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## Original Article

Identification of phenolic compounds in *Myricaria bracteata* leaves by high-performance liquid chromatography with a diode array detector (HPLC-DAD) and liquid chromatography with tandem mass spectrometry (LC-MS/MS)Alexander A. Chernonosov<sup>a,\*</sup>, Evgeniya A. Karpova<sup>b</sup>, Elena M. Lyakh<sup>b</sup><sup>a</sup> Institute of Chemical Biology and Fundamental Medicine, Siberian Branch of Russian Academy of Sciences, Novosibirsk, Russia<sup>b</sup> Central Siberian Botanical Garden, Siberian Branch of Russian Academy of Sciences, Novosibirsk, Russia

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## ABSTRACT

*Myricaria bracteata* Royle, Tamaricaceae, is a species with a wide geographic range that encompasses Eastern Europe, Western and Central Siberia, Central Asia, and the Himalayas. This plant is used in traditional folk medicine in Russia (Siberia) and in China typically as an analgesic and for the treatment of some infections and certain types of intoxication. The aim of this study was to identify phenolic constituents of the leaves of *M. bracteata* from two considerably distant populations. Chromatographic profiles of the leaves of *M. bracteata* were analyzed for the first time. Seventeen compounds, mainly methyl ethers of quercetin (isorhamnetin, rhamnazin), kaempferol (kaempferide, rhamnocitrin), and ellagic acid as well as quercetin, quercetin 3-glucoside, kaempferol, luteolin, chrysoeriol, citric acid, gallic acid, methyl gallate, ethyl gallate, and ferulic acid were identified in hydrolyzed aqueous ethanol extracts of the leaves. Flavonols and ellagic acid were the major compounds in both samples. Isorhamnetin was the main flavonoid constituent. Kaempferide and rhamnazin were also abundant in the flavonoid complex of the leaves of *M. bracteata* from the Altai. This study shows that *M. bracteata* leaves are a source of flavonoids with possible biological activities.

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## Introduction

*Myricaria* Desv., Tamaricaceae, is a genus of herbs with a potential for applications to conventional medicine. *Myricaria* species are used in folk medicine in Russia (Siberian regions) and in many Asian countries (more commonly in China, Mongolia, and India) for the treatment of some infections, certain types of intoxication, liver diseases, scalds, and arthritis (Semenova, 1993; Gewali, 2008; Kletter et al., 2008; Kirbag et al., 2009; Singh, 2012). Some species were reported to have high antimicrobial and acetylcholinesterase inhibitory activities and thus can be considered natural sources of antibiotics and drugs for the treatment of neurological disorders such as Alzheimer's disease (Mukherjee et al., 2007).

High antimicrobial activity of the volatiles of intact *Myricaria bracteata* plants against *Staphylococcus epidermidis* as well

as a pronounced antifungal activity against *Candida albicans* were also revealed (Lyakh and Tsybulya, 2009). Crude ethanol extract from the above-ground part of *M. bracteata* and its fractions have been shown to have a strong antimicrobial activity against *Staphylococcus aureus*, *Enterococcus faecalis*, *Micrococcus luteus*, and *Pseudomonas aeruginosa* (Gonchig et al., 2008).

The literature lacks detailed information on regular constituents of *M. bracteata*, their geographic variability, and the key active ingredients. The aim of this study was to identify the phenolic constituents of the leaves of *M. bracteata* from two considerably distant populations.

## Materials and methods

All chemicals were mass spectrometry (MS) and analytical grade. Chemical reference standards of gallic and ferulic acids were purchased from Serva (Heidelberg, Germany); quercetin, kaempferol, rhamnetin, isorhamnetin, luteolin, citric and ellagic acids were purchased from Sigma (St. Louis, MO, USA).

\* Corresponding author.

E-mail: [alexander.chernonosov@niboch.nsc.ru](mailto:alexander.chernonosov@niboch.nsc.ru) (A.A. Chernonosov).

The samples of *M. bracteata* Royle, Tamaricaceae, leaves were collected in the fruiting period of 2011. Sample A was collected at 1800 m above the sea level (a.s.l.) on a pebble floodplain of the Bolshoi Yaloman River, 1 km from Bolshoi Yaloman village, Ongudaysky Region, the Altai Republic. Sample B was collected at 3100 m a.s.l. on a pebble and sandy floodplain of the Tokuz-Bulak River (the left tributary of the Gunt River), 1.5 km from the Kuygantukoy village, Shugnon Region, the Republic of Tajikistan. The collected *Myricaria* samples were dried and ground into powder using a household mill.

A precisely weighed sample of air-dried plant material (0.3 g) was exhaustively extracted with an ethanol:water mixture (70:30, v/v) in a water bath at 60–70 °C. The aqueous ethanol extract was hydrolyzed with 2 N HCl for 2 h in a boiling water bath. The hydrolysate was purified by means of a C16 Diapack cartridge and redissolved in ethanol.

Mass spectrometric analysis was carried out at the Core Facility of Mass Spectrometric Analysis (ICBFM SB RAS). An Agilent 1200 liquid chromatography system, with an Agilent 6410 QQQ mass spectrometer (Agilent Technologies, USA) equipped with an electrospray ionization (ESI) source, served as an LC-MS/MS system. The chromatographic separation was carried out at 25 °C on a Zorbax Eclipse XBD-C18 Column (4.6 mm × 150 mm, 5 μm i.d.) with an Eclipse XBD-C18 guard column (4.6 mm × 12.5 mm, 5 μm i.d.).

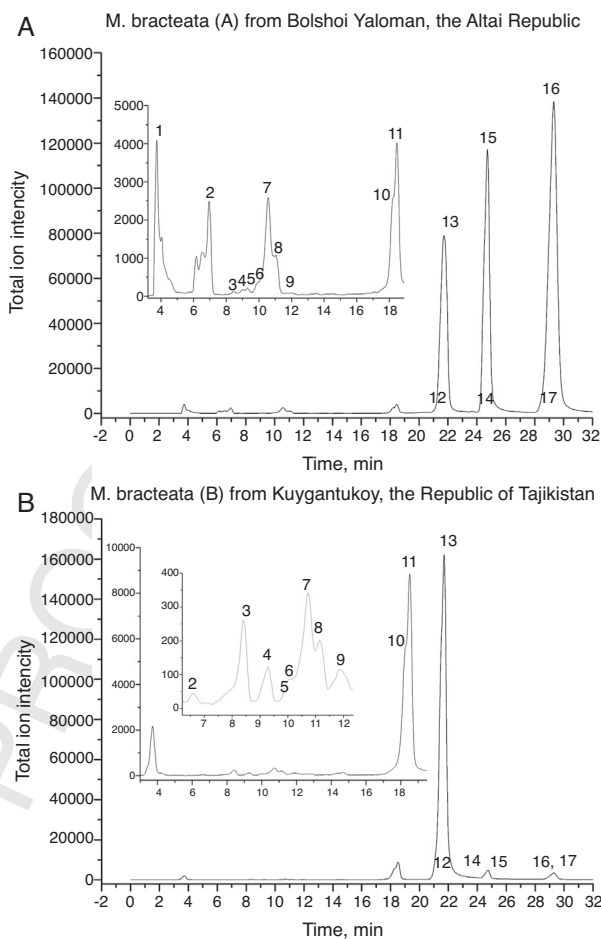
For the gradient elution, 10 mM NH<sub>4</sub>COOH in water (pH 4.0, adjusted with formic acid), i.e., solvent A, and 10 mM NH<sub>4</sub>COOH (pH 4.0 adjusted with formic acid) in acetonitrile–water 95:5 (v/v), i.e., solvent B, were used. The run was started with 0.6 ml min<sup>−1</sup> flow rate at a solvent A:solvent B ratio of 100:0 (v/v) followed by a linear gradient to 50:50 (v/v) for the first 5 min, then to 48:52 (v/v) from min 5 to min 10, then to 0:100 (v/v) from min 10 to min 20; and returned to 35:65 (v/v) from min 20 to min 35, then to 100:0 (v/v) from min 35 to min 40. The sample injection volume was 30 μl. All data acquisition and peak integration tasks were performed in the MassHunter software (version 1.3) from Agilent Technologies.

At the first step of the analysis, the samples were analyzed in full-scan negative mode from 100 to 1500 Da. The multiple-reaction monitoring (MRM) transitions for possible molecular structures that have been proposed for a detected ion (Supplementary data A) were based on the RIKEN MSn spectral database for phytochemicals (<http://spectra.psc.riken.jp>), MassBank (<http://www.massbank.jp>) and the Human Metabolome Database (HMDB) (<http://www.hmdb.ca>).

The HPLC system for absolute quantification of phenolic consisted of an Agilent 1200 with a diode array detector (DAD) and the ChemStation (Agilent Technologies, USA) software for data processing. The chromatographic separation was carried out at 25 °C on a Zorbax SB-C18 Column (4.6 mm × 150 mm, 5 μm i.d.) with an 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). The run was started at 1 ml min<sup>−1</sup> rate with a solvent A–solvent B mixture at 50:50 (v/v) followed by a linear gradient to 52:48 (v/v) for the first 15 min, then to 100:0 (v/v) from min 15 to min 17. It was returned to 50:50 (v/v) from min 17 to min 20. The sample injection volume was 10 μl.

The quantification of phenolic compounds was conducted as previously reported (Karpova and Khranova, 2014) (see Supplementary data B). All the data were reported as mean ± standard deviation (SD) of three replicates. The results were compared by Student's *t* test. The data analysis was performed using the Statistica 7.0 software (Statsoft Inc., Tulsa, OK, USA), and differences between the means were considered statistically significant at the 5% level (*p* < 0.05).



**Fig. 1.** LC-MS/MS base peak chromatograms of MS in negative ion mode for the hydrolyzed aqueous ethanol extracts of the leaves of *Myricaria bracteata* from the Altai (A) and from the Tajikistan (B).

## Results and discussion

Seventeen constituents, mainly flavonoids, were identified in the hydrolyzed extracts of each geographic sample of the leaves of *M. bracteata*. The composition of the leaves was identical (Fig. 1).

At the beginning of the analysis, 3 phenolic acids were identified in *M. bracteata* leaf extract (Table 1). Citric (1), gallic (2), ellagic (7), and ferulic (9) acids were directly identified by comparison of their retention time with that of standards and were confirmed in the MS/MS spectrum. Citric acid is being suggested as a constituent of *M. bracteata* for the first time and characterizes this plant as alkali-tolerant (Nawwar et al., 2013).

Ethyl gallate (8) was tentatively identified according to [M–H]<sup>−</sup> at *m/z* 197 and MS/MS fragment ions at *m/z* 124 and 169, where the latter corresponds to gallic acid. Compounds 3 and 4 with [M–H]<sup>−</sup> at *m/z* 183 and an MS/MS fragment ion at *m/z* 124 (Rendekova et al., 2015) were tentatively identified as methyl gallate isomers. An additional fragment ion at *m/z* 140 was detected for 3 (Rendekova et al., 2015); however, this fragment ion was not revealed for 4, probably because of low quantity. Methyl gallate and other derivatives of hydroxybenzoic acids have been reported to be frequent constituents of *M. bracteata* (Zhang et al., 2011; Zhao et al., 2005).

A total of ten flavonoid derivatives were identified in *M. bracteata* leaves. Quercetin (11), luteolin (10), kaempferol (12) and isorhamnetin (13) were identified by comparison of retention time and the MS/MS spectra with those of authentic standards.

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