruitlets, leaf blade shape and colour, and the position of the flower (Tamura, 1995). They have different geographic ranges: *E. sibirica* is widespread in Eastern and Western Siberia, whereas *E. stellata* grows in the east of China, in Korea, and in the Far East of Russia (Shipchinskij, 1937). *Eranthis tanhoensis* was described recently and is an endemic species; it is morphologically similar to *E. sibirica* and *E. stellata* because it has white sepals, tubular two-lipped petals with bilobate or forked lips, apically acute lobes with an abaxial lip, and globular yellow protuberances (nectaries) at the top or in the central part (Erst et al., 2020). *Eranthis tanhoensis* can be found at 350–2400 m above sea level (a.s.l.), where it grows in fir, Siberian pine, spruce, and birch forests, on riverbanks, near streams (up to 1500 m a.s.l.), and in subalpine meadows (at higher altitudes) (Erst et al., 2020).

The set and biological activities of compounds from the underground parts of *Eranthis* plants are being actively studied. Triterpene glycosides of cycloartan and oleanan series and triterpene saponins have been discovered in tubers of *E. cilicica* Schott. & Kotschy. These substances exert a cytotoxic effect on human promyelocytic leukaemia (HL-60) cells (Watanabe et al., 2003, 2019). Antioxidant properties of chromones isolated from *E. cilicica* tubers were revealed (Kuroda et al., 2009). Chromones and lectins with anticancer, insecticidal, antifungal, and antiviral effects have been found in tubers of *E. hyemalis* (L.) Salisb. (Junior, 1979; Kopp et al., 1991; Kumar et al., 1993; George et al., 2011; McConnell et al., 2015; Djafari et al., 2018). The ethanol extract from the roots of *E. hyemalis* has anti-inflammatory effects (Malik et al., 2017). The whole *Eranthis* plant is used to treat diuresis and urolithiasis (Hao, 2018). Data on the profile and levels of phenolic compounds in plants of the genus *Eranthis* are still scarce. We previously revealed the uniqueness of phenolic compounds profile in leaves of *Eranthis* plants, section *Shibateranthis* (Erst et al., 2020).

The present study aimed to evaluate the levels of phenolic compounds identified by high-performance liquid chromatography (HPLC) in leaves of *E. sibirica*, *E. stellata*, and *E. tanhoensis*.

Materials and methods

Leaves of *E. sibirica*, *E. stellata*, and *E. tanhoensis* from natural populations were analyzed for phenolic compounds (Table 1). The material was collected in Irkutsk Oblast, Republic of Buryatia and Primorsky Krai during fruiting in May 2019.

The phenolic compounds were studied in 70% aqueous ethanol extracts of leaves; these extracts were prepared by water bath extraction. A certain portion (0.200 g) of the crushed air-dried material was extracted twice: first, with 30 ml for 30 min, then with 20 ml for 20 min. After filtration, the residue in the flask and on the filter was washed with 5 ml of 70% ethyl alcohol. The mixed extract was then concentrated in porcelain cups to 10–15 ml (exact volume). The analysis was performed in duplicate (two biological replicates) (Vysochina, 2004).

An aqueous ethanol extract in the amount of 1 ml was diluted with double-distilled water to 5 ml and passed through a Diapak C16 concentrating cartridge (ZAO BioKhimMak). Substances were washed off the cartridge with a small amount (3 ml) of 70% ethanol and then with 2 ml of 96% ethanol. The mixed eluate was passed through a membrane filter with a pore diameter of 0.45 μm.

Phenolic compounds in the eluate were investigated on an analytical HPLC system that consisted of an Agilent 1200 liquid chromatograph (USA) (column: Zorbax SB-C18, 4.6 × 150 mm, 5 μm) equipped with a diode array detector, an autosampler, and a ChemStation system for collecting and processing chromatographic data by the method of T.A. van Beek (2002), with modifications. The separation was carried out under the following conditions: a gradient from 31% to 33% methanol acidified with orthophosphoric acid (0.1%) for 27 min, then the methanol level in the mobile phase in the aqueous solution of orthophosphoric acid (0.1%) was raised from 33% to 46% within 11 min; next, it was changed from 46% to 56% over the next 12 min and from 56% to 100% within 4 min. The flow rate of the eluent was 1 ml/min, column temperature 26 °C, and injected-sample volume was 10 μl. Detection was performed at λ = 255, 270, 290, 340, 360, and 370 nm.

Quantitative determination of individual phenolic compounds in the plant samples was conducted by the external-standard method at λ = 255 nm. To prepare the standard samples, we used salicylic and chlorogenic acids, quercetin, kaempferol, orientin (Sigma-Aldrich), gentisic and caffeic acids (Serva), and vitexin (Fluka). The concentration of the standard solutions was 10 μg/ml. Comparative analysis of the levels of individual-group of phenolic compounds in the plant leaf extracts was performed by the integrated intensity of the chromatographic signal of the phenolic compound at λ = 360 nm. Quantitative processing of the data was performed in Microsoft Excel.

Table 1. Sites of collection of the studied *Eranthis* specimens

Sample number*	Locality, date, and collector
	E. sibirica
S1	Russia, Irkutsk Oblast, Slyudyansky Distr., Burovshina Riv.; 51°37'06.00''N, 103°49'16.17''E; alt. 470 m; 02.05.2019. A.S. Erst, D.A. Krivenko, O.A. Chernysheva
S2	Russia, Irkutsk Oblast, Slyudyansky Distr., vicinity of Slyudyanka town; 51°38'02.94''N, 103°41'13.90''E; alt. 531 m; 02.05.2019.A.S. Erst, D.A. Krivenko, O.A. Chernysheva
	E. stellata
St3	Russia, Primorsky Krai, Vladivostok City, Malaya Sedanka Riv.; 43°12'37.9"N131°59'39.1"E; alt. 59 m. 16.05.2018. V.Yu. Nikulin, A.Yu. Nikulin
St4	Russia, Primorsky Krai, Vladivostok city, 13 km St.; 43°11'32.3"N,131°55'49.0"E; alt. 103 m; 14.05.2019. V.Yu. Nikulin, A.Yu. Nikulin
St5	Russia, Primorsky Krai, Vladivostok City, Ostrov Russkij; 42°59'05.0"N, 131°51'51.5"E; alt. 74 m; 12.05.2019. V.Yu. Nikulin, A.Yu. Nikulin
	E. tanhoensis
T6	Russia, Irkutsk Oblast, Slyudyansky Distr., Malye Mangaly (Kuytun) Riv.; 51°26'48.17''N, 104°34'16.62''E; alt. 471 m; 02.05.2019.A.S. Erst, D.A. Krivenko, O.A. Chernysheva
T7	Russia, Buryatia Republic, Kabansky Distr., Tolbazikha Riv. 51°26'21.06''N, 104°41'09.82''E; alt. 471 m; 01.05.2019. A.S. Erst, D.A. Krivenko, O.A. Chernysheva

Note. Leaves are taken from 5 – 6 plants.

Results and discussion

In 70% aqueous ethanol extracts from leaves of the three studied plant species of the genus *Eranthis*, 24 phenolic compounds were found previously (Erst et al., 2020) 24 phenolic compounds were identified here. The identified compounds included chlorogenic, gentisic, caffeic, and salicylic acids, quercetin, kaempferol and hyperoside flavonols, orientin, and vitexin flavones. Hyperoside was found only in the aqueous ethanol leaf extracts of the plants in the flowering phase. The studied species turned out to be very similar in the set of phenolic compounds in leaves; however, they contain phenolic compounds specific for each

taxon in question. It was revealed that *E. sibirica* and *E. tanhoensis* are closer in terms of secondary metabolism. *Eranthis stellata* is well distinguished from the other two species. In contrast to *E. tanhoensis*, the chromatographic profile of *E. sibirica* contains caffeic acid, orientin, vitexin, and flavone 6 in the aqueous ethanol leaf extracts. Leaves of *E. tanhoensis* from almost all the studied populations were found to contain quercetin, which was not detectable in *E. sibirica*. The compounds identified in *E. stellata* leaf extracts specifically were gentisic acid, phenolic acid 4, and flavone 21 (i.e., they were not found in the other two species). Vitexin, hyperoside, and salicylic acid were not observed previously in leaves of *E. stellata* (Erst et al., 2020). Samples from different populations of the same *Eranthis* species could be distinguished by the presence of minor phenolic compounds in leaf extracts (Table 2). For example, salicylic acid was detectable in leaves of the *E. sibirica* growing in the Slyudyanka town vicinity (S2). *Eranthis sibirica* from another population growing in Burovshina River valley (S1) does not contain salicylic acid. Flavone 20 was identified only in aqueous ethanol leaf extracts of *E. sibirica* sample S1. Phenolic acid 14 was found in leaves of *E. stellata* growing in the vicinity of Vladivostok city and in Malaya Sedanka River valley, whereas in St5 plants, this phenolic acid was not observed. Besides, *E. stellata* specimens from Malaya Sedanka River valley (St3) are distinguished by the presence of flavonol 18 in leaves (Table 2). Leaves of *E. tanhoensis* specimens from Tolbazikha River valley (T7) contain phenolic acid 14 and kaempferol. *E. tanhoensis* specimens collected in Malye Mangaly River valley (T6) contain quercetin in their leaves (Table 2). The observed species specificity of the chromatographic profiles of *E. sibirica*, *E. stellata*, and *E. tanhoensis* indicates their species independence, as proved by morphological and molecular data (Wahlsteen, 2016; Park et al., 2019; Erst et al., 2020). At the same time, the similarity of biochemical patterns and different geographic ranges of *E. stellata* and *E. sibirica* favour the opinion of some scientists that these species have developed in parallel from a common ancestor. Several taxa closely related to these species grow in China and Japan (Popov, 1959, Kiseleva, 1978).

Table 2. Description of the phenolic compounds found in leaf extracts of *Eranthis* species

Peak No.	Phenolic compound	Retention time (t_R), min	Spectral characteristic λmax, nm	Sample number		
				E. sibirica	*E. stellata*	*E. tanhoensis*
1	Chlorogenic acid	3.2	244, 300 sh., 325	S1, S2	St3, St4, St5	T6, T7
2	Gentisic acid	4.1	240, 330	–	St3, St4, St5	–
3	Caffeic acid	5.2	240, 290 sh., 320	S1, S2	St3, St4, St5	–
4	Phenolic acid 4*	7.1	250, 300	–	St3, St4, St5	–
5	Orientin	8.9	250, 350	S1, S2	St3, St4, St5	–
6	Flavone 6*	9.4	270, 310	S1, S2	–	–
7	Phenolic acid 7	10.0	250, 290 sh., 335	S1, S2	St3, St4, St5	T6, T7
8	Vitexin	12.1	270, 340	S1, S2	–	traces
9	Flavonol 9*	15.1	255, 270 sh., 300	S1, S2	St3, St4, St5	T6, T7
10	Hyperoside	18.4	255, 268 sh., 355	during flowering	–	during flowering
11	Salicylic acid	19.8	240, 305	S2	–	T6, T7
12	Phenolic acid 12*	20.9	240, 290 sh., 335	S1, S2	St3, St4, St5	T6, T7
13	Flavonol 13*	22.3	255, 360	–	St3, St4, St5	T6, T7
14	Phenolic acid 14*	25.4	255, 300	S1, S2	St3, St4	T7
15	Flavonol 15*	32.7	270, 310, 365	S1, S2	St3, St4, St5	T6, T7
16	Phenolic acid 16*	34.5	250, 290, 330	S1, S2	St3, St4, St5	T6, T7
17	Flavone 17*	36.9	250, 325	S1, S2	St3, St4, St5	T6, T7
18	Flavonol 18*	38.7	255, 305, 360	S1, S2	St3	T6, T7
19	Quercetin	40.1	255, 372	–	St3, St4, St5	T6
20	Flavone 20*	41.6	265, 320	S1	–	–
21	Flavone 21*	42.2	270, 310	–	St3, St4, St5	–
22	Flavanone 22*	43.4	275, 315	S1, S2	–	T6, T7
23	Phenolic acid 23*	44.3	255, 300, 330	S1, S2	St3, St4, St5	T6, T7
24	Kaempferol	47.3	266, 370	–	St3, St4, St5	T7

Note. * – group of compounds identified by spectral characteristics (Zaprometov, 1993; Klyshev et al., 1978; Zaprometov, 1993); "–" compound was not detected; sh. – shoulder (traces of a signal)

Among the studied *Eranthis* species, a quantitative comparison of the phenolic compounds we identified in the aqueous ethanol leaf extracts showed that the levels of chlorogenic (0.34–0.96 mg/g), caffeic (0.29–0.32 mg/g), and salicylic acids (0.25 mg/g) are

higher in leaves of *E. sibirica*, and the level of kaempferol (0.42 mg/g) is higher in leaves of *E. tanhoensis* (Table 3). In aqueous ethanol leaf extracts of *E. stellata*, concentrations of orientin (1.19–4.99 mg/g) and quercetin (0.12–0.20 mg/g) are higher than those in the other two species; vitexin (1.84–3.63 mg/g) was found in leaves of *E. sibirica* only (Table 3).

Table 3. Concentrations of phenolic compound in leaves of three *Eranthis* species (mg/g of air-dried material)

Compound	Sample number						
	E. sibirica		*E. stellata*			*E. tanhoensis*	
	S1	S2	St3	St4	St5	T6	T7
Chlorogenic acid	0.96±0.04	0.34±0.01	0.19±0.01	0.37±0.01	0.11±0.00	0.34±0.01	0.71±0.03
Gentisic acid	–	–	0.19±0.01	0.24±0.01	0.02±0.00	–	–
Caffeic acid	0.32±0.01	0.29±0.01	0.09±0.00	0.32±0.01	0.20±0.01	–	–
Orientin	0.69±0.03	2.94±0.11	2.50±0.09	4.99±0.18	1.19±0.04	–	–
Vitexin	1.84±0.07	3.63±0.13	–	–	–	–	–
Salicylic acid	–	0.25±0.01	–	–	–	0.18±0.01	0.19±0.01
Quercetin	–	–	0.20±0.01	0.12±0.00	0.17±0.01	0.07±0.00	–
Kaempferol	–	–	0.31±0.01	0.15±0.01	0.20±0.01	–	0.42±0.02

Note. The data presented like means and standard errors (n = 3).

The main phenolic compounds in the plant extracts under study are phenolic acid 7, phenolic acid 12, and flavonol 9. Analysis of concentration ratios of the main phenolic compounds uncovered significant differences among studied *Eranthis* species (Fig. 1). In leaves of *E. sibirica*, the level of phenolic acid 12 in sample S1 and phenolic acid 7 in sample S2 exceeds that of flavonol 9. Flavonol 9 is dominant in leaf extracts of *E. stellata* and *E. tanhoensis* (Fig. 1). The flavonol 9–to–phenolic acid 12 ratio in *E. sibirica* sample S1 is 0.69; in sample S2, it amounts to 0.72; and among *E. stellata* samples, it varies from 1.49 to 1.85. In *E. tanhoensis*, the ratio of the main phenolic compounds is much higher than that in the other two species: 5.48 in sample T6 and 5.66 in sample T7. This ratio may be a species marker for the studied *Eranthis* species. Nonetheless, the ratio should be verified on a larger number of samples.

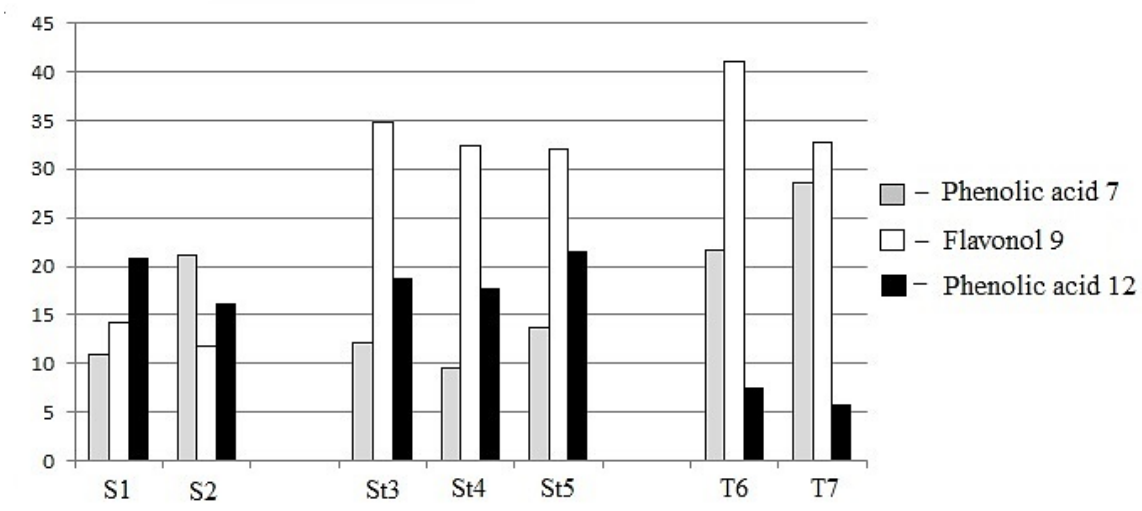

Fig. 1. A comparison of concentrations of the main phenolic compounds in aqueous ethanol extracts of *Eranthis sibirica* (samples S1 and S2), *E. stellata* (St3, St4, and St5), and *E. tanhoensis* (T6 and T7). The X-axis is the sample number; the Y-axis indicates the integrated intensity of a chromatographic signal of a phenolic compound, %.

The three analyzed *Eranthis* species differ in the concentration ratio of total phenolcarboxylic acids to total flavonols in leaves (Fig. 2). In leaves of *E. sibirica*, the total concentration of phenolcarboxylic acids exceeds almost 2-fold that of flavonols (Fig. 2). On the contrary, the total level of flavonols in leaves of *E. stellata* and *E. tanhoensis* is higher or almost equal to that of phenolic acids (Fig. 2). It is important to note that the total level of flavones in these plant leaves is less than that of phenolcarboxylic acids and flavonols. The only exception is *E. sibirica* sample S2 collected in the vicinity of Slyudyanka town because its leaves contain more flavones than flavonols (Fig. 2).

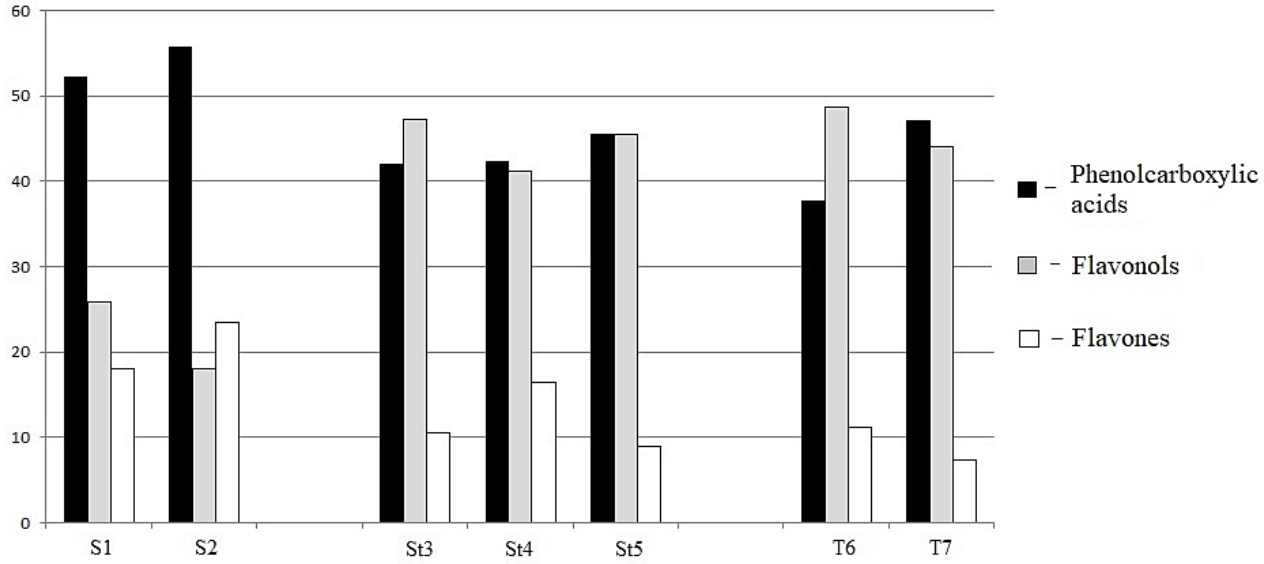

Fig. 2. A comparison of levels of phenolic-compound classes in leaves of *Eranthis sibirica* (samples S1 and S2), *E. stellata* (St3, St4, and St5), and *E. tanhoensis* (T6 and T7). The X-axis is the sample number; the Y-axis indicates the integrated intensity of a chromatographic signal of a phenolic compound, %.

These differences in the levels of phenolic compounds may be due to dissimilarity of growth conditions of the plants in question. Phenolic compounds play a crucial role in plant adaptation to unfavourable environmental conditions and increase ecological plasticity of plants. These compounds also protect against UV radiation and other stress factors (Zaprometov, 1993, Vysochina, 2004, Karakulov et al., 2018). *Eranthis sibirica* grows in the continental climate of the mountainous terrain in Irkutsk Oblast. Specimens of *E. sibirica* were collected at an altitude of 470–531 m a.s.l. Probably, these conditions promote the accumulation of phenolcarboxylic acids in leaves. E. stellata grows under milder conditions of the moderate monsoon climate in Primorsky Krai, and the plant specimens were collected at a relatively low altitude of 59–103 m a.s.l. The increased level of insolation in Primorsky Krai may facilitate the accumulation of flavonols in *E. stellata* leaves. Nevertheless, the concentration ratio of total phenol carboxylic acids to total flavonols in the leaves of *E. tanhoensis*, which like *E. sibirica* grows in the continental climate of the mountainous terrain in Irkutsk Oblast, indicates species specificity of the levels of phenolic compounds, as demonstrated in other plant species (Khramova & Andysheva, 2014, Raal et al., 2015). In leaves of *E. tanhoensis*, the level of flavonols is higher or almost equal to that of phenolcarboxylic acids, thus showing its similarity to *E. stellata* growing in Primorsky Krai in terms of the levels of these substances.

Conclusion

Phenolic compounds identified in aqueous ethanol leaf extracts by HPLC are quantitated for the first time in plants of the genus *Eranthis*, namely, white-flowered species of the section *Shibateranthis*: *E. sibirica, E. stellata,* and *E. tanhoensis*. Among the three species, concentrations of chlorogenic (0.34–0.96 mg/g), caffeic (0.29–0.32 mg/g), and salicylic acids (0.25 mg/g) were found to be higher in *E. sibirica* leaves, and the highest concentration of kaempferol (0.42 mg/g) was observed in leaves of *E. tanhoensis*. *Eranthis stellata* leaves contain more orientin (1.19–4.99 mg/g) and quercetin (0.12–0.20 mg/g) as compared to the other two species. Vitexin (1.84–3.63 mg/g) was detectable in leaves of *E. sibirica* only. A species-specific ratio of levels of the main phenolic compounds in leaves was identified in the three studied species of the genus *Eranthis*. The total concentration of phenolcarboxylic acids in *E. sibirica* leaves exceeds almost 2-fold that of flavonols. On the contrary, the total concentration of flavonols in *E. stellata* and *E. tanhoensis* leaves is higher or almost equal to that of phenolcarboxylic acids. In terms of the chromatographic profile, *E. tanhoensis* is closer to *E. sibirica*, and in terms of phenolic compounds levels, to *E. stellata*.

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