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Component Composition of Flavonols and Their Content in *Aconogonon alpinum* (All.) Schur Growing in the Altay

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Abstract

Results of the investigation of composition and content of the flavonol complex in *Aconogonon alpinum* (All.) Schur which is widespread in the Altay are reported. The above-ground part of the plants contains glycosides: astragalin, quercetrin, avicularin, hyperoside, quercetin-3,7-diglucoside, rutin, myricitrin, and aglycones: kaempferol, quercetin and myricetin. The concentration and qualitative composition of flavonols in the plants collected in the phase of mass blooming in the Ust-Kan District of the Republic of Altay (meadow steppe, 293 km along the road to Tuekta village) were investigated by means of HPLC. Due to the high content of flavonols (up to 10.35 %) and the rich qualitative composition, *A. alpinum* can be used as a source of these very valuable compounds with high biological activity and low toxicity.

Key words: *Aconogonon alpinum* (All.) Schur, flavonols, glycosides, aglycones, HPLC, the Republic of Altay

INTRODUCTION

Plants containing flavonoids are a source of valuable anti-inflammatory, capillary tonic, choleretic, antitumor, immunomodulating and other medicinal agents. The extensive data on radioprotective, spasmolytic, anti-oxidant effect of flavonoids and on their influence on the alimentary tract and liver are published [1–7]. The main feature of flavonoids and other plant polyphenols is their action to the capillaries, resulting in a decrease of capillary wall permeability [3, 8]. In the recent decades, special attention of researchers has been concentrated on the antioxidant effects of flavonoids and their ability to trap the free radicals causing many serious humans diseases and to remove them from the body [9, 10]. Rather low toxicity of flavonoids along with their selective pharmacological effects on the human organism allow one to increase the use of this group of compounds for the development of new medications.

Flavanols comprise the most numerous and abundant class of flavonoids. Flavonols in the free form are seldom in plants, they are represented mainly by O and C glycosides. The most common flavonol glycosides are derivatives of quercetin, kaempferol and isorhamnetin. Due to the high biological activity, flavonols are subjected to various biochemical changes and participate in several physiological processes in plant tissues. It is revealed that together with ascorbic acid, they are involved in enzymatic processes of oxidation and reduction [3].

A wide range of biological activities of flavonoids attracts attention of researchers to the taxons rich in these substances, for example the species of the *Aconogonon* L. (Polygonaceae). In extratropical Asia and western part of North America there are about 35 species of this genus, some of them are used as ornamental, fodder and technical plants [11], and 13 species grow in Siberia [12].

The object of our investigation is a *Aconogonon alpinum* (All.) Schur (= *Polygonumalpinum* All., *P. undulatum* Murr., *P. polymorphum* Ledeb.). This is a perennial grassy plant with panicle-shaped inflorescence up to 120 cm

found in various planting zones in dry meadows, forest glades, stony slopes and cliffs, in the steppes, on the banks of rivers and streams, in the mountains – from the forest zone to the alpine one. *A. alpinum* has an extensive disjunctive area, covering almost the entire territory of the former USSR, Mongolia, Japan, China, Western Europe mountains, North America [13].

Flavonol glycosides constitute the main part of phenolic compounds set of *A. alpinum* [14–17]. These substances are considered to determine the anti-inflammatory and antioxidant action of *A. alpinum* [17]. A little information about the presence of flavonoids in the plants of this species is known, especially for the part of its range located in Siberia. To identify flavonols, in our previous studies we used the methods described in [18]: two-dimensional chromatographic separation of the components of the flavonol complex on the Filtrak No. 15 paper, the extraction of different substances, acid hydrolysis, UV spectroscopy in the presence of complexing and ionizing additives, as well as comparison of the physical constants of substances with the data in literature [16]. Identification of aglycones was performed according to standard methods.

The goal of the present work is to study flavonols composition and content in some organs and over ground parts of *Aconogonon alpinum* growing in the Altay by the chromatographic methods.

EXPERIMENTAL

The *A. alpinum* plants, collected in different years (1995–2008) during the expeditions to the Altay were the test material. The organs of the plants were separated, dried in ventilated compartments and analyzed for the content of flavonols.

Plants gathered in the Ust-Kan District of the Republic of Altay (meadow steppe, 293 km along the road to the village Tuekta) in July 2008, were studied for identification of aglycones and main flavonol glycosides, and the determination of their contents.

The methods used for the quantitative determination of flavonoids were the following: chromato-spectrophotometric method based on preliminary separation of flavonols by two-dimensional chromatography on the paper [19], the method of V. V. Belikov and M. S. Shraiber [20] and high performance liquid chromatography (HPLC) [21] with an Agilent 1100 liquid chromatograph (Agilent Technologies, USA).

Chromato-spectrophotometric method

Air-dry raw material accurately weighed (0.1–0.5 g) and shredded to 1 mm particles was placed into 100 mL flask, 30 mL of 40 % ethanol was added, then the mixture was heated in a water bath under reflux for 30 min. The extract was filtered. The second extraction was performed with 20 mL of ethanol for 15 min. After filtration, the residue in the flask and on the filter was washed with 5 mL of ethanol. Extraction of flavonols is nearly exhaustive under these conditions. The joint filtrate was thickened in porcelain cups in a draft hood or in a rotary evaporator to the volume of 2–3 mL. The obtained extracts were examined by chromatography on the paper (Filtrak No. 15) in solvent systems: direction I – isopropyl alcohol/formic acid/water (2 : 5 : 5), direction II – *n*-butyl alcohol/acetic acid/water (40 : 12 : 28) – with subsequent spectrophotometry of eluates. Then 0.10–0.15 mL of the extract was applied on a sheet of chromatographic paper, depending on the weight of raw material sample and extract quantity. Each sample was assayed in triplicate. Flavonols were eluted by 40 % ethanol in 0.5 mL portions up to a final volume of at least 3 mL. The optical density of eluates was determined with a SF26 spectrophotometer at the wavelength of 360 nm, because the absorption maxima of flavonols in the species under investigation are within the wavelength region 355–365 nm of the UV range. Ethyl alcohol (40 %) was used as the reference.

Quantity calculation of flavonol glycosides (in percent by mass of air-dry raw material) was performed using equation

$$X = DV_1V_2\,100/MV_3 \cdot 1000$$

where D is flavonol content in 1 mL of test solution, found from the calibration graph constructed for rutin, mg; V_1, V_2, V_3 are the volumes of extract, eluate and extract placed on

the chromatogram, respectively, mL; *M* is the mass of air-dry raw material, g.

To construct calibration plot, we used the solution of rutin (1 mg/mL) in 40 % ethanol. It was subjected to chromatography and elution under the conditions described above for the separation of flavonol complex in extracts under investigation. The total content of flavonols was calculated by summing the individual components of the flavonol complex of the sample. The relative error of this method is ±1.39 % [19].

High performance liquid chromatography

A liquid chromatograph Agilent 1100 (Agilent Technologies, USA) with UV spectrophotometric detector and ChemStation software for processing the chromatographic data [21] was used.

Chromatographic conditions: column filled with reversed-phase sorbent Diasfer 110 C16, 2.0×150 mm, isocratic elution in methanol–0.1 % H_3PO_4 system (32 : 68) for 27 min. Further chromatography was performed using a gradient mode of elution. In the mobile phase, methanol content in the aqueous solution of orthophosphoric acid (0.1 %) varied from 32 to 46 % in 11 min, then from 46 to 56 % in the next 12 min. Eluent flow rate was 0.25 mL/min, column temperature 35 °C, the volume of injected sample 3 μL. Detection was performed at λ = 360 nm. Detailed description of the sample preparation method, analysis and calculations is given in [22].

To determine the content of flavonol glycosides (glycosides of quercetin, kaempferol, and myricetin separately) by the HPLC method, the analysis of aglycones – quercetin, kaempferol and myricetin formed after acid hydrolysis of the corresponding glycosides was performed. For the acid hydrolysis of glycosides, 0.5 mL of HCl (with a concentration of 2 mol/L) was added to 0.5 mL of ethanol/water extract and heated in a boiling water bath for 2 h. After cooling, the diluted extract was passed through the concentrating cartridge; aglycones were washed with 96 % ethanol. Then chromatography with gradient elution was carried out. In the mobile phase, methanol content in the aqueous solution of orthophosphoric acid (0.1 %) varied from 47 to 49 % during 18 min. The total content of flavonol glycosides (glycosides of quercetin, kaempferol and myricetin separately) in plant samples was calculated according to the content of aglycones obtained after acid hydrolysis, using the known coefficients of the conversion of aglycones to proper glycosides: 2.504 for quercetin and 2.588 for kaempferol [21, 23]. The content of myricetin glycosides was calculated according to quercetin.

Standard samples (Fluka) were used for quantitative analysis and identification of flavonols by HPLC.

RESULTS AND DISCUSSION

As a result of our investigations, flavonols are shown to be one of the leading groups of natural compounds of *A. alpinum*, and the range of their content variability is 2.14–10.35 % by mass of air-dry raw material (in the flowers), 1.98–6.36 % (in the leaves), and 1.50–6.16 % (in aboveground part of the plants) (Table 1).

Under high insolation *A. alpinum* is able to accumulate significant amounts of flavonols. Flavonoids are considered to be the pigments functionally related to plant protection from UV radiation [24].

Particularly large amount of flavonols is revealed in the plants of subalpine meadows and other meadow phytocenoses – grass meadow forest edges, grass meadows, meadow steppe communities. And in all the cases investigated, the flowers are richer in flavonols than the leaves, and aboveground plant parts are typically less rich than the leaves. This is because of the presence of stems in a sample; they usually are much less rich in flavonols compared with flowers and leaves.

At the Kurai Ridge of the Altay Mountains (Gorny Altay) the upper boundary of *A. alpinum* distribution area corresponds to the mountain tundra zone at a height of 2550 m above sea level, where this plant is quite rare along the rocky bank of a cold mountain stream. According to [25], the soil is only weakly heated there (in July, the temperature at a depth of 5–10 cm hardly reaches 10 °C), soil humidity is high. The average temperature of surface air layer in July is only 6.1 °C; humidity is at

TABLE 1

Content of flavonoids in *Aconogonon alpinum* plants growing in the Republic of Altay, % by mass of air-dry material

Collecting ground, vegetative stage	Leaves	Flowers	Aboveground part
The environs of Kurai settlement, the Kurai Ridge, mountain-tundra zone, alpine meadow plots at the altitude of 2550 m; blooming	2.14	1.98	1.50
In the same place, subalpine zone, subalpine meadow at the altitude of 2350 m; blooming	10.35	4.89	4.16
In the same place, mountain forest zone, larch forest at the altitude of 2050 m; blooming	4.22	3.45	2.94
In the same place, Kurai steppe, wormwood-grasses association, at the altitude of 1600 m; blooming	5.16	4.10	4.24
The environs of Meny settlement, motley grass meadow plots in mixed forest at the altitude of 1450 m; blooming	9.70	6.36	4.10
The environs of Uvazhan settlement, excessively moist stream banks at the altitude of 400 m; blooming	6.10	4.17	3.65
The environs of Elanda settlement, motley grass-grasses meadow along the road at the altitude of 450 m; blooming	8.74	4.13	6.16
The Ust-Kan District, the environs of Yakonur settlement, Ligularia-iris meadow steppe; blooming	7.72	5.19	4.60
The environs of Soldatovo settlement, in the crops; blooming	6.12	4.27	2.80
The environs of Chegan-Uzun settlement, flood-land of Chegan-Uzun River, alkali soils; blooming	5.56	4.45	3.19
The Severo-Chuya Ridge, the north face, steppe meadow at the altitude of 1850 m; the end of blooming	5.30	4.86	3.10
The Chikhachev Ridge offsets, south-west side, subalpine meadow at the altitude of 2000 m; the end of blooming	9.68	5.17	4.12
The environs of Kokorya settlement, steppe meadow near the sheep-fold at the altitude of 1900 m; the end of blooming	5.08	3.12	2.17
The environs of Kokorya settlement, pebbles along the stream bank at the altitude of 1900 m; the end of blooming	6.67	4.19	4.36
The environs of Tashanta village, the foot of the north side exposition, meadow steppe; blooming	7.80	4.19	2.85
Kosh-Agach aymak, The environs of Tarkhatty village, the stony sedge motley grass steppe at the altitude of 2600 m; blooming	4.50	3.47	2.86

least 40 %. The diurnal range of temperature fluctuation in summer is 12–20 °C. The amount of liquid precipitation during vegetative period is about 300 mm on average. Under so harsh living conditions (alpine grass-plots along the stream) the plants contain only about 2 % of flavonols. At the same time, the flavonol content was quite high in other extremely humid ecotopes with more favourable air and soil temperature. Low air and soil temperature of the mountain tundra zone of the Kurai Ridge probably does not contribute to the biosynthesis of flavonols (see Table 1).

In a subalpine meadow of the Kurai Ridge (altitude 2350 m) *A. alpinum* grows in small clumps. The soils there are mightier than those in the rocky tundra areas. Microclimatic conditions in the alpine meadow cenoses of the Kurai Ridge are softer compared to the rocky tundra. The vegetation period is short. This is compensated by quick plant development. Under these conditions, the highest content of flavonols is observed. Most of the plants of *A. alpinum* we analyzed, which grow under the conditions of high insolation in the Altay Mountains (Gorny Altay) accumulate considerable

TABLE 2

Content of flavonoids in the plants organs of *Aconogonon alpinum* (the Republic of Altay, Ust-Kan District, 293 km along to the road to Tuekta village, meadow steppe, July 2008), % by mass of air-dry material

Flavonol	Sample No. 1			Sample No. 2		
	Flowers	Leaves	Stems	Flowers	Leaves	Stems
1	0.01	0.01	0.003	0.02	0.01	0.004
2	0.01	0.07	0.003	0.05	0.02	0.01
3	0.03	0.03	0.01	0.03	0.14	
4	0.01	0.02	0.02	0.02	0.01	0.01
5	0.05	0.13		0.04	0.00	
6	0.17	0.22	0.41	0.12	0.05	0.13
Hyperoside **7**	0.59	0.46	0.22	0.62	0.19	0.08
Rutin **8**	0.35	0.49	0.08	0.35	0.52	0.10
9	0.02	0.03	0.02	0.02	0.91	0.01
10	0.02	0.03	0.01	0.39	0.02	0.04
11	0.03	0.01	0.02	0.09	0.71	0.05
12	0.02	0.01	0.01	0.10	0.23	0.04
13	1.19	1.57	0.24	0.80	0.19	0.11
Quercetrin **14**	0.33	0.58	0.02	0.26	0.19	0.004
15	0.01	0.02	0.01	0.03	0.04	0.01
16	0.01	0.03	0.004		0.04	0.01
17			0.01	0.01	0.04	0.01
18				0.01	0.01	0.004
19	0.06	0.05	0.01	0.05	0.02	0.004
Quercetin **20**	0.03	0.03	0.01	0.04	0.01	0.004
Kaempferol **21**	0.004	0.001		0.003		
Total amount of free aglycones	0.03	0.03	0.01	0.04	0.01	0.004
Aglycones after hydrolysis:						
Myricetin	0.06	0.18	0.05	0.07	0.15	0.03
Quercetin	1.90	3.26	0.71	2.11	2.76	0.37
Kaempferol	0.15	0.13	0.06	0.22	0.18	0.04
Total amount of flavonol glycosides	2.10	3.57	0.82	2.40	3.09	0.44
Content of flavonoids (all the peaks)	2.94	3.78	1.07	3.06	3.35	0.61
Content of flavonoids (according to the method of V. V. Belikov and M. S. Shraiber, 1970)	3.48	4.25	1.20	3.90	3.35	0.79

amounts of flavonols; this fact indirectly confirms the protective function of these substances. The flavonoid pigments very likely acts as a filter protecting the plant tissues from harmful effects of ultraviolet rays, and their amount in plants depends on daylight illuminance of their habitat.

In *A. alpinum* plants gathered in the Ust-Kan District of the Republic of Altay (meadow steppe, 293 miles along the road to Tuekta village) in July 2008 in the phase of mass flowering, aglycones and main glycosides of flavonols are identified. Identification based on UV spectrography, acid hydrolysis (for glycosides) and comparative chromatography [18] showed that aglycones of the examined plants are kaempferol, quercetin and myricetin, and the main flavonol glycosides are astragalin, quercetrin, avicularin, hyperoside, quercetin 3,7-O-diglucoside, rutin, myricitrin. This conclusion agrees with the results of our previous studies as well as data published [16, 17].

Identification of flavonols by HPLC chromatography (Agilent 1100) is limited by the set of high-purity glycoside standards at our disposal. Flavonol glycosides hyperoside **7**, rutin **8** and quercetrin **14**, and aglycones: quercetin **14** and kaempferol **21** are identified (Table 2). All three aglycones – quercetin, kaempferol, and myricetin – were found only after hydrolysis. Before it, the free myricetin was not identified. The main aglycone of *A. alpinum* is quercetin (up to 3.26 %). Kaempferol and myricetin glycosides are found in small quantities, so *A. alpinum* can be used as a producer of quercetin glycosides. Figure 1 shows the chromatogram of the ethanol extract of *A. alpinum* flowers (sample No. 2).

HPLC methods allow us to detect minute differences in the qualitative content of the components of individual plant organs, plants and samples from different sampling sites. In our work, two samples gathered at almost the same place, a few meters from each other, contain various components of glycoside complex. Thus, the flowers of the first sample contain glycoside **13**, hyperoside, rutin, quercetrin, and the flowers of the second one (see Fig. 1) – **13**, hyperoside, **10**, rutin, quercetrin (according to content decreasing). The leaves of the first sample contain main glycosides – **13**, quercetrin, rutin and hyperoside, the leaves of the second one – **9**, **11**, rutin and **12**. Hyperoside, quercetrin and glycoside **13** are found in equal amounts (0.19 %). The main glycosides in the stems are the same (**6–8** and **13**). Earlier we noted variability of the content of main glycosides of *A. alpinum* [16].

The samples slightly differ in total content of glycosides in the individual plant organs. Taking into account all the peaks observed in the chromatograms, an assumption can be made that flavonoids are mainly represented by flavonol glycosides. These data obtained by HPLC method differ from those obtained by V. V. Belikov and M. S. Shraiber [20] using the method based on the reaction of flavonoids with aluminium chloride, which is mainly used in biochemical studies of resource plant species. The results obtained by this method proved to be significantly higher in most cases.

CONCLUSIONS

Our investigations of the structure and content of flavonols of *A. alpinum* (All.) Schur growing in the Republic of Altay demonstrated its extraordinary value as a producer of flavonols – bioactive substances with a wide-range therapeutic action. Flavonols constitute one of the main groups of natural compounds of *A. alpinum*. The content of flavonols is up to 10.35 % in flowers, 6.36 % in leaves and 6.16 % in the aerial part. And the plants of subalpine meadows and other meadow communities are particularly rich in these substances. The flavonol complex of *A. alpinum* includes astraga-

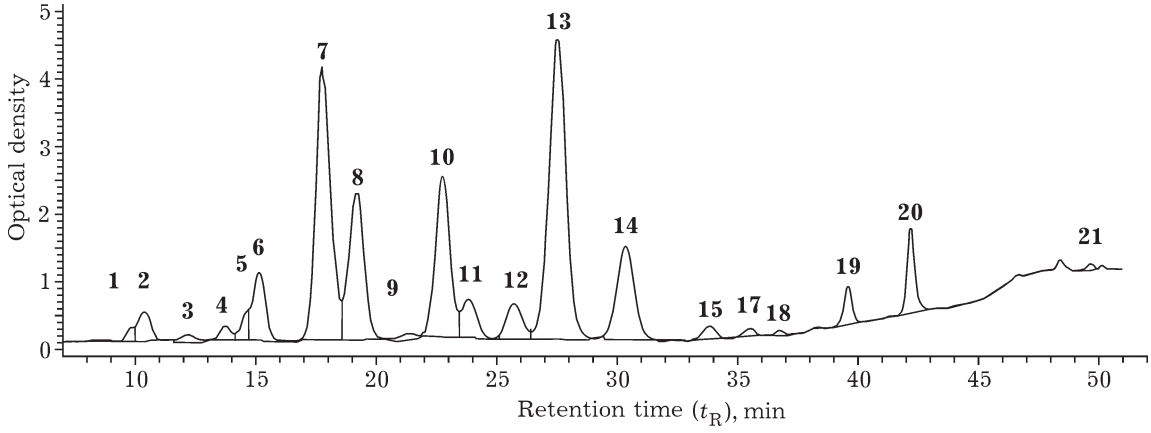

Fig. 1. Chromatogram of *Aconogonon alpinum* blossom extract (sample No. 2) (the Ust-Kan District of the Republic of Altay, 293 km 293 km along the road to Tuekta village, meadow steppe, July 2008): **1–6**, **9–13**, **15–19** – unidentified constituents, **7** – hyperoside (t_R = 17.76 min), **8** – rutin (t_R = 19.18 min), **14** – quercetrin (t_R = 30.33 min), **20** – quercetin (t_R = 42.16 min), **21** – kaempferol (t_R = 49. 63 min).

lin, quercetrin, avicularin, hyperoside, quercetin-3,7-diglucoside, rutin, myricitrin. The qualitative composition of flavonols was determined according to the method described in [18] and HPLC comparing with reliable samples. To determine the content of substances we used chromato-spectrophotometric method [19], that of V. V. Belikov and M. S. Shraiber [20] and HPLC method [21] on an Agilent 1100 liquid chromatograph (Agilent Technologies, USA). It is shown that the use of HPLC methods allows one to detect minute differences in the components revealed in individual organs of plants, individual plants, and samples from different sampling sites. We established that V. V. Belikov's method gives overestimated results.

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