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PHENOLIC COMPOUNDS FROM *Rhododendron dauricum* FROM THE BAIKAL REGION

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Rhododendron dauricum L. (Ericaceae) is a mountainous half-evergreen shrub indigenous to Central Siberia and the Baikal region. The phenolic complex of *R. dauricum* contained simple phenols (hydroquinone, arbutin, 4-*O*-methylfloroacetophenone, orcinol, grifolin) [1], phenolcarboxylic acids (*p*-hydroxybenzoic, anisic, protocatechoic, vanillic, syringic, 3-methyl ether of gallic acid) [1, 2], coumarins (umbelliferone, scopoletin) [3], chromanes (rhododaurichromanic acids A and B) [4], chromenes (daurichromenic acid, daurichromenes A–D, confluentin) [4, 5], and flavonoids (poriol, farrerol, matteucinol, kaempferol, 5-methylkaempferol, quercetin, azaleatin, hyperoside, isohyperoside, avicularin, quercitrin, myricetin, 5-methylmyricetin, gossipetin) [1, 3, 6, 7]. *R. dauricum* growing in China, Khabarosk Territory, and in the Altai have been studied previously.

Our goal was to study the qualitative and quantitative content of phenolic compounds in *R. dauricum* from the Baikal region.

Leaves of *R. dauricum* were collected in Irkutsk Oblast (Irkutsk, test plot of SIFIBR, SB RAS, June 28, 2008, raw material I; August 28, 2008, II; Olkha village, September 3, 2008, III; Orlenok station, September 9, 2008, IV), in the Lake Baikal basin (Olkhon Island, September 13, 2008, V; November 4, 2008, VI), and in the Republic of Buryatiya (Mukhorshibir Region, Barsk Ridge, July 25, 2009, VII; Soviet Region, Ulan-Ude, August 25, 2009, VIII; Pribaikal Region, Goryachinsk village, September 17, 2009, IX). The speciation was determined by Cand. Pharm. Sci. N. K. Chirikova (IGEB, SB RAS). Raw material samples are preserved in the herbarium of the Division of Biologically Active Compounds, IGEB, SB RAS.

Dried ground raw material (0.2 kg, VII) was extracted with EtOH (80%, 5×, 1:25) at 80°C. The EtOH extract was concentrated to a watery residue that was extracted with hexane, $CHCl_3$, EtOAc, and *n*-BuOH to produce five fractions: hexane (20.07 g, 10.04% of air-dried mass), $CHCl_3$ (2.59 g, 1.30%), EtOAc (16.29 g, 8.15%), *n*-BuOH (8.25 g, 4.13%) and an aqueous residue (25.22 g, 12.61%). The total yield of extracted compounds was 36.23% of the air-dried mass.

The $CHCl_3$ fraction (2.4 g) was separated by column chromatography over SiO_2 (2 × 30 cm) using hexane:$CHCl_3$ (100:0→70:30) and $CHCl_3$:Me_2CO (100:0→50:50) with subsequent rechromatography by preparative TLC (toluene:EtOAc:HCOOH 3:3:1) and HPTLC (petroleum ether:Et_2O:HCOOH 9:4:1). This afforded **1** (10 mg, farrerol, 6,8-dimethyl-5,7,4′-trihydroxyflavanone) [8, 9], **2** (31 mg, scopoletin) [10], **3** (6 mg, umbelliferone) [10], **4** (4 mg, kaempferol) [11], **5** (10 mg, quercetin) [12], and **6** (11 mg, hyperoside) [12]. The EtOAc (10 g) and *n*-BuOH extracts (7 g) were chromatographed over columns of SiO_2 (3.5 × 40 cm) using $CHCl_3$:Me_2CO (100:0→70:30), Me_2CO:EtOH (100:0→50:50), and EtOH:H_2O (95:5→40:60). Subfractions were separated over columns of polyamide (Woelm, 2 × 20 cm, EtOH:H_2O, 0:100→95:5, Me_2CO) with subsequent preparative TLC (toluene:EtOAc:HCOOH, 5:4:1; EtOAc:MeOH:H_2O, 9.6:1.9:1).

Chromatographic separation of the EtOAc fraction isolated **7** (67 mg, gallic acid 3-methyl ether) [13], **8** (18 mg, gallic acid) [13], **9** (31 mg, ellagic acid) [14], **10** [11 mg, (+)-catechin], **11** [4 mg, (–)-epicatechin] [15], **6** (214 mg), **12** [2 mg, (–)-epicatechin-3-gallate] [16], **13** (82 mg, avicularin) [12], **14** (41 mg, quercitrin) [12], and **15** (8 mg, procyanidin B1) [17]. HPLC (conditions 1) of the EtOAc fraction also detected *p*-hydroxybenzoic (**16**), gentisic (**17**), and syringic (**18**) acids. The *n*-BuOH fraction afforded **8** (10 mg), **10** (5 mg), **12** (6 mg), **15** (12 mg), and **17** (8 mg).

The study found in leaves of *R. dauricum* 18 compounds. Of these, **1**-**7**, **13**, **14**, **16**, and **18** were isolated earlier from this species whereas **8**-**12**, **15**, and **17** were isolated for the first time.

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TABLE 1. Content of Phenolic Compounds in *R. dauricum* Leaves, mg/g Air-dried Raw Material

Compound	Raw material								
	I	II	III	IV	V	VI	VII	VIII	IX
2	0.67	2.80	1.40	0.98	0.36	1.67	0.21	1.59	1.74
3	0.21	1.26	0.29	0.12	0.04	0.26	0.03	0.38	0.51
5	0.06	0.04	0.02	0.04	0.05	0.03	0.03	сл.	сл.
6	4.73	3.90	4.93	4.37	3.60	8.02	3.00	5.81	4.52
7	0.81	2.08	–	–	0.70	0.85	2.49	1.23	0.73
8	1.89	0.78	0.25	1.50	3.26	1.48	0.94	1.22	0.71
9	–	6.45	2.74	6.32	1.74	17.89	1.77	–	10.18
13	4.77	3.57	3.32	3.17	3.17	7.20	1.63	3.47	2.98
14	2.23	2.09	1.69	1.17	2.14	3.43	0.76	1.28	1.37

Next we used HPLC (conditions 1) to determine the quantitative content of the dominant constituents of the phenolic complex in raw material of nine cenopopulations of *R. dauricum* from Baikal Region. The marker compounds were **2**, **3**, **5–9**, **13**, and **14** (Table 1). The results showed that the composition of phenolic compounds from *R. dauricum* was variable. As a rule, hyperoside dominated in the flavonoids whereas the scopoletin content was always 2.2–9.0 times greater than that of umbelliferone. *R. dauricum* leaves in autumn typically had higher contents of coumarins, ellagic acid, and flavonoids.

R. dauricum leaves collected on Olkhon Island (VI) had the highest content of phenolic compounds. This was probably explained by the more favorable habitat. Raw materials III and IV were collected in regions of Irkutsk Oblast with similar natural conditions. However, they differed in the level of man-made contamination. Orlenok station (IV) is located very close to Irkutsk aluminum plant (affected zone) whereas Olkha village (III) is not affected by it (buffer zone). Because of the different ecological conditions, raw material IV typically had lower contents of coumarins (**2** and **3**) and flavonoid glycosides (**6**, **13**, and **14**) whereas the concentrations of gallic (**8**) and ellagic (**9**) acids were increased by 6 and 2.3 times.

The presence of arbutin in *R. dauricum* was reported [1]. However, we did not observe it in this plant species in preliminary experiments. In order to resolve this contradiction, we analyzed extracts of *R. dauricum* by HPLC (conditions 2). This also did not detect arbutin and hydroquinones in raw material of the studied cenopopulations. Analogous results were obtained earlier for other *Rhododendron* species, i.e., *R. aureum* [18], *R. ferrugineum*, and *R. hirsutum* [19].

The isolated compounds were identified using melting points, specific rotation, chemical transformations, UV and ^{13}C NMR spectroscopy, spectrophotometric studies on a UV-Vis mini (Shimadzu) spectrophotometer, optical rotation on an SM-3 polarimeter (Zagorsk Optico-Mechanical Plant), ^{13}C NMR spectra on a VXR 500S (Varian) NMR spectrometer at operating frequency 125.7 MHz with solutions (1%) of compounds in DMSO-d_6, HPLC using conditions 1 on a Summit liquid chromatograph (Dionex) with a Prodigy ODS 3 column (5 μm, 250 × 4.6 mm, Phenomenex) and gradient elution (A, 0.1% TFA; B, MeCN) at flow rate 0.2 mL/min and 20°C with a UVD 170 S UV detector set at λ 254 and 280 nm and using conditions 2 on a Milikhrom A-02 liquid chromatograph (Ekonova) with a Nucleosil 100-5 C18 column (5 μm, 75 × 2 mm) and gradient elution (A, 0.05 M KH_2PO_4:MeCN 95:5; B, MeOH) at flow rate 0.15 mL/min and 35°C with a UV detector at wavelengths 202, 224, 270, and 278 nm. The chromatographic mobility, spectrum in a stopped-flow solvent, and spectral ratios were measured during the analysis. Experiments with additions of standard compounds were also performed.

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