

Phenolic compounds of *Dasiphora fruticosa* plants from natural populations in the south of the Russian Far East

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Abstract. The profile and amounts were determined for phenolic compounds in the leaves of *Dasiphora fruticosa* plants growing in natural populations in the south of the Russian Far East. It was found that the phenolic profiles of the plants are similar among all the analyzed populations, but there are differences in the concentrations of the phenolic compounds. Total concentrations of phenolic compounds, flavonols, rhamnetin glycosides, and free quercetin are 12–46% higher in the leaves of plants of three populations from Shikotan Island. The highest total concentration of ellagic compounds (12.6 mg/g) was noted in the population from Primorsky Krai.

Potential usefulness of *Dasiphora fruticosa* (L.) Rydb. (\equiv *Pentaphylloides fruticosa* (L.) O. Schwarz) from the family Rosaceae Juss. [1], when applied as a medicinal plant, has aroused much interest among pharmacologists, resource specialists, and innovation specialists. There is evidence of anti-inflammatory, hypoglycemic, antiallergic, immunomodulatory, antioxidant, antibacterial, analgesic, and antimicrobial properties of the plant material from *D. fruticosa* [2-8]. These broad-spectrum therapeutic activities result from the presence of phenolic compounds, mostly flavonols, which are largely responsible for the therapeutic effect of this plant material. The concentration of flavonols varies from 0.7% to 6.0%, thus making the research on their profile and levels relevant and important. The phenolic profile of *D. fruticosa* has been studied in sufficient detail [9-12]. Nonetheless, the pattern and amounts of biochemical compounds in *D. fruticosa* plants growing on the territory of the Russian Far East are poorly studied, even though this species is widespread in this part of its areal.

The aim of this study was a comparative analysis of the profiles and levels of phenolic compounds in the leaves of *D. fruticosa* plants among populations growing in the south of the Russian Far East.

The biological material for the study consisted of specimens of *D. fruticosa* collected from four coenotic populations (CPs) in the south of the Russian Far East: from Primorsky Krai (CP 1) and from Shikotan Island of the Smaller Kuril Archipelago (CPs 2–4; Table 1). Meteorological parameters of the study sites are different. Primorsky Krai is characterized

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by a moderate monsoon climate with dry cold winter and warm humid summer. The average air temperature in January is -12°C and in July $+17.5^{\circ}\text{C}$; annual precipitation is 840 mm/year, and sunshine 1414 h/year [13]. Shikotan Island is characterized by a mild moderately marine climate affected by monsoons, with warm winter and mild summer; the high humidity does not have a negative effect because of the mild temperature regimen: the average air temperature in January is -4.6°C and in July $+16.3^{\circ}\text{C}$; annual precipitation is 894 mm/year, with sunshine at 1606 h/year [14].

Table 1. Sites of sampling of *D. fruticosa* specimens.

Coenotic populations	Sampling site, habitat, coordinates
CP 1: Ol'khovaya	Primorsky Krai, Partizansky District, slope of mountain Ol'khovaya, 43.20364°N, 133.39275°E, 1581 m above sea level
CP 2: Otradnaya	Sakhalin Oblast, Yuzhno-Kurilsky District, northeastern coast of Shikotan Island, Otradnaya Bay, 43.52180°N, 146.47037°E, 54 m above sea level
CP 3: Malokuril'skaya	Sakhalin Oblast, Yuzhno-Kurilsky District, northeastern coast of Shikotan Island, near Malokuril'skoe village, 43.52531°N, 146.49215°E, 24 m above sea level
CP 4: Gorobetskaya	Sakhalin Oblast, Yuzhno-Kurilsky District, northwestern coast of Shikotan Island, Gorobets bay, 43.4936°N, 146.4224°E, 55 m above sea level

To measure the concentrations of phenolic compounds, representative sampling was conducted involving 30 specimens from each population at the mass flowering stage. Year-old foliated shoots 15–20 cm long were cut down uniformly according to the crown surface, were separated into organs, and air-dried completely. Aqueous ethanolic extracts of the plant samples were prepared by a standard method [15]. To eliminate hydrophilic compounds, solid-phase extraction was performed [16]. A detailed description of the method for sample preparation is provided in a study by Khramova E.P. and colleagues [6]. The quantitation of phenolic compounds was carried out by high-performance liquid chromatography (HPLC) on an Agilent 1100 liquid chromatograph (Agilent Technologies, USA) equipped with a UV spectrophotometric detector and the ChemStation software for chromatographic-data analysis, according to a modified T.A. van Beek procedure [17], at the Central Siberian Botanical Garden SB RAS [18]. All the data were normalized to absolutely dry weight of the starting plant material. Because standard samples were unavailable for the quantitation of flavonol glycosides in *D. fruticosa* leaf extracts by HPLC, we analyzed free aglycons forming after acid hydrolysis of respective glycosides, with subsequent conversion of the data to the amounts of flavonol glycosides [17, 19].

The results showed that the leaf extracts from the plants contain at least 21 phenolic compounds. Ten compounds were identified, among them, six flavonol glycosides (hyperoside, isoquercitrin, avicularin, quercitrin, astragaline, and kaempferol-3-O-rutinoside), two aglycons (quercetin and kaempferol), and two ellagic tannins (ellagic acid and its glycoside). According to the UV spectra recorded in on-line mode, the other phenolic components (ID numbers 1–3, 9, 13, 15–19) were assigned flavonoid structures (Table 2).

It was found that the phenolic profiles of *D. fruticosa* leaves are similar among the populations being studied. In the leaf samples from all the analyzed populations, we detected hyperoside, isoquercitrin, ellagic acid and its glycoside, avicularin, astragaline, kaempferol-3-O-rutinoside, and quercetin. Additionally, in the samples from population Ol'khovaya (CP 1), quercitrin was present as a minor phenolic component, and the same was true for kaempferol in the samples from population Gorobetskaya (CP 4).

Quantitative differences in the accumulation of phenolic compounds were observed too. For instance, the highest concentration of hyperoside (5.10 mg/g) was detected in the leaves of plants from population Ol'khovaya (CP 1); the highest concentrations of isoquercitrin (3.84 mg/g), avicularin (4.10 mg/g), kaempferol-3-O-rutinoside (0.45 mg/g), and quercetin (0.21 mg/g) were registered in the leaves of plants from population Otradnaya (CP 2); and the highest concentration of astragalín (0.37 mg/g) was found in the leaves of plants from population Malokuril'skaya (CP 3; Table 2).

Table 2. Concentrations of phenolic compounds in the leaves of *D. fruticosa* (mg/g of absolutely dry weight).

Phenolic compounds	Coenotic populations			
	Primorsky Krai	Shikotan Island		
	Ol'khovaya, CP 1	Otradnaya, CP 2	Malokuril'skaya, CP 3	Gorobetskaya, CP 4
In aqueous ethanol extracts:				
Compound 1	1.67	– ¹	–	–
Compound 2	3.69	0.40	0.91	0.14
Compound 3	0.42	–	–	0.20
Hyperoside	5.10	2.16	0.85	0.48
Isoquercitrin	2.19	3.84	2.83	1.20
Ellagic acid	5.97	2.92	0.16	0.29
Ellagic acid glycoside	6.63	7.78	10.14	9.91
Avicularin	0.23	4.10	3.40	3.14
Compound 9	0.18	4.94	5.28	2.32
Quercitrin	0.05	–	–	–
Astragalín	0.31	0.29	0.37	0.25
Kaempferol-3-O-rutinoside	0.32	0.45	0.19	0.38
Compound 13	–	0.18	–	–
Quercetin	0.13	0.21	0.19	0.06
Compound 15	–	–	–	0.14
Compound 16	–	0.12	–	–
Compound 17	0.26	0.99	1.28	1.29
Compound 18	0.16	0.15	–	–
Compound 19	–	0.49	0.42	0.35
Kaempferol	–	–	–	0.06
Compound 21	–	2.19	4.94	7.70
Total content of phenolic compounds	27.3	31.6	31.0	27.9
Sum of tannins	12.6	10.70	10.30	10.20
<i>In hydrolysates:</i>				
Sum of flavonols ²	12.1	22.44	15.16	15.25
Including glycosides:				
quercetin	11.3	18.4	10.3	9.5
kaempferol	0.71	1.09	0.64	0.60
rhamnetin	–	2.69	4.04	5.02

Notes: ¹ concentration below the detection limit (0.1 mg/g), ² total concentration of flavonols is a sum of concentrations of flavonol glycosides and free aglycons after acid hydrolysis.

The total concentrations of phenolic compounds in the leaves were almost identical among the studied populations, and the highest level (31.6 mg/g) was detected in the

specimens from population Otradnaya on Shikotan Island (CP 3), whereas the lowest (27.3 mg/g) in population Ol'khovaya from Primorsky Krai (CP 1).

To analyze the concentrations of flavonol glycosides individually, we performed acid hydrolysis of the aqueous ethanolic extracts from *D. fruticosa* leaves.

The results showed that the total concentration of flavonols (consisting of a sum of flavonol glycosides and free aglycons after the acid hydrolysis) in the leaves of plants from population Otradnaya (CP 2) was 32–46% higher as compared with the other populations and was 22.4 mg/g, with the lowest level found in population Ol'khovaya (CP 1): 12.1 mg/g.

The differences among the plants from different habitats also manifested themselves in unequal proportions of aglycons in the leaf hydrolysates (Table 3). According to the analysis of aglycons that formed after the acid hydrolysis of *D. fruticosa* leaf extracts, three aglycons were identified in the hydrolysates: quercetin, kaempferol, and rhamnetin. Glycosides of quercetin and kaempferol were present in all the hydrolysates, whereas rhamnetin glycosides were undetectable in the leaf extract hydrolysates from population Ol'khovaya (CP 1, Primorsky Krai), where we noticed the highest concentration of quercetin glycosides: 95%. All the studied populations of *D. fruticosa* contain quercetin glycosides, and in the leaves of plant populations Malokuril'skaya (CP 3) and Gorobetskaya (CP 4), aside from the dominant levels of quercetin derivatives (69% and 63%), there were substantial amounts of rhamnetin glycosides: 26% and 33%, respectively. Concentrations of kaempferol glycosides proved to be nearly equal among the populations: 4% to 5% (Table 3).

The highest concentrations of glycosides of quercetin (18.4 mg/g) and of kaempferol (1.09 mg/g) were detected in the leaves of plant population Otradnaya (CP 2), i.e., 1.5–2.0-fold higher than that in the other populations. By contrast, the concentration of rhamnetin was higher in two populations: Malokuril'skaya (CP 3) and Gorobetskaya (CP 4) from Shikotan Island (4.04 and 5.02 mg/g, respectively; Table 2).

Table 3. The quercetin:kaempferol:rhamnetin ratio (%) in the leaf extract hydrolysates from *D. fruticosa*.

Ol'khovaya	Otradnaya	Malokuril'skaya	Gorobetskaya
95:5:0	83:5:12	69:4:26	63:5:33

Among the studied populations, there were also differences in the accumulation of ellagic tannins (total and individual tannins). For example, the total concentration of ellagic compounds in the *D. fruticosa* leaves from population Ol'khovaya (CP 1) (12.6 mg/g) was 1.2-fold higher than that in the three Shikotan Island populations, among which, the total levels of ellagic compounds were roughly the same (10.20–10.70 mg/g). The highest concentration of ellagic acid was registered in *D. fruticosa* leaves from population Ol'khovaya (CP 1): 5.97 mg/g. This is 2- to 37-fold higher relative to the three Kuril populations from Shikotan Island. In the plants of the Kuril populations, the concentration of a glycoside of ellagic acid turned out to be 1.1–1.5-fold higher than that in the samples from population Ol'khovaya (CP 1, Primorsky Krai; Table 2).

Thus, our results indicate that leaf phenolic profiles of *D. fruticosa* are similar among the four habitats and are represented by 21 compounds, but there are differences in the concentrations of the phenolic compounds. Six flavonol glycosides, two aglycons, and two ellagic tannins were identified. The highest total concentrations of phenolic compounds and of flavonols were revealed in the specimens of *D. fruticosa* present on Shikotan Island: population Otradnaya (CP 2) and Malokuril'skaya (CP 3). The highest total concentration of ellagic compounds was noted in the plants from population Ol'khovaya (CP 1, Primorsky Krai). These findings may be explained by differences in habitat conditions. Our study

shows that quercetin glycosides are dominant in leaf samples from all four analyzed populations in the south of the Russian Far East.

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