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RESEARCH PAPER

A chemotaxonomic study of phenolic compounds in the species of the genus *Dasiphora* (Rosaceae) from the Russian Far East and Eastern Siberia

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ABSTRACT

The profile and concentrations of phenolic compounds were determined in the leaves of five species and one variety of the genus *Dasiphora* Raf. from natural habitats of the Russian Far East and Eastern Siberia. It was found that the phenolic profile is specific for each taxon under study. The largest number of phenolic components was detected in *D. davurica* and *D. mandshurica*, and the lowest in *D. parvifolia* and *D. davurica* var. *flava*. For each studied taxon, a distinct set of highly accumulated phenolic components was identified. According to paired-affinity and group affinity calculations, species *D. mandshurica* and *D. davurica* are the closest relatives. Species specificity was evaluated on the basis of aglycon structure of flavonol glycosides. *D. fruticosa*, *D. parvifolia*, and *D. davurica* showed a tendency of increased accumulation of quercetin glycosides, whereas *D. davurica* var. *flava*, *D. gorovoii*, and *D. mandshurica* mainly accumulate the glycosides of rhamnetin. *D. parvifolia* and *D. gorovoii* are characterized by the highest content of phenolic compounds (in total and by groups), whereas *D. davurica* is distinguished by the high content of ellagic compounds.

Keywords: *Dasiphora*, Russian Far East, Eastern Siberia, chemotaxonomic markers, phenolic compounds

РЕЗЮМЕ

Андышева Е.В., Храмова Е.П. Хемотаксономический анализ фенольных соединений представителей рода *Dasiphora* (Rosaceae) российского Дальнего Востока и Восточной Сибири. Приведены результаты исследования состава и содержания фенольных соединений в листьях растений пяти видов и одной разновидности рода *Dasiphora* Raf. Установлено, что для каждого исследованного вида характерен свой фенольный профиль. Наибольшее число компонентов фенольной структуры присутствовало у растений *D. davurica* и *D. mandshurica*, наименьшее – у *D. parvifolia* и *D. davurica* var. *flava*. Для каждого исследованного вида установлено повышенное накопление определенного набора компонентов. По коэффициентам парного и группового сходства наиболее близки виды *D. mandshurica* и *D. davurica*. Отмечена видоспецифичность в зависимости от агликоновой структуры флавонолгликозидов. *D. fruticosa*, *D. parvifolia* и *D. davurica* свойственно повышенное накопление гликозидов кверцетина, *D. davurica* var. *flava*, *D. gorovoii* и *D. mandshurica* – гликозидов рамнетина. По наибольшему содержанию фенольных соединений (суммарному и по группам) выделены виды *D. parvifolia* и *D. gorovoii*, по суммарному содержанию эллаговых соединений – *D. davurica*.

Ключевые слова: род *Dasiphora*, фенольные соединения, хемотаксономия, российский Дальний Восток, Восточная Сибирь

Genus *Dasiphora* Raf. (≡ *Pentaphylloides* Hill) belongs to the family Rosaceae Juss. In the Asian part of Russia, there are five species and one variety: *D. fruticosa* (L.) Rydb., *D. parvifolia* (Fisch. ex Lehm.) Juz., *D. mandshurica* (Maxim.) Juz., *D. davurica* (Nestler) Kom. (Wolf 1908, Cherepanov 1995, Baikov 2012, IPNI 2015), *D. gorovoii* Pshennikova, and *D. davurica* var. *flava* (Vorosch.) Gorovoj, Pshenn. et S. Volkova (Pshennikova 2006, 2016, Baikov 2012).

Despite the low species diversity of the genus, the problems of its classification are still unsolved. The taxonomic details of the genus are always specified due to the revision of the number of affiliated species. Chemical methods may be used in addition to the classical methods of systematics to resolve its classification.

Phenolic compounds are valuable chemotaxonomic markers and may be utilized in systematics to resolve disputes and determine phylogenetic connections among taxa (Harborne 1977, Julkunen-Tiitto et al. 1996, Robards & Antonovich 1997, Laitinen 2003, Williams et al. 2003, Wink 2003, Vysochina 2004, Raucher 2006).

The research on flavonol aglycones of the genus *Potentilla* showed species specificity of their biochemical profile and the suitability of their use for more accurate determination of taxonomic affiliations of these plants on different levels (Bate-Smith 1961), as confirmed later by Tril' (1977). Among the Siberian species of the genus *Dasiphora*, the differences in the concentrations and profile of phenolic compounds were found by Khramova (2013).

This paper focus on comparison of the phenolic compositions within the genus *Dasiphora* across the large part of the eastern Asian Russia, to find species with high phenolic content, and to test if the phenolic composition can serve as chemotaxonomic markers.

MATERIAL AND METHODS

The materials for this chemotaxonomic study were specimens (leaves) of five species and of one variety of the genus *Dasiphora* from 62 coenotic populations (CPs) of the Russian Far East and Eastern Siberia. *D. fruticosa* specimens were collected in 52 geographically remote habitats: two in the Magadan Region (CPs 1 and 2), seven in the Sakha Republic (Yakutia) (CPs 3–9), two in the Irkutsk region (CPs 10 and 11), four in the Republic of Buryatia (CPs 12–15), five in the Zabaykalsky Territory (CPs 17–20), 20 in the Amur region (CPs 22–41), two in the Jewish Autonomous region (CPs 42 and 43), five in the Khabarovsk Territory (CPs 44–48), four on the Kuril Islands (CPs 60–62), one specimen in the Primorsky Territory (CP 49), and one on the Sakhalin Island (CP 59). *D. davurica* specimens were collected in four (CPs 50–53) and *D. mandshurica* specimens in three natural populations (CPs 56–58). The specimens of *D. gorovoii* (CP 55) and *D. davurica* var. *flava* (CP 54) were represented by a single population each from the Primorsky Territory. The single population of *D. parvifolia* (CP 16) is located in the Republic of Buryatia (Fig. 1).

To identify and quantify phenolic compounds (total content, by groups, and individual components), a sample from 30 individual plants on average was taken in the mass blooming phase. Annual shoots 15–20 cm long with leaves were cut off evenly across the crown, and the leaves were separated and air dried completely. Aqueous ethanol extracts of the vegetation samples were prepared according to the method described by Ermakov et al. (1987). Then, to rid the extracts of hydrophilic impurities, the solid-phase extraction method was used (Sychyov 2005). The detailed description of sample preparation may be found in the study by Khramova et al. (2013). The analysis of the profile and concentrations of phenolic compounds in the samples under study was carried out by high-performance liquid chromatography (HPLC) on an Agilent 1200 liquid chromatograph (Agilent Technologies, USA) with a diode-matrix detector, an autosampler, and the chromatography data processing software ChemStation by the method of van Beek (2002) with a modification by Khramova (2014). The chromatographic separation was conducted at 26°C on a Zorbax SB-C18 Column (4.6 × 150 mm, 5 µm internal diameter) with the Agilent Guard Column Hardware Kit. Isocratic elution in a methanol – 0.1 % aqueous orthophosphoric acid (H_3PO_4) system (31:69) lasted for 27 min. The chromatographic analysis was performed in gradient elution mode. In the mobile phase (methanol plus the 0.1 % aqueous solution of H_3PO_4), the proportion of methanol was changed from 33 % to 46 % during 11 min, then from 46 % to 56 % during the next 12 min, and from 56 % to 100 % during the next 4 min. The flow rate was set to 1 ml/min. The sample injection volume was 5 µl, and the analytical wavelengths were 254, 270, 290, 340, 360 and 370 nm. The total content of phenolic compounds was calculated as the sum of chromatographic peak areas at λ = 360 nm, because for many most active flavonoids, the maxima of absorption are in the long-wavelength area (362 ± 14 nm), which allows us to distinguish them easily from other classes of plant substances. The total content of tannins was calculated by summing up the concentrations of ellagic acid and of its glycoside. Because of the absence of available standard samples and complicated conditions for separation of flavonol glycosides (quercetin, kaempferol, and rhamnetin glycosides were isolated separately) from the leaf extracts by the HPLC method, the analysis of free aglycones was carried out instead. Free aglycones were produced by the acid hydrolysis of the corresponding glycosides with subsequent recalculation (van Beek 2002, Yuriev et al. 2003). The chromatographic analysis was performed in gradient elution mode. In the mobile phase (methanol plus the 0.1 % aqueous solution of H_3PO_4), the proportion of methanol was changed from 45 % to 48 % during 18 min. For recalculation of aglycone concentration in a corresponding glycoside, some coefficients from literary data were employed: 2.504 for quercetin and 2.588 for kaempferol (van Beek 2002, Yuriev et al. 2003). The recalculation of rhamnetin concentration was done likewise for quercetin.

All the data were processed in Excel 7.0 and Statistica 10.0 software. For species *D. fruticosa*, *D. davurica*, and *D. mandshurica*, the means and standard deviations were calculated for all the specimens of the selected populations. For taxa *D. parvifolia*, *D. davurica* var. *flava*, and *D. gorovoii*, which are represented by a single population each, the standard deviations were calculated to evaluate reproducibility of the measurements. The relative standard deviation of repetition (or reproducibility of the measurements) in the phenolic-compound analysis was σ = 0.011.

Based on chromatographic data, the coefficients of paired affinity (PA)
and group affinity (GA)

$$PA = \frac{\text{spots in common for species 1 and 2}}{\text{total spots in 1+2}} \times 100$$

$$GA = \Sigma PA + 100$$

(Ellison et al. 1962, Suresh & Pandey 2003) were calculated.

To determine the similarity in the aglycone content of hydrolysates of leaf extracts, cluster analysis of the plants was conducted by Ward's method. Euclidean distance was calculated as a measure of variation.

RESULTS AND DISCUSSION

Quantitation of phenolic content showed that in the leaf extracts of the species under study, the concentration of at least 34 compounds was determined successfully (Table 1). Based on the spectral data (ultraviolet spectrophotometry and mass spectrometry), a comparison of the substances' peak retention times with those of the standard samples and with literary data (Khramova & Shkel 1999, Miliauskas et al. 2004, Khramova 2016) detected seven flavonol glycosides (hyperoside, isoquercitrin, rutin, avicularin, quercetrin, astragalin,

and kaempferol-3-O-rutinoside), three aglycones (quercetin, kaempferol, and rhamnetin), and two ellagic compounds (ellagic acid and its glycoside). The rest of the compounds (1–4, 10, 12, 16–21, 23–28, 30–33) were not identified, but during the chromatography run in "on-line" mode, their ultraviolet spectra were registered. For the unidentified compounds, absorption in the ultraviolet-visible region was typical; at the same time, the absorption spectrum contained two bands: one of them in a shorter-wave region (250–290 nm: band II), and the other in a longer-wave region (340–380 nm: band I). According to these data, all these leaf extract components belong to the flavonol family (Khramova 2016).

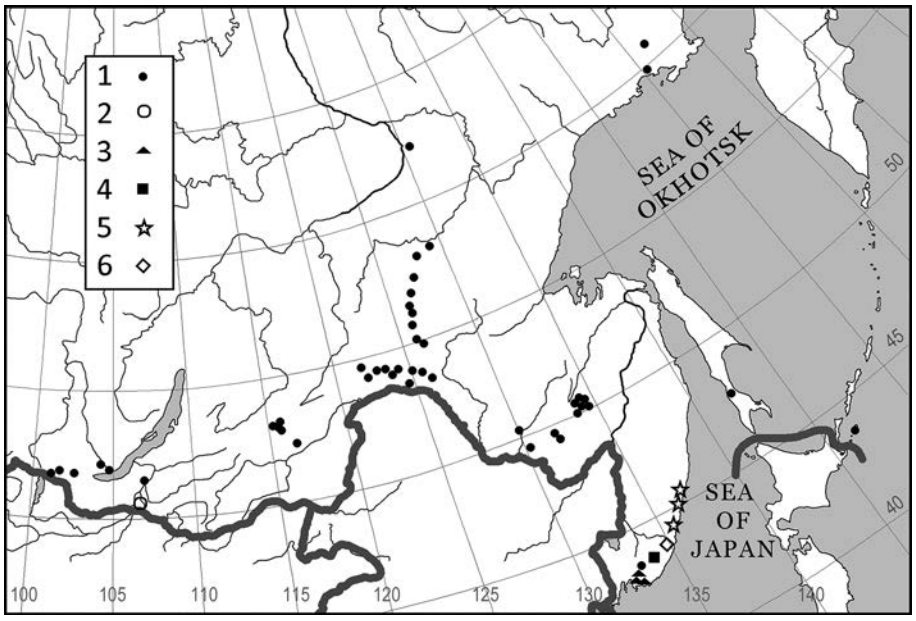

Figure 1 The scheme of specimen collections in the eastern part of Asian Russia: 1 – *Dasiphora fruticosa*, 2 – *D. parvifolia*, 3 – *D. davurica*, 4 – *D. gorovoii*, 5 – *D. mandshurica*, 6 – *D. davurica* var. *flava*

A chromatographic comparison of the aqueous ethanol extracts from the leaves revealed that the greatest number of phenolic components was present in *D. fruticosa* (33 compounds), and the lowest number in *D. parvifolia* and *D. davurica* var. *flava* (19 phenolic compounds; Table 1). Components 1, 2, 12, and 25 as well as hyperoside, isoquercitrin, ellagic acid and its glycoside, avicularin, quercitrin, astragalin, and quercetin were detected in all the species under study, whereas component 3 was not found only in *D. gorovoii* leaves. In *D. fruticosa*, *D. davurica*, and *D. davurica* var. *flava*, an extra component (number 4) was discovered, and in *D. fruticosa*, components 10, 17, and 18. In *D. parvifolia*, components 23, 26, and 31 were not detected. Rutin, kaempferol-3-O-rutinoside, and component 16 were not found in the samples of *D. davurica* var. *flava* and in leaves of *D. gorovoii* in contrast to the rest of the taxa. Components 19 and 20 were found only in *D. fruticosa* and *D. gorovoii* leaves; component 21 only in the leaves of *D. fruticosa*, *D. gorovoii*, and *D. mandshurica*; component 28 in *D. fruticosa* and *D. mandshurica* leaves; and component 33 in *D. fruticosa* and *D. parvifolia* leaves. Component 24 was not detected in either *D. davurica* var. *flava* leaves or *D. davurica* leaves, component 27 in the leaves of *D. davurica* var. *flava*, and component 30 in either *D. parvifolia* leaves or *D. gorovoii* leaves. Free kaempferol in trace amounts was detected in the leaves of *D. fruticosa*, *D. davurica*, and *D. mandshurica*. Free rhamnetin was not found in either *D. fruticosa* leaves or *D. parvifolia* leaves, and component 32 in either *D. parvifolia* samples or *D. davurica* var. *flava* samples (Table 1).

On the basis of the chromatographic data, the coefficients of paired and group affinity (Ellison et al. 1962, Suresh & Pandey 2003) were calculated next.

The highest similarity judging by 34 components (86 %) was observed between species *D. davurica* and *D. mandshurica* (Table 2). High paired affinity (76 %) was noted within two pairs of taxa: *D. fruticosa*–*D. mandshurica* and *D. davurica*–*D. davurica* var. *flava*. The other taxon pairs had a coefficient of similarity less than 76 %. The lowest similarity in phenolic content (52 %) was observed between *D. parvifolia* and two taxa: *D. gorovoii* and *D. davurica* var. *flava*. Group affinity of the species was the lowest in *D. parvifolia* (489) and the highest in *D. mandshurica* (559). Thus, the closest relation was noted between *D. mandshurica* and *D. davurica* according to the coefficient of paired affinity for the concentrations of phenolic components (Table 2).

Analysis of individual phenolic compounds' concentrations in the leaves of all the analyzed plants also uncovered species specificity (Table 1). Thus, in *D. fruticosa* leaves, the dominant phenolic compounds were rutin, kaempferol-3-O-rutinoside, components 3, 4, 10, 17, 18, 25, 30, and 33, whereas in *D. parvifolia* leaves, the dominant phenolic compounds were hyperoside, ellagic acid, and components 1, 2, and 12 (Table 1). *D. davurica* var. *flava* leaves accumulated substantial quantities of quercetin (up to 0.19 mg/g), component 26 (2.10 mg/g), and component 31 (1.16 mg/g). In *D. davurica*, we detected high concentrations of kaempferol-3-O-rutinoside and component 16 (0.33 and 0.48 mg/g, respectively). In *D. gorovoii* leaves, the dominant phenolic compounds were isoquercitrin (2.62 mg/g), avicularin (2.68 mg/g), quercitrin (1.93 mg/g), quercetin (0.19 mg/g), and components 19–21, 23, 27, and 32 (Table 1). *D. mandshurica* was noteworthy because of high accumulation of astragalin (0.49 mg/g), kaempferol (0.10 mg/g), rhamnetin (0.31 mg/g), component 24 (0.94 mg/g) and component 28 (1.60 mg/g). A relatively high concentration of ellagic acid glycoside was detected in two species: *D. fruticosa* (9.00 mg/g) and *D. davurica* (9.47 mg/g; Table 1).

There were substantial differences in the total phenolic content of leaves among the analyzed taxa (Table 3). The highest accumulation of phenolic compounds in the leaves was observed in *D. fruticosa* (27 mg/g) and *D. parvifolia* (26 mg/g), and the lowest in *D. mandshurica* and *D. davurica* var. *flava* (14 mg/g each). It was found that in *D. davurica* and

Table 1. The content of phenolic compounds in the leaves of *Dasiphora* species (mg/g of absolutely dry weight).

Phenolic compounds	*D. fruticosa* (52 CPs)	*D. parvifolia* (1 CP)	*D. davurica* var. *flava* (1 CP)	*D. davurica* (4 CPs)	*D. gorovoii* (1 CP)	*D. mandshurica* (3 CPs)
Compound 1	0.64±0.42	1.93±0.02	0.37±0.00	0.25±0.09	0.33±0.00	0.18±0.02
Compound 2	1.72±1.06	2.40±0.03	0.19±0.00	0.31±0.11	0.43±0.00	0.14±0.02
Compound 3	0.87±0.56	0.49±0.01	0.11±0.00	0.30±0.13	b.l.	0.05±0.00
Compound 4	0.30±0.07	b.l.	0.15±0.00	0.30±0.13	b.l.	b.l.
Hyperoside	2.04±1.22	4.47±0.05	0.90±0.01	1.69±0.54	1.20±0.01	0.53±0.32
Isoquercitrin	2.30±1.06	0.73±0.01	1.01±0.01	1.18±0.33	2.62±0.03	0.90±0.14
Rutin	0.34±0.20	0.18±0.00	b.l.	0.21±0.04	b.l.	0.12±0.00
Ellagic acid	3.00±1.80	7.10±0.08	1.22±0.01	3.01±0.84	2.05±0.02	0.46±0.15
Ellagic acid glycoside	9.00±4.12	1.32±0.01	0.63±0.01	9.47±2.00	0.15±0.00	0.78±0.33
Compound 10	0.86±0.43	b.l.	b.l.	b.l.	b.l.	b.l.
Avicularin	1.53±0.91	1.66±0.02	2.19±0.02	0.84±0.14	2.68±0.03	2.32±1.29
Compound 12	0.93±0.56	2.36±0.03	1.31±0.01	0.77±0.13	1.27±0.01	0.73±0.15
Quercitrin	0.31±0.19	0.46±0.01	0.62±0.01	0.49±0.29	1.93±0.02	0.33±0.02
Astragalin	0.32±0.20	0.39±0.00	0.08±0.00	0.22±0.15	0.22±0.00	0.49±0.30
Kaempferol-3-O-rutinoside	0.33±0.19	0.12±0.00	b.l.	0.33±0.20	b.l.	0.10±0.06
Compound 16	0.23±0.08	0.19±0.00	b.l.	0.48±0.24	b.l.	0.12±0.00
Compound 17	0.25±0.13	b.l.	b.l.	b.l.	b.l.	b.l.
Compound 18	0.21±0.09	b.l.	b.l.	b.l.	b.l.	b.l.
Compound 19	0.18±0.09	b.l.	b.l.	b.l.	0.25±0.00	b.l.
Compound 20	0.22±0.08	b.l.	b.l.	b.l.	0.34±0.00	b.l.
Compound 21	0.39±0.23	b.l.	b.l.	b.l.	0.74±0.01	0.63±0.00
Quercetin	0.13±0.08	0.14±0.00	0.19±0.00	0.12±0.03	0.19±0.00	0.12±0.06
Compound 23	0.23±0.11	b.l.	0.24±0.00	0.16±0.01	0.38±0.00	0.19±0.04
Compound 24	0.40±0.24	0.16±0.00	b.l.	b.l.	0.13±0.00	0.94±0.01
Compound 25	1.70±1.02	1.39±0.02	0.78±0.01	0.33±0.06	1.64±0.02	1.57±0.94
Compound 26	0.63±0.38	b.l.	2.10±0.02	0.20±0.09	0.15±0.00	0.39±0.14
Compound 27	0.53±0.31	0.53±0.01	b.l.	0.33±0.15	2.12±0.02	1.63±0.68
Compound 28	1.29±0.77	b.l.	b.l.	b.l.	b.l.	1.60±0.00
Kaempferol	0.06±0.04	b.l.	b.l.	0.06±0.00	b.l.	0.10±0.03
Compound 30	1.67±1.00	b.l.	0.27±0.00	0.19±0.08	b.l.	0.42±0.08
Compound 31	0.30±0.18	b.l.	1.16±0.01	0.18±0.07	0.50±0.01	1.00±0.13
Compound 32	0.13±0.01	b.l.	b.l.	0.15±0.00	3.65±0.04	0.13±0.01
Compound 33	0.43±0.26	0.36±0.00	b.l.	b.l.	b.l.	b.l.
Rhamnetin	b.l.	b.l.	0.23±0.00	0.08±0.00	0.18±0.00	0.31±0.03
Number of compounds	**33**	**19**	**19**	**25**	**22**	**27**

Notes: the data are presented as the mean value ± standard deviation; C ≥ 1.0 mg/g: the main component; 1.0 ≤ C ≤ 0.1 mg/g: a minor component; C ≤ 0.1 mg/g: trace amounts; b.l.: concentration below the detection limit (0.01 mg/g).

Table 2. Paired affinity (%) and group affinity (GA [%], last column) of *Dasiphora* species according to the profiles of phenolic compounds.

Species	*D. fruticosa*	*D. parvifolia*	*D. davurica* var. *flava*	*D. davurica*	*D. gorovoii*	*D. mandshurica*	GA
D. fruticosa	100	58	53	71	62	76	520
D. parvifolia		100	52	63	52	64	489
D. davurica var. *flava*			100	76	64	64	509
D. davurica				100	62	**86**	558
D. gorovoii					100	69	509
D. mandshurica						100	**559**

Table 3. The concentration of phenolic compounds (total and by groups) in the leaves of *Dasiphora* species (mg/g of absolutely dry weight).

Phenolic compounds	*D. fruticosa*	*D. parvifolia*	*D. davurica* var. *flava*	*D. davurica*	*D. gorovoii*	*D. mandshurica*
Total content of phenolic compounds	27±7	26±0.3	14±0.2	21±3	23±0.3	14±5
including glycosides:						
quercetin	13.2±4.5		4.2±0.0	7.4±1.2	7.7±0.1	3.3±1.8
kaempferol	1.1±0.5	8.9±0.1	0.1±0.0	0.3±0.1	0.3±0.0	1.6±1.0
rhamnetin	2.9±1.7	1.5±0.0	3.8±0.0	0.4±0.3	11.3±0.1	3.8±1.3
Sum of flavonols	17.0±5.2	9.3±0.1	8.6±0.1	8.2±1.1	19.7±0.2	9.2±4.8
Sum of tannins	11.8±5.0	8.4±0.1	1.8±0.0	12.5±2.7	2.2±0.0	1.2±0.3

D. gorovoii leaves, the total phenolic content was at an intermediate level (from 21 to 23 mg/g).

In the plant leaves, the proportion of some flavonols in the total phenolic content varied from 34 % to 85 % among the taxa being studied. The highest concentration of flavonols was found in *D. gorovoii* leaves (19.7 mg/g), with the close second being *D. fruticosa* leaves (17.0 mg/g). The total content of flavonols in the leaves of three taxa – *D. parvifolia*, *D. mandshurica* and *D. davurica* var. *flava*, was virtually the same (9.3–8.6 mg/g, respectively). *D. davurica* leaves showed the lowest total flavonol content (8.2 mg/g; Table 3).

Furthermore, among the studied taxa, there were differences in the aglycone profile of leaf extract hydrolysates. In the analysis of aglycones, which are generated by the acidic hydrolysis of glycosides, three compounds were detected: quercetin, kaempferol, and rhamnetin. They were identified virtually in all the taxa under study except for *D. parvifolia*, in which rhamnetin was not detectable (Table 4). Quercetin derivatives were dominant in *D. davurica*, *D. parvifolia*, and *D. fruticosa* leaves (91 %, 87 %, and 78 %, respectively), whereas rhamnetin derivatives were dominant in the samples of *D. gorovoii* and *D. mandshurica* (58 % and 47 %). Meanwhile, it was noted that in *D. gorovoii* and *D. mandshurica* leaves, the proportion of quercetin derivatives was also high (40 % and 37 %, respectively). In *D. mandshurica* and *D. parvifolia*, kaempferol derivatives were present in considerable amounts (17 % and 13 %, respectively). The proportion of rhamnetin glycosides was high in the leaves of *D. davurica* var. *flava* (47 %) but low in *D. davurica* (only 5 %). It is worth noting that in *D. gorovoii* leaves, rhamnetin derivatives were dominant (58 %), thus confirming the similarity of *D. gorovoii* with *D. mandshurica*. On the other hand, in *D. gorovoii* leaves, the proportion of kaempferol derivatives was minimal (2 %), making this species similar to *D. davurica*. This finding may be a confirmation of the hybrid origin of *D. gorovoii*: from *D. mandshurica* and *D. davurica*. Thus, in the leaves, the differences in the profile of quercetin, kaempferol, and rhamnetin derivatives reflect interspecies differences among the plants under study.

To determine similarities in the aglycone content of leaf extract hydrolysates, cluster analysis of the plants by Ward's method was performed next. Euclidean distance was calculated as a measure of variation. In the dendrogram, the leaf samples of the studied species yielded two clusters (Fig. 2).

The first cluster included *D. mandshurica*, *D. gorovoii*, and *D. davurica* var. *flava*. The latter and *D. gorovoii* were found to be the closest species by aglycone content, and *D. mandshurica* was close to them. The second cluster included *D. fruticosa* and *D. parvifolia*, and *D. davurica* showed the least similarity with them. Perhaps the differences of these three species from the others are related to the high concentration of quercetin and relatively low concentration of rhamnetin in *D. fruticosa* and *D. davurica* or to the absence of rhamnetin (methylated quercetin) in *D. parvifolia*. In the two species and one variety in the first cluster (*D. mandshurica*, *D. gorovoii*, and *D. davurica* var. *flava*), a relatively high concentration of the methylated form (rhamnetin) was found. The presence of the methylated form is an indication of evolutionary species superiority (Bate-Smith 1976, Harborne 1977, Vysochina 2004).

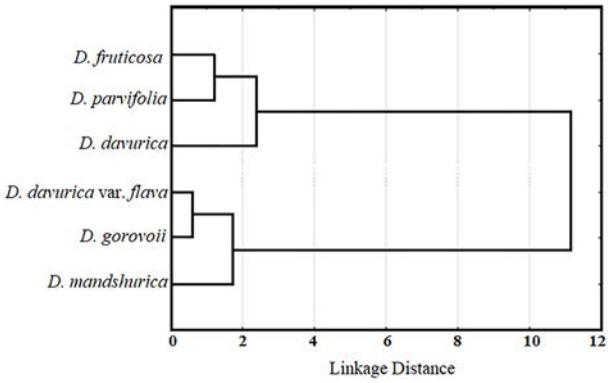

Figure 2 The dendrogram of *Dasiphora* species similarity in the aglycone content of leaf extract hydrolysates (Ward's method, Euclidian distances)

Table 4. The quercetin : kaempferol : rhamnetin ratio (%) in the leaf extract hydrolysates from *Dasiphora* species.

Species	Ratio
D. fruticosa	78:06:16
D. parvifolia	87:13:00
D. davurica var. *flava*	51:01:47
D. davurica	91:04:05
D. gorovoii	40:02:58
D. mandshurica	37:17:47

In addition, there were differences in the accumulation of ellagic compounds. The highest total ellagic content was found in *D. davurica* leaves (12.5 mg/g). Slightly lower total ellagic content was noted in the leaves of two species: *D. fruticosa* and *D. parvifolia* (11.8 and 8.4 mg/g, respectively), whereas the lowest was detected in the samples of *D. mandshurica*, *D. davurica* var. *flava*, and *D. gorovoii* leaves (1.2, 1.8, and 2.2 mg/g, respectively; Table 3). The analysis of individual ellagic compounds indicated that in *D. fruticosa* leaves, the content of ellagic acid (3.0 mg/g) was lower than that of ellagic acid glycoside (9.0 mg/g). In *D. parvifolia*, a different pattern was observed: dominance of ellagic acid among ellagic compounds (7.1 mg/g) and the opposite situation for its glycoside (1.3 mg/g). In *D. davurica* var. *flava* and *D. gorovoii*, ellagic acid content was higher (1.2 and 2.0 mg/g) than the content of ellagic acid glycoside (0.6 and 0.2 mg/g, respectively). On the contrary, in *D. davurica* (the highest total ellagic content) and in *D. mandshurica* (the lowest total ellagic content), the concentration of ellagic acid was the lowest among ellagic compounds (3.0 and 0.5 mg/g), whereas the concentration of ellagic acid glycoside was the highest (9.5 and 0.8 mg/g; Table 1).

CONCLUSION

The phenolic profiles of *Dasiphora* species show species specificity and may be used to determine taxonomic affiliation of the plants. The largest numbers of phenolic compounds were detected in *D. davurica* and *D. mandshurica* (25 and 24 compounds, respectively), and the lowest in *D. parvifolia* and *D. davurica* var. *flava* (19 compounds). The highest paired affinity in terms of the group of phenolic compounds was noted between *D. mandshurica* and *D. davu-*

rica. The highest group affinity (in terms of concentrations of phenolic components in the taxa under study) was observed between *D. mandshurica* and *D. davurica*. The lowest group affinity was observed between two pairs of taxa: *D. parvifolia–D. gorovoii* and *D. parvifolia–D. davurica* var. *flava*.

In the leaves, the differences in the concentration and profile of phenolic compounds were also studied in detail among the plants. Each analyzed taxon was characterized by high accumulation of specific components: rutin and kaempferol-3-O-rutinoside in *D. fruticosa*; hyperoside and ellagic acid in *D. parvifolia*; quercetin in *D. davurica* var. *flava*; ellagic acid glycoside in *D. davurica*; isoquercitrin, avicularin, quercitrin, and quercetin in *D. gorovoii*; and astragalin, kaempferol, and rhamnetin in *D. mandshurica*. Species specificity turned out to be based on the aglycone structure of flavonol glycosides. In *D. fruticosa*, *D. parvifolia*, and *D. davurica*, there was high accumulation of quercetin glycosides, whereas in *D. davurica* var. *flava*, *D. gorovoii*, and *D. mandshurica*, there was high accumulation of the glycosides of rhamnetin. The highest content of phenolic compounds (total and by groups) was detected in *D. parvifolia* and *D. gorovoii*, and the highest total ellagic content in *D. davurica*.

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