

Phenolic compounds in taxonomy of *Myricaria longifolia* and *Myricaria bracteata* (Tamaricaceae)

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Abstract. For the first time, the phenolic profile of poorly studied Siberian species of the genus *Myricaria* Desv. *Myricaria longifolia* (Willd.) Ehrenb. was investigated by HPLC and LC-MS/MS in comparison with that of wide-ranging species *M. bracteata* Royle. 65 quantitative parameters of the phenolic profiles were processed by ANOVA and principal component analysis (PCA). The results suggest that the distinction between the species is mainly determined by the variance in total flavonoids and free quercetin in the leaves. Free gallic and ferulic acids, hyperoside and total phenolics in aqueous ethanol extract, as well as kaempferol and rhamnazin in the hydrolyzed extract contributed to the difference between the species. The significant differences justify the positions of these species in two individual series of the genus *Myricaria* established before. The statistical analysis of the biochemical data allowed us to identify both the characters that determined the distinction between species, and indicators of heterogeneity of the species that varies abnormally (the concentrations of isorhamnetin and rhamnazin, and their ratio).

1 Introduction

Myricaria Desv. (Tamaricaceae) is a genus of shrubs growing on mountain river banks in Asia and Eastern Europe. Himalayan region was considered to be the origin of the distribution of this genus [1]. By now, 11 to 13 *Myricaria* species were identified, and their taxonomy is very complicated [2]. E.G. Bobrov (1967) attributed the genus *Myricaria* to the ancient genera of the desert flora of the Eastern Hemisphere whose origin and development were associated with Central Asia. He regard *M. bracteata* Royle as a species the closest to the original form. It is widely distributed in extensive territories in the West Himalayas, Central and Northern Asia. Meanwhile, *M. longifolia* (Willd.) Ehrenb. occurs mainly in the mountains of southern Siberia and represents a line of further development of the genus [3]. The genus *Myricaria* has a long history of taxonomic study. So far, there is no consensus on taxonomy of *Myricaria* species in botanical literature. For instance, various researchers counted 2 to 5 *Myricaria* species in the Siberian flora [3, 4]. According

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to recent data in our investigation, we consider two species: *M. bracteata* and *M. longifolia*. In their identification, shape and size of leaves were accepted as the main diagnostic characters. *M. bracteata* was shown to belong to small-leaved species assigned to series *Germanicae* Gorschk., whereas *M. longifolia* is large-leaved species, which was placed in another series *Dahuricae* Gorschk. or series *Elegantes* Bobr. [3]. Another diagnostic character, flower racemes arrangement, was revealed to vary during plant development, and its diagnostic value is questionable [4].

The difficulties of species delimitation associated with high polymorphism and a substantial spatial and temporal variation of many morphological characters induce us to increase the reliability of taxonomic analysis due to biochemical features. In this regard, secondary metabolite profiling creates additional opportunities for the identification of the phylogenetic relations between plant taxa. Along with morphological parameters, phenolic compounds are indicators of the biological diversity of plants. These compounds were regularly used as taxonomic markers and facilitated the solution of taxonomic problems at different levels [5]. Their considerable structural diversity is the basis of taxon-specificity of phenolic profiles. Phenolic compounds of *Myricaria* species are poorly studied. The investigation of phenolic aglycon composition in *M. bracteata* leaves by LC-MS/MS have revealed the diversity of methylated flavonols and the presence of flavones in the leaves of this species [6]. The aim of this study is to determine phenolic profile of *M. longifolia* in comparison to the profile of *M. bracteata*, and to identify their species-specificity.

2 Material and methods

The samples of *M. bracteata* and *M. longifolia* leaves from nature were collected during the fruiting periods of 2011–2020 on pebble floodplains of mountain rivers in areas with moderate and high aridity (*Myricaria bracteata*: Maliy Yaloman village, Ongudaysky Region, Altai Republic, Russian Federation, Kuygantukoy village, Shugnon Region, Republic of Tajikistan, The Morj village, Shugnon Region, the Republic of Tajikistan; *Myricaria longifolia*: Aktal village, Kosh-Agachsky Region, Altai Republic, Russian Federation, Khandagaty village, Republic of Tyva, Erzin village, Republic of Tyva). The leaves of field-grown plants were gathered from “Collections of living plants indoors and outdoors” USU 440534 of Central Siberian Botanical Garden of Siberian Branch of Russian Academy of Sciences (CSBG SB RAS) from plants obtained by rooting of green cuttings from the plants of the Altai populations. The CSBG SB RAS garden plot is located in a forest-steppe region with gray forest soil. The plants were grown on rock drainage.

For LC-MS/MS and HPLC analyses, precisely weighed samples of air-dried plant material (0.3 g) were exhaustively extracted with an ethanol:water mixture (70:30, v/v) in a water bath at 60–70°C. Dry-weight concentration in the samples was calculated by the gravimetric method. A hydrolyzed extract was obtained by hydrolysis of the aqueous ethanol extract with 2N HCl for 2 h in a boiling water bath, followed by purification by means of a C16 Diapack cartridge and redissolution in ethanol. It was used for identification and quantification of the aglycones. The identification was performed by liquid chromatography with tandem mass spectrometry. MS analysis was carried out at the Core Facility of Mass Spectrometric Analysis (ICBFM SB RAS) as described before [6]. The quantification of phenolic compounds was conducted by the external-standard method. Chromatographic analysis for absolute quantification of phenolics was carried out with an Agilent 1200 chromatograph with a diode matrix detector and the system for processing the chromatographic data ChemStation (Agilent Technologies, USA). The separation was performed with a Zorbax SB-C18 column 4.6 × 150 mm in size, with particle diameter 5 mm. The mobile phase consisted of MeOH (solvent A) and 0.1% orthophosphoric acid in water (solvent B). Chromatographic separations of aglycones in hydrolyzed extracts with

gradient 1 and native compounds (phenolic acids, flavonoid glycosides and free aglycones) with gradient 2 was described previously [6, 7]. To construct calibration curves, chemical reference standards of gallic and ferulic acids from Serva (Heidelberg, Germany), ellagic acid, ascorbic acid, quercetin, kaempferol, rhamnetin, isorhamnetin, luteolin, apigenin, naringenin, astragalol, isoquercitrin, and isorhamnetin 3-*O*-rutinoside from Sigma (St. Louis, MO, USA), and hyperoside from Fluka (Sigma-Aldrich Chemie GmbH, Munich, Germany) were used. The sum of organic acids was calculated as ascorbic acid equivalents at 250 nm, and flavonol glycosides 18, 23, 25–27 were calculated as hyperoside equivalents at 350 nm. All the data were processed in the Statistica 10.0 software (Statsoft Inc., Tulsa, OK, USA). The significance of difference between datasets was determined by one-way analysis of variance (ANOVA). Data from the quantification of phenolic compounds were expressed as mean \pm SE of 9 (3 biological \times 3 technical) replicates and were compared by Duncan's multiple range test. Differences between the means of any parameters were considered statistically significant at the 5% level ($p < 0.05$). To evaluate the variations among the *Myricaria* species and to identify major compounds contributed to delimiting species, principal components analysis (PCA) was performed. PCA was applied to 64 biochemical parameters, including the concentrations, ratios, and number of the compounds.

3 Results

Aglycon compositions of *M. bracteata* and *M. longifolia* determined by LC-MS/MS were rather similar. In the hydrolyzed extracts of the leaves, citric acid, gallic acid, methyl gallate, ethyl gallate, ferulic acid, ellagic acid, quercetin, quercetin 3-glucoside, kaempferol, luteolin, chrysoeriol, rhamnocitrin, isorhamnetin, rhamnazin, kaempferide, tartaric acid, quercetin pentoside, isoferulic acid, kaempferol hexoside, naringenin, apigenin, and rhamnetin were detected.

Quantification of these compounds in hydrolyzed extracts of the leaves by HPLC showed the substantial variation in levels of most of the compounds. Organic acids and ellagic acid were permanent major organic constituents of both species, and gallic acid, isorhamnetin, and rhamnazin levels varied between populations from high to negligible. Apigenin and rhamnetin occurs only in traces in both species.

M. longifolia exceeded *M. bracteata* in the concentrations of ferulic acid and quercetin, but the concentration of kaempferol in the leaves of *M. longifolia* was lower (Table 1). The differences in the average concentrations of isorhamnetin and rhamnazin were not statistically significant due to substantial variation in their concentrations.

Table 1. Concentrations of major phenolic compounds ($\text{mg}\cdot\text{g}^{-1}$ dry weight) in the hydrolyzed extracts of the leaves of *M. bracteata* and *M. longifolia*

Compound	T_R (min)	<i>Myricaria bracteata</i>		<i>Myricaria longifolia</i>	
		Range	Average	Range	Average
Organic acids	1.4	2.45-17.21	6.69 ± 2.70^a	2.61-25.07	10.87 ± 5.20^a
Gallic acid	1.7	0.96-6.20	2.40 ± 0.96^a	1.37-11.94	4.24 ± 2.57^a
Ferulic and isoferulic acids	2.9	0.11-0.24	0.17 ± 0.02^a	0.72-1.38	1.04 ± 0.15^b
Ellagic acid	3.8	2.42-8.67	5.56 ± 1.06^a	0.67-11.81	6.28 ± 2.32^a
Quercetin	6.4	0.12-1.64	0.74 ± 0.27^a	1.44-2.70	1.78 ± 0.33^b
Kaempferol	11.2	0.10-0.42	0.28 ± 0.06^b	0.04-0.08	0.03 ± 0.01^a
Isorhamnetin	12.4	0.60-11.73	5.36 ± 2.08^a	0.14-0.55	1.07 ± 0.64^a

Rhamnazin	18.7	0.00-1.10	0.26 ± 0.21 ^a	0.35-1.50	1.27 ± 0.47 ^a
Rhamnazin/ Isorhamnetin ratio		0.00-0.49	0.10 ± 0.10	0.92-17.00	5.72 ± 3.78

Here and in Table 2, means in rows followed by the same letter do not differ significantly according to Duncan's test ($p < 0.05$).

Among intact compounds in the aqueous ethanol extracts of the leaves, organic acids, free gallic and ferulic acids, free ellagic acid, flavonol glycosides (including hyperoside, isoquercitrin, astragalol, and isorhamnetin 3-*O*-rutinoside), and free aglycones (including quercetin, naringenin, luteolin and apigenin) were detected (Table 2).

Table 2. Concentrations of major intact phenolic compounds (mg·g⁻¹ dry weight) in the leaves of *M. bracteata* and *M. longifolia*

Compound	T _R (min)	λ max, nm	<i>Myricaria bracteata</i>	<i>Myricaria longifolia</i>
Organic acids	1.3	250	7.25 ± 1.71 ^a	7.30 ± 2.44 ^a
Gallic acid	1.8	272	7.04 ± 2.33 ^a	22.70 ± 7.39 ^b
Ferulic acid	9.2	325	1.98 ± 0.26 ^b	0.15 ± 0.05 ^a
Hyperoside	18.3	255, 355	1.79 ± 0.85 ^a	6.06 ± 1.35 ^b
Ellagic acid	22.0	250	0.72 ± 0.51 ^a	0.83 ± 0.22 ^a
Astragalol	32.4	265, 346	0.10 ± 0.02 ^a	1.50 ± 0.44 ^b
Isorhamnetin 3- <i>O</i> -rutinoside	35.7	255, 355	0.08 ± 0.05 ^a	0.28 ± 0.08 ^b
Quercetin	40.2	260, 370	0.08 ± 0.02 ^a	1.20 ± 0.29 ^b
Total compounds			33.86 ± 5.97 ^a	57.54 ± 7.13 ^b
Total flavonoids			4.21 ± 0.77 ^a	13.44 ± 1.23 ^b
Average number of compounds			20.60 ± 1.03 ^a	24.75 ± 3.61 ^a

ND = not detected.

Organic acids, free gallic acid and hyperoside were major components in both species. *M. longifolia* surpassed *M. bracteata* in the concentrations of most of components, as well as in total phenolic compounds and total flavonoids. On the other hand, *M. bracteata* had an increased level of ferulic acid. The results of PCA confirmed this finding (Table 3).

Table 3. The results of PCA for 12 from 64 biochemical parameters of *Myricaria longifolia* and *Myricaria bracteata*

Parameters	Factor 1	Factor 2	Factor 3	Factor 4
Free gallic acid	-0.86	-0.07	0.26	0.29
Free ferulic acid	0.88	0.35	-0.02	0.23
Hyperoside	-0.85	-0.07	-0.16	-0.11
Free quercetin	-0.94	0.14	0.07	0.13
Total phenolics	-0.86	0.01	0.45	0.04
Total flavonoids	-0.97	0.01	-0.07	0.03
Number of flavonoids	-0.71	0.53	0.09	0.30
Ferulic acid	-0.74	-0.37	-0.36	-0.22
Kaempferol	0.74	0.14	0.34	-0.29
Rhamnazin	-0.80	0.15	-0.04	0.04

Isorhamnetin	0.71	0.38	-0.26	0.35
	PC1	PC2	PC3	PC4
Eigenvalues	18.89	13.54	8.19	7.03
Cumulative %	28.63	49.15	61.56	72.21

According to PCA, the main parameters that determine the difference between the phenolic profiles of the species were total flavonoids in aqueous ethanol extract and free quercetin, strongly correlated with PC1. Free gallic and ferulic acids, hyperoside, total phenolics in aqueous ethanol extract, as well as kaempferol and rhamnazin in the hydrolyzed extract showed a high correlation with PC1 too.

Thus, the statistical analysis of the biochemical data allowed us to identify both the characters that determine the distinction between species, and abnormally varying characters indicating the variability within the species. Proportions of phenolic compounds are common in plant taxonomy [8]. Previously, we dealt with a high ratio of kaempferol to quercetin as taxonomic character of the series *Elegantes* Pojark. in the genus *Spiraea* (Rosaceae) [9]. In this case, the ratio of rhamnazin to isorhamnetin seems to reflect a differentiation within the studied species. Considerable variation in this ratio between two significantly distant of each other populations of *M. bracteata* we have discovered before [6].

4 Conclusions

For the first time, the phenolic profile of poorly studied Siberian species *Myricaria longifolia* was investigated by HPLC and LC/MS/MS in comparison with that of wide-ranging species *M. bracteata*. The results suggest that the distinction between the species is mainly determined by the variance of total flavonoids and free quercetin in the leaves. The significant differences justify the positions of these species in two individual series of the genus *Myricaria* in the classifications of S.G. Gorshkova (1949) and E.G. Bobrov (1967). High variation in the concentrations of major flavonoid aglycones between the populations within the species confirms a possible subdivision of *M. longifolia* and *M. bracteata* into species or subspecies. Our study fills the gap in knowledge about differentiation and relationships in the genus *Myricaria*.

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References

1. Y. Chen, JEE, **3** (2017)
2. Y. Wang, Y. Liu, S. Liu, H. Huang, Bot. Stud. **50** (2009)
3. E. G. Bobrov, Botanical journal **52** (1967)
4. E. M. Lyakh, Eurasian Journal of Forest Research **16** (2013)
5. B. Lemma et al., Plants (Basel) **8** (2019)
6. A. A. Chernonosov, E. A. Karpova, E. M. Lyakh, Rev. Bras. Farmacogn. **27** (2017)
7. E. A. Karpova, E. P. Khranova, Chemistry for sustainable development **27** (2019)
8. V. Míka et al., Plant, Soil and Environment **51** (2005)

9. E. A. Karpova, N. P. Lapteva, *Turczaninowia* **17**(1) (2014)