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Leaf surface secretion of flavonoids in *Myricaria bracteata* and *M. longifolia* (Tamaricaceae)

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ABSTRACT: Flavonoids of the salt glands and tissues of *Myricaria bracteata* and *Myricaria longifolia* were studied by histochemical assays and chromatographic analysis. Histochemical staining indicated flavonoid accumulation in the salt glands, within vascular bundles, and in subepidermal parenchyma layers of the leaves. Exudate flavonoids of the leaves were characterized for the first time. Phenolic profiles of these species proved to be similar and showed high levels of ellagic acid and total flavonoids, including hyperoside, isoquercitrin, astragalin, avicularin, isorhamnetin 3-O-rutinoside, quercetin, naringenin, and luteolin. Astragalin and hyperoside are major phenolic compounds in the leaves. The latter were found to constitute ~0.2−0.3% of total phenolics of the leaves; meanwhile, exudate flavonoids make up ~0.5% of total flavonoids. The high similarity of phenolic composition between the whole-leaf extracts and the exudates indicates the homogeneous origin of these compounds. The observed tissue distribution of flavonoids and their presence in the salt glands confirm the role of these glands in leaf surface secretion of flavonoids.

KEY WORDS: *Myricaria*, Tamaricaceae, leaf exudate, salt glands, flavonoids.

INTRODUCTION

The genus *Myricaria* Desv. (Tamaricaceae) is widely distributed in Eastern Europe and Asia. *Myricaria bracteata* Royle is a component of folk remedies (Zhao *et al.,* 2005; Wang *et al.,* 2008) and contains compounds with a pronounced anti-inflammatory effect (Liu *et al.,* 2015). *Myricaria longifolia* (Willd.) Ehrenb. (*M. dahurica* (Willd.) Ehrenb., *Tamarix longifolia* Willd.) (Lyakh, 2006) is an ingredient of traditional Mongolian and Tibetan herbal mixtures for treating fever and poisoning. Choleretic and antitumor activities of branches of this species have been demonstrated in previous experimental studies (Obmann *et al.,* 2010).

It is now well established that biological activities are closely associated with phenolic constituents in general and flavonoids in particular (Cowan, 1999). These compounds of the genus *Myricaria* are considerably diverse in their structure and are subdivided into several classes. Compounds like gallic and ellagic acids and their derivatives, flavonols (quercetin and kaempferol), their methyl ethers (isorhamnetin, rhamnazin, kaempferide, and tamarixetin), glycosides as well as a flavone (chrysoeriol) have been identified in the aerial parts of *M. bracteata* and *M. longifolia* (Zhou *et al.,* 2006; Li *et al.,* 2010; Zhang *et al.,* 2011; Chernonosov *et al.,* 2017). The above works deal only with the identification of a few individual compounds, and phenolic profiles of representatives of the genus have not yet been determined.

A review of the literature reveals that there is a lack of knowledge about the tissue distribution of phenolic compounds in the leaves of the genus *Myricaria*, including the leaf surface. Morphology, ultrastructure, and histochemistry of surface structures of a plant leaf (glandular and non-glandular trichomes) as well as accumulation of phenolics in some other tissues have been investigated in many taxa (Matias *et al.,* 2016; Muravnik *et al.,* 2016; Soukupová *et al.,* 2000; Tattini *et al.,* 2000; Valkama *et al.,* 2004). On the surface of *Myricaria* leaves, trichomes are absent, but salt glands secreting sulfates and chlorides are often present (Calquist, 2010). The structure of the leaf salt glands was characterized for one species of the genus *Myricaria* (*M. germanica*) (Dörken *et al.,* 2017). Kuster *et al.,* (2020) have examined the leaf salt glands in *Jacquinia armillaris* (Primulaceae) and revealed flavonoids by histochemical test with Wilson's reagent. The current study is concerned with similar glands in *M. longifolia* and *M. bracteata* leaves in terms of the localization of phenolic compounds, in particular flavonoids.

Another way to investigate leaf surface secretion is the profiling of exudate compounds. Such profiles have been studied in detail in representatives of a few families including Asteraceae, Betulaceae, Boraginaceae, Empetraceae, Lamiaceae, Salicaceae, Oleaceae, Scrophulariaceae, Ranunculaceae, Rubiaceae, and Solanaceae (Greenaway, 1992; Tattini *et al.,* 2000; Nikolova *et al.,* 2003; Valant-Vetschera *et al.,* 2003; Valkama *et al.,* 2003; Wollenweber *et al.,* 2005b; Muravnik *et al.,* 2016). Nevertheless, reports on the quantification of exudate compounds are still few relative to the number of the plant species (Muravnik and Shavarda,

Table 1. The origin of the field populations of ***M. bracteata*** and ***M. longifolia***.

Species	Population origin	Voucher specimen
Myricaria bracteata	Bolshoi Yaloman River floodplain; The Maliy Yaloman village (50º52'08"N, 86º56'10"E), Ongudaysky Region, the Altai Republic, Russian Federation.	NSK 3001194
Myricaria longifolia	Yustyt River floodplain; The Aktal village (49º91'56"N, 88º91'06"E), Kosh-Agachsky Region, the Altai Republic, Russian Federation.	NSK 3001195

2012; Tattini *et al.,* 2000; Valkama *et al.,* 2004). There is lack of information about the exudate profiles of species of the genus *Myricaria*.

Therefore, the aim of this study was to reveal flavonoids in salt glands and in the tissues of two *Myricaria* species, *M. bracteata* and *M. longifolia*, and to determine their phenolic and flavonoid profiles (including leaf exudate profiles).

MATERIALS AND METHODS

Plant Materials

The leaves of *M. bracteata* and *M. longifolia* were collected from the field from Unique Scientific Unit (USU) No. 440534 of CSBG SB RAS ("Collections of living plants indoors and outdoors"; Table 1). Each sample represented the leaves from 10 twigs from each of 10 individuals. The twigs were collected at a height of ~0.7 m, in the middle part of the branches.

For microscopic examination, the twigs during flowering and fruiting were collected to determine the dynamics of flavonoid accumulation. The leaves and stems (five leaves with stems from each of 10 individuals at each stage) were analyzed by light and fluorescence microscopy immediately after sampling. For SEM studies, freshly collected leaves were dried within 4–5 hours between layers of filter paper and cut into small pieces (10 × 10 mm^2). For LC-MS/MS and HPLC analyses, fresh leaves from the garden plot were pulped up in a household mill immediately after sampling. Precisely weighed samples of fresh plant material (0.5 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. The extracts were filtered, diluted with the ethanol:water mixture (70:30, v/v) up to the volume of 25 mL in a graduated flask, and used for quantification of glycosides and free aglycones.

Hydrolyzed extracts were obtained by hydrolysis of the aqueous ethanol extracts 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. Hydrolyzed extracts of fresh *M. bracteata* and *M. longifolia* leaves were analyzed by liquid chromatography with tandem mass spectrometry (LC-MS/MS) for identification of phenolic aglycones.

For extraction of exudate compounds, fresh (not ground) leaves of flowering field-grown plants were weighed and briefly rinsed with acetone to dissolve the surface constituents. The acetone solution was evaporated to dryness, and the residue was dissolved in a small volume of ethanol. These ethanol extracts were subjected to the quantification of compounds in leaf exudates (Wollenweber *et al.,* 2005a).

Scanning Electron Microscopy (SEM), Fluorescence Microscopy, and Histochemistry

The samples of the leaves were placed on Standard Carbon Conductive Tabs and examined without sputter coating by means of a Hitachi TM-1000 tabletop scanning electron microscope (Hitachi High-Technologies Corporation, Japan) at 15 kV accelerating voltage. The SEM control software associated with the microscope was used to produce digital images.

Whole mounts of fresh leaves and cross-sections of stems and leaves were subjected to light and fluorescence microscopy. For preparation of cross-sections, plant material was transversely sectioned on a sledge microtome. Fresh leaves and anatomical sections were studied under a light microscope, Primo Star iLED (Carl Zeiss Microscopy GmbH, Germany), with a 470 nm light-emitting diode and filter set 09 (excitation BP 450–490 nm, emission LP 515 nm), a 5-megapixel microscope camera (AxioCamMRc), and imaging software AxioVision 4.8 (Carl Zeiss Microscopy GmbH, Germany).

Localization of phenolic compounds was investigated by a 5 min histochemical test with 0.05% Toluidine Blue 0 in water (Muravnik *et al.,* 2016). Polyphenolic compounds stained dark blue (Ranocha *et al.,* 2002). For fluorescence microscopy, fresh sections were stained with Wilson's reagent [5% citric acid (w/v) and 5% boric acid (w/v) in absolute methanol] for 15 min (Muravnik *et al.,* 2016) to detect flavonoids, inducing yellow–green fluorescence at 450 nm. The fluorescence intensity of the reagent-treated samples was compared with the untreated controls to take into account autofluorescence of lignin and flavonoids.

LC-MS/MS

In order to identify aglycones in the hydrolyzed extracts, the MS analysis was carried out (at the Core Facility of Mass Spectrometric Analysis), as described by (Chernonosov *et al.,* 2017). In brief, gradient separation chromatography was carried out on an Agilent 1200 HPLC system (Agilent Technologies, USA) using a Zorbax Eclipse XBD-C18 Column (4.6 × 150 mm, 5 μm i.d.) with an Eclipse XBD-C18 guard column (4.6 × 12.5

Table 2. Phytochemicals identified in the hydrolyzed extracts of leaves of ***M. bracteata*** and ***M. longifolia***.

Peak	Compound	T_R (min)	$[M-H]^-$ *m/z*	Molecular fragments *m/z*	Relative peak area (%)	
					M. bracteata	*M. longifolia*
1	Tartaric acid	5.35	149	87	0.05 ± 0.02	0.36 ± 0.11
4	Quercetin pentoside	14.67	452	301, 151	0.97 ± 0.33	ND
6	Isoferulic acid	15.22	193	134, 178	3.05 ± 0.12	0.34 ± 0.04
7	Ferulic acid[a]	15.26	193	178, 149, 134	2.69 ± 0.05	0.48 ± 0.05
9	Kaempferol 3-O-hexoside	15.99	447	284	0.17 ± 0.02	ND
10	Ellagic acid[a]	22.41	301	284, 257, 229, 185	0.41 ± 0.04	ND
11	Quercetin[a]	22.46	301	245, 229, 179, 151, 121, 107	5.43 ± 0.61	2.31 ± 0.60
12	Rhamnocitrin	22.49	299	271, 256, 255, 227, 211, 199, 151	1.33 ± 0.23	0.86 ± 0.12
14	Naringenin	24.83	271	151, 119	0.66 ± 0.12	0.25 ± 0.01
15	Apigenin	24.92	269	117, 151, 107	0.14 ± 0.04	ND
18	Isorhamnetin[a]	25.99	315	300, 283, 271, 255, 243, 227, 164, 151, 148, 136, 107, 83, 63	70.94 ± 8.76	61.99 ± 0.51
20	Rhamnetin [a]	28.88	315	165, 121	0.07 ± 0.03	ND
21	Rhamnazin	29.70	329	314, 299, 271, 227	0.98 ± 0.15	22.79 ± 0.76
22	Kaempferide	32.60	299	284, 255, 227, 164, 163, 132, 107, 83, 63	10.50 ± 0.78	6.67 ± 0.47
	Total [b]				100	100

[a] Identified by comparison with a standard. [b] Total peak area of all 22 compounds. ND = not detected.

mm, 5 μm i.d.). MS detection was performed on an Agilent 6410 QQQ mass spectrometer (Agilent Technologies, USA) equipped with an electrospray ionization (ESI) source. The ESI-MS detection was carried out in the negative multiple-reaction monitoring (MRM) mode. Typical ESI-MS conditions were: spray voltage at 4000 V, turbo spray temperature at 300 °C, and nitrogen as nebulizer gas at 40 psi and 9.2 l/min flow. The precursor-to-product ion pairs, the optimized fragmentor voltage, and the collision energy for each analyte were set as described elsewhere (Chernonosov *et al.,* 2017). The detected compounds were characterized by a comparison of retention time and the MS/MS spectra from the LC-MS/MS mass analyzer with those of authentic standards when available. The other identification procedures involved interpreting the MS and MS/MS spectra of the detected compounds via the literature and databases. Such samples were analyzed in product ion mode with optimized parameters.

HPLC Analysis

The analysis of phenolic compounds was performed on an Agilent 1200 HPLC system equipped with a diode array detector (Agilent Technologies, USA). The chromatographic separation was conducted at 25°C on a Zorbax SB-C18 Column (4.6 × 150 mm, 5 μm i.d.) with the Agilent Guard Column Hardware Kit (p.n. 820888-901).

The mobile phase consisted of MeOH (solvent A) and 0.1% orthophosphoric acid in water (solvent B). Separation was performed with a gradient. The run via gradient was started with a solvent A–solvent B mixture at 32:68 (v/v) followed by a linear gradient to 33:67 (v/v) for the first 27 min, then to 46:64 (v/v) from minute 28 to minute 38, then to 56:44 from minute 39 to minute 50, and then to 100:0 from minute 51 to minute 54. The mobile phase was returned to 32:68 (v/v) from minute 55 to minute 56. The flow rate was set to 1 $mL \cdot min^{-1}$. The sample injection volume was 10 μL, and the absorbance was measured at 210, 255, 270, 290, 325, 340, 360, and 370 nm.

The quantification of phenolic compounds was conducted by the external-standard method. Validation of the analytical procedures was performed in accordance with ICH guidelines (ICH, 2005). To construct calibration curves, chemical reference standards of gallic and ferulic acids from Serva (Heidelberg, Germany), ellagic and citric acids, quercetin, astragalin, isoquercitrin, isorhamnetin 3-*O*-rutinoside from Sigma (St. Louis, MO, USA), and hyperoside from Fluka (Sigma-Aldrich Chemie GmbH, Munich, Germany) were used. Standard stock solutions at a concentration of 1 $mg \cdot ml^{-1}$ in methanol were prepared to construct calibration curves in the concentration range of 2–100 $\mu g \cdot ml^{-1}$. The sum of low-molecular-weight compounds (LMWCs) was calculated as citric acid equivalents at 220 nm (Brent *et al.,* 2014), and flavonol glycosides 17, 19, and 21–23 were quantified as hyperoside equivalents at 350 nm.

Statistical Analysis

The data were processed in the Statistica 10.0 software (Statsoft Inc., Tulsa, OK, USA), are reported as mean ± standard deviation (SD) of nine replicates (3 biological and 3 technical ones), and were compared by Duncan's multiple range test. Differences between the means were considered statistically significant at the 5% level ($p < 0.05$).

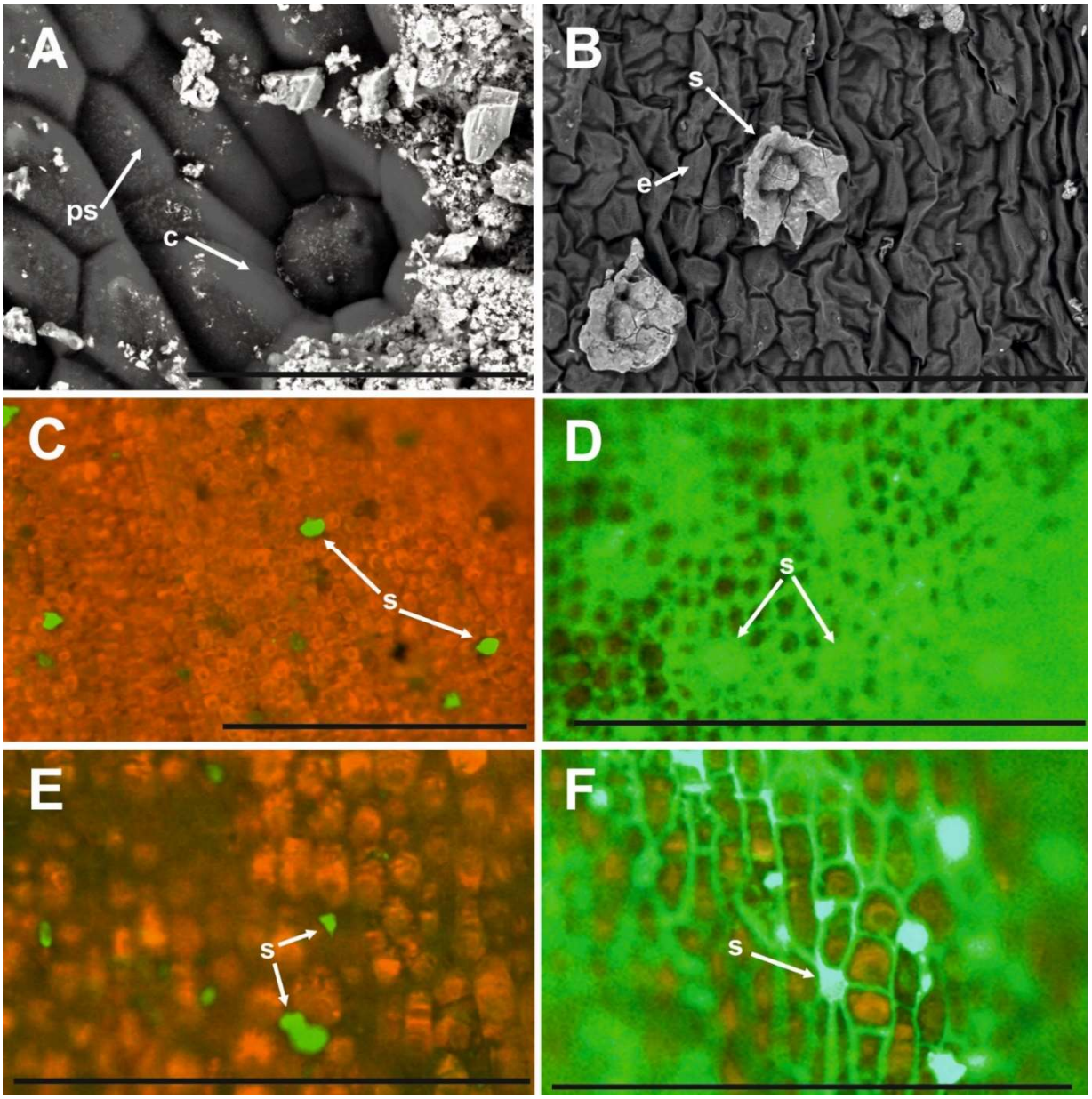

Fig. 1. Scanning electron microscopy (**A**, **B**) and fluorescence microscopy (**C**–**F**) of the leaf surface of ***M. bracteata*** and ***M. longifolia***. **A:** Secretory glands with secretion on the surface of a developed leaf of *M. bracteata*. **B:** Glands filled with salt on mature leaves of *M. bracteata*. **C:** Autofluorescence of flavonoids on the leaf surface of *M. bracteata*. **D:** Fluorescence of flavonoids induced by Wilson's reagent on the leaf surface of *M. bracteata*. **E:** Autofluorescence of flavonoids on the leaf surface of *M. longifolia*. **F:** Fluorescence of flavonoids induced by Wilson's reagent on the leaf surface of *M. longifolia*. Abbreviations: c, rosette of epidermal cells; e, epidermal cells; ps, papillae-shaped epidermal cells; s, secretory glands. Scale bars: A = 100 μm; B = 300 μm; C–F = 500 μm.

RESULTS

Location of Phenolic Compounds

General patterns of the anatomical structure of the leaves and stems of *M. bracteata* and *M. longifolia* are very similar and typical for Tamaricaceae. Salt glands are found on the abaxial surface of the leaves. The sunken glands have a secreting area ~30 μm in diameter and are surrounded by epidermal cells accommodated in a rosette (Fig. 1A). Taking these cells into account, the glands reach 60–80 μm in diameter. Crystal deposits were most pronounced on the surface of mature leaves (Fig. 1B).

Fluorescence microscopy with Wilson's reagent revealed the localization of flavonoids: in the salt glands and in the cell wall of epidermal cells of the leaves (Fig. 1C–F) and stems (Fig. 2H). Fluorescent signals in the cross-sections of stems collected during flowering were concentrated within stand-alone vascular bundles (Fig. 2B). During the development and expansion of vascular tissues in the fruiting stage, flavonoids were found to be

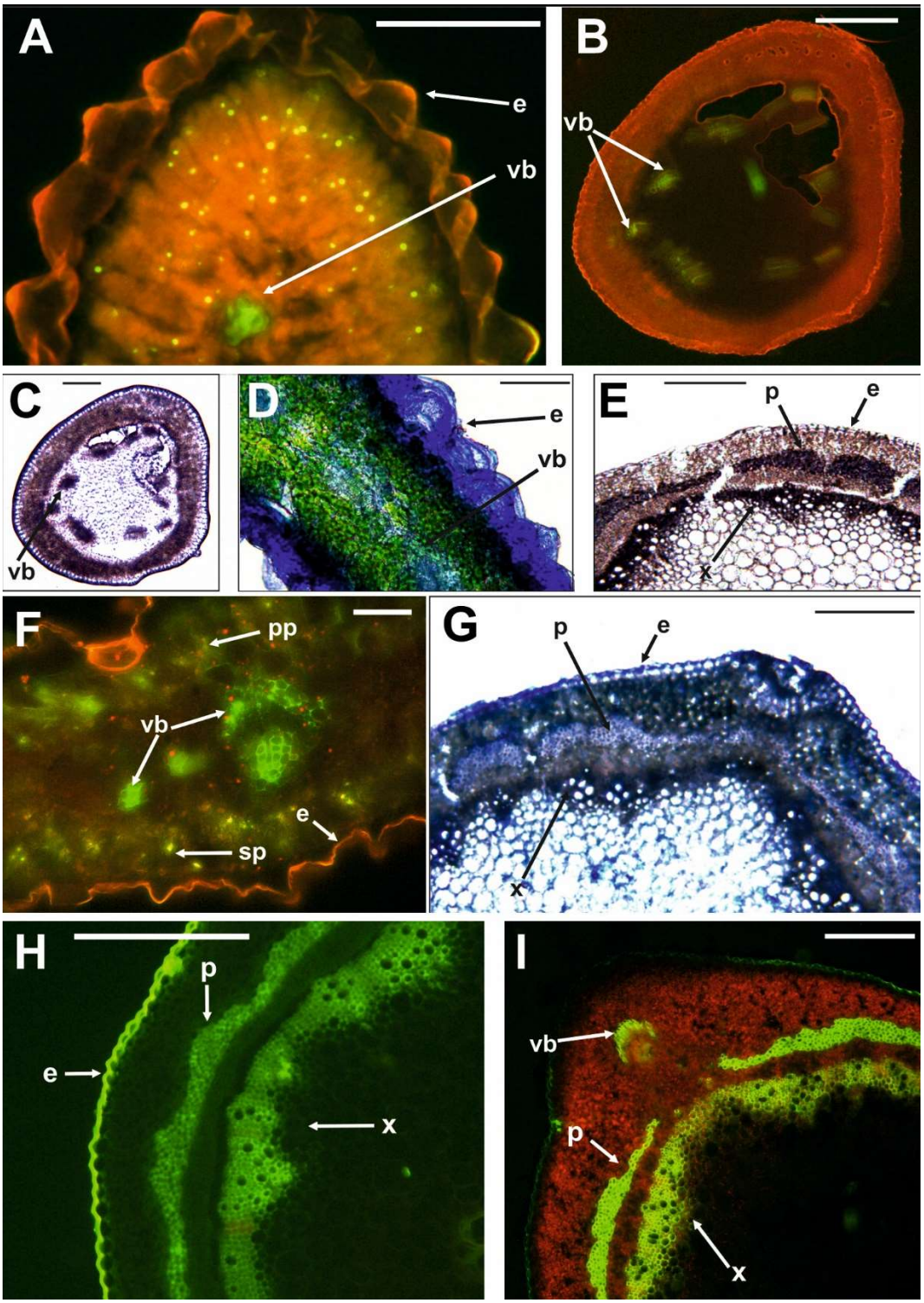

Fig. 2. Histochemical visualization of phenolic compounds and flavonoids in cross-sections of leaves (A, D and F) and stems (B, C, E, G, H, I) of ***M. longifolia***. **A:** Green fluorescence of flavonoids with Wilson's reagent in parenchyma and vascular bundles (flowering). **B:** Green fluorescence of flavonoids with Wilson's reagent in the vascular bundles (flowering). **C:** Vascular bundles in a cross-section of a stem without staining (flowering). **D:** Phenolic compounds in the vascular bundles and epidermis stained with Toluidine Blue (fruiting). **E:** Vascular bundles in a cross-section of a stem without staining (fruiting). **F:** Green fluorescence of flavonoids with Wilson's reagent in parenchyma and vascular bundles (fruiting). **G:** Phenolic compounds in vascular bundles with Toluidine Blue staining (fruiting). **H:** Green fluorescence of flavonoids with Wilson's reagent in vascular bundles and in the epidermis (fruiting). **I:** Green fluorescence of flavonoids with Wilson's reagent in vascular bundles (fruiting). Abbreviations: e, epidermal cells; p, phloem; pp, palisade parenchyma; sp, spongy parenchyma; vb, vascular bundles; x, xylem. Scale bars: A, D, F = 50 µm and B, C, E, G, H, I = 200 µm.

Table 3. Concentrations of phenolic compounds (μg·g^{-1} dry weight) in the leaves (A) and on the leaf surface (B) of ***M. bracteata*** and ***M. longifolia*** and ratio B/A (%).

Compound	T_R (min)	λ max, nm	*M. bracteata*			*M. longifolia*		
			A	B	B/A, %	A	B	B/A, %
LMWCs	1.3	220	24659 ± 219^{c***}	50.5 ± 1.3^{a}	0.2 ± 0.0^{b}	77888 ± 181^{d}	54.3 ± 0.3^{b}	0.1 ± 0.0^{a}
Ferulic acid	9.2	325	486 ± 5^{d}	9.3 ± 0.5^{b}	1.9 ± 0.0^{b}	221 ± 1^{c}	0.5 ± 0.0^{a}	0.2 ± 0.0^{a}
Hyperoside	18.3	255, 355	1237 ± 16^{c}	3.7 ± 0.3^{a}	0.3 ± 0.0^{a}	5873 ± 52^{d}	17.6 ± 0.2^{b}	0.3 ± 0.0^{a}
Isoquercitrin	19.0	255, 355	79.0 ± 4.2^{c}	1.4 ± 0.1^{b}	1.8 ± 0.0^{b}	1346 ± 10^{d}	0.9 ± 0.0^{a}	0.1 ± 0.0^{a}
Ellagic acid	22.0	250	1002 ± 7^{c}	ND	ND	995 ± 7^{b}	11.15 ± 0.1^{a}	1.1 ± 0.0^{a}
Avicularin	29.5	255, 355	50.0 ± 6.1^{c}	4.0 ± 0.3^{a}	8.0 ± 0.1^{b}	2163 ± 10^{d}	9.3 ± 0.1^{b}	0.4 ± 0.0^{a}
Astragalin	32.4	265, 346	1451.3 ± 8.2^{d}	4.7 ± 0.4^{a}	0.3 ± 0.0^{a}	1309 ± 8^{c}	16.0 ± 0.2^{b}	1.2 ± 0.0^{b}
Isorhamnetin 3-O-rutinoside	35.7	255, 355	79.0 ± 2.9^{a}	ND	ND	526 ± 25^{b}	ND	ND
Flavonol glycoside 19	38.4	250, 350	39.5 ± 2.2^{a}	ND	ND	201 ± 10^{b}	ND	ND
Quercetin	40.2	260, 370	15.8 ± 0.5^{c}	3.2 ± 0.3^{a}	20.5 ± 1.6^{b}	1534 ± 80^{d}	12.4 ± 0.1^{b}	0.8 ± 0.0^{a}
Naringenin	41.1	289, 326 sh.	165.3 ± 5.4^{b}	ND	ND	532.4 ± 22.5^{c}	8.5 ± 0.1^{a}	1.6 ± 0.0^{a}
Flavone 22	42.3	255, 340	106.1 ± 6.4^{b}	ND	ND	420.1 ± 15.3^{c}	1.0 ± 0.0^{a}	0.3 ± 0.0^{a}
Luteolin	44.2	253, 350	ND	ND	ND	335.4 ± 6.8^{a}	ND	ND
Sum of the compounds (including flavonoids)			29304 ± 284^{c} (3222 ± 18)	76.8 ± 3.2^{a} (17.0 ± 0.2)	0.3 ± 0.00^{b} (0.5 ± 0.00)	93343 ± 457^{d} (14240 ± 77)	132 ± 1^{b} (65.7 ± 0.3)	0.1 ± 0.00^{a} (0.5 ± 0.00)

Means in rows followed by the same letter do not differ significantly according to the *t* test ($p < 0.05$); the differences between the means of the concentrations and the ratios (B/A) were estimated separately. ND = not detected.

distributed throughout the vascular system (Fig. 2I). Flavonoid accumulation was also detected in subepidermal spongy and palisade parenchyma layers of the leaves (Fig. 2A and F).

Toluidine Blue staining indicated the accumulation of phenolic compounds inside the vascular bundles and the epidermal cells (Fig. 2D and G).

Phenolic compounds analyzed by LC-MS/MS

In hydrolyzed extracts of the leaves of *M. bracteata*, 22 compounds were detected, including 15 previously identified and 7 new ones: tartaric acid **(1)**, quercetin pentoside **(4)**, isoferulic acid **(6)**, kaempferol hexoside **(9)**, naringenin **(14)**, apigenin **(15)**, and rhamnetin **(20)**. In hydrolyzed extracts of the leaves of *M. longifolia*, 15 compounds were identified. All of them were found in *M. bracteata*. In Table 2, parameters of the major constituents are represented. Contributions of citric acid (**2**), gallic acid (**3**), ethyl gallate (**5**), methyl gallate (**8**), chrysoeriol (**13**), luteolin (**16**), kaempferol (**17**), and myricetin (**19**) to total peak areas were minimal. The main aglycone component of the leaves in the analyzed species was flavonoid isorhamnetin. Its relative peak areas were 70.94% and 61.99% in the total phenolics of *M. bracteata* and *M. longifolia*, respectively. In the leaves of *M. longifolia*, rhamnazin was abundant (22.79%). Therefore, the LC-MS/MS data are suggestive of similarity of the leaf flavonoid aglycone profiles between *M. longifolia* and *M. bracteata*; this similarity can be expressed as 15 out of 22 compounds (68%).

HPLC Analysis

In the leaves, free phenolic acids (including gallic and ferulic acids), free ellagic acid, flavonol glycosides (including hyperoside, isoquercitrin, avicularin, astragalin, and isorhamnetin 3-O-rutinoside), free quercetin, naringenin, luteolin, and one unidentified flavone were detected. Most of these constituents were also found in the exudates of the leaves (Table 3). Concentrations of the phenolic constituents in the exudate ranged from 0.2% to 20.5% of each compound's total concentration in the leaves of *M. bracteata* and from 0.1% to 1.6% of each compound's total concentration in the leaves of *M. longifolia*. Ratios of the sum of phenolic compounds on the leaf surface to the total of leaf phenolic compounds were 0.3% and 0.1% in *M. bracteata* and *M. longifolia*, respectively. Ratios of the sum of flavonoids on the leaf surface to the total of leaf flavonoids were significantly higher (0.5% for both species).

Quercetin was detected in the leaves of *M. bracteata* only in minute amounts, but its large amount (20.5%) was located on the surface. In addition, significant percentages of avicularin were located on the surface of *M. bracteata* leaves. In the leaves, astragalin and hyperoside were the main constituents. The former, along with LMWCs and ferulic acid, predominated among exudate phenolic constituents of *M. bracteata* leaves. Concentrations of most of compounds in the leaves and on the leaf surface of *M. longifolia* were higher than those in *M. bracteata*. Hyperoside and astragalin, just as in *M. bracteata*, along with avicularin and quercetin, were the main constituents in the leaves of *M. longifolia*. Among the major exudate constituents of *M. longifolia* leaves, LMWCs and astragalin were also dominant. In addition, concentrations of hyperoside and quercetin were substantial.

DISCUSSION

The present study reveals that exudate phenolic constituents represent a modest proportion of phenolics of the leaves in *Myricaria* species. Their total amount in

the exudates constituted less than 0.5% of the total amount of phenolics in the leaves, but certain compounds accumulated on the surface in substantial quantities (quercetin). In the leaves of *M. bracteata*, the percentage of exudate phenolics in relation to total leaf phenolics was higher in comparison to *M. longifolia*. Nonetheless, absolute amounts of several compounds (hyperoside, astragalin, quercetin, and ellagic acid) in *M. longifolia* leaf exudates exceeded their amounts in the exudates of *M. bracteata* (Table 3). The higher proportion of exudate flavonoids in total flavonoids in comparison to the corresponding proportion of other phenolics (≥2-fold) reflects the importance of flavonoids as secreted compounds.

The same components as in the whole-leaf extracts were detected in the leaf exudates, where mainly phenolic acids, flavonol glycosides, and free quercetin were present (Table 3). These results are consistent with the literature data, showing the moderate abundance of flavonoid aglycones and the presence of phenolic acids and flavonoid glycosides in secretory structures on the leaf surface of several plant taxa (Valant-Vetschera & Brem, 2006; Muravnik *et al.,* 2016), in contrast to the high aglycone abundance and chemodiversity in representatives of Lamiaceae and Asteraceae (Wollenweber *et al.,* 2005a).

The microscopy results revealed the presence of flavonoids in salt glands and inside the leaf. Flavonoids and other phenolics are located in the cells of spongy and palisade parenchyma under the epidermis as well as in vascular bundles and epidermal cells. These findings match the data (Soukupová *et al.,* 2000; Donaldson and Williams, 2018) about the localization of phenolic compounds in mesophyll cells close to the epidermis and in the epidermis. Other researchers (Oliveira Ribeiro *et al.,* 2014) detected tannins in the epidermis and in spongy and palisade parenchyma. Another research group (Agati *et al.,* 2007) provided a number of references on the detection of quercetin and kaempferol derivatives in the cell wall of epidermal cells of various plants. (Silva *et al.,* 2015) revealed fluorescence of flavonoids in the epidermis, parenchyma, and vessels in response to pathogen infection. The localization of flavonoids in the salt glands and throughout the vascular system indicates the pathway of their secretion into the phyllosphere. A similar flavonoid transport pathway through non-glandular trichomes has been described by others (Tattini *et al.,* 2007; Karpova *et al.,* 2019). It appears that the path involves collecting and secretory cells of salt glands in *Myricaria* leaves (Dörken *et al.,* 2017).

Results of HPLC indicated a high concentration of total phenolics (29.30 and 93.34 mg·g^{-1} dry weight) in the leaves of *M. bracteata* and *M. longifolia*, respectively, and wide structural diversity of flavonoids. They included free quercetin and glycosides of quercetin, kaempferol, and isorhamnetin. According to LC-MS/MS, glycosides of flavones (chrysoeriol, luteolin, and apigenin), flavonols (rhamnazin, rhamnetin, rhamnocitrin, and kaempferide), and a flavanone (naringenin) also contribute to the total phenolic contents of these species. Among LMWCs, organic acids (citric and tartaric) were detected. Tartaric acid **(1)**, naringenin **(14)**, and apigenin **(15)** were detected for the first time in the genus *Myricaria*. Previously, they have been identified in the genus *Tamarix* (Parmar *et al.,* 1994; Karker *et al.,* 2016).

Phenolic profiles of these species of *Myricaria* are overall similar to those of *Tamarix* species (Iwashina, 2013). Nevertheless, the ratios of the compound concentrations in previously investigated species are difficult to compare with our findings owing to the incompleteness of their data, especially for flavonoids. The ellagic acid content was found to be substantial in both species. For this reason, they can be regarded as a good source of ellagic acid, one of the most investigated health-promoting phytochemicals with antioxidant, antimutagenic, and anticancer properties (Usta *et al.,* 2013).

Thus, the presence of phenolic compounds and flavonoids in the leaves and on the leaf surface of the studied *Myricaria* species shows great value of these plants for human health and for the environment, e.g., for indoor air cleaning (Brilli *et al.,* 2018; Turner *et al.,* 2013). Herewith, salt glands are a part of the system of transport and distribution of phenolic compounds, including flavonoids, in plant organs, and these glands play essential roles in flavonoid accumulation on the leaf surface.

CONCLUSION

Microscopy and histochemical assays revealed the presence of flavonoids in salt glands and the spread of these compounds throughout the vascular system. The phenolic profiles are similar between *M. bracteata* and *M. longifolia*, with high levels of ellagic acid and total flavonoids. Quantification of phenolic compounds in the leaves of these two *Myricaria* species adds information to the sparse data on the patterns of phenolic compounds in the genus *Myricaria.* The comparative profiling of the leaves and leaf exudates uncovered a similarity of phenolic composition between the leaf interior and leaf surface. These findings provide insights into the secretion of phenolics via salt glands.

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