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Flavonoids of Wild and Introduced Plants of Several Species of the *Hedysarum* L. Genus

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Abstract

Flavonoid content was studied in the organs of the top part of plants of seven species of *Hedysarum* L. genus (*H. alpinum* L., *H. flavescens* Regel et Schmalh., *H. austrosibiricum* B. Fedtsch., *H. neglectum* Ledeb., *H. theinum* Krasnob., *H. gmelinii* Ledeb. and *H. tschuense* A. I. Pjak *et* A. L. Ebel) growing in the forest-steppe zone of West Siberia (Novosibirsk) and in natural populations in the Altai Republic and Northern Kazakhstan. The species peculiarity of the accumulation of flavonoids was observed: the largest amount of flavonoids was detected in the leaves of *H. flavescens*, *H. alpinum* and *H. theinum* species (up to 15.0, 13.2 and 11.1 %, respectively). In general, all the studied species of *Gamotion* section are distinguished by exclusively high flavonoid content independently of age. The representatives of *Multicaulia* section *H. gmelinii* and *H. tschuense* are distinguished by somewhat lower (in comparison with the above-indicated species) flavonoid content (2.6 and 1.8 %, respectively). The species of *Gamotion* section are to be recommended as the source of flavonoids because the introduced and wild plants are characterized by the exclusive ability to synthesize the large amount of these compounds.

Key words: flavonoids, tick trefoil *Hedysarum* L., *Gamotion* section, legumes Fabaceae

INTRODUCTION

Search for flavonoid-containing plants is an urgent task for modern resource science. Flavonoid-containing plants are used in medicine to obtain medicinal preparations with broad action ranges: anti-inflammatory, capillary strengthening, bile-expelling, radioprotective, anti-tumour, immunomodulatory *etc.* [1–5]. Special attention of researchers is attracted to the antioxidant action of flavonoids, their ability to bind free radicals and remove them from human organism [6–8].

Medicine has accumulated substantial positive experience of treating diseases with the help of medi9cinal plants including those of *Hedysarum* L. (Fabaceae). Representatives of *Hedysarum* genus are bushes, stemless or tigellum plants [9]. There are 285 species of this genus on the Earth [10]. They grow in forests (in wet meadows, along river banks), in alpine and subalpine meadows, stony fields, steppes. Within the boundaries of the former USSR the *Hedysarum* genus includes more than 100 species [11], and the flora of Siberia includes 23 species [12].

The plants of *Hedysarum* genus have been long used to treat cardiac, chronic lung diseases, some chill and skin diseases of viral nature. The curative properties of these plants are due to the unique set of biologically active substances – flavonoids, xanthones, polysaccharides *etc.* [13]. The component composition of the plants of *Hedysarum* genus was considered in detail in [14]. According to the data reported in that work, flavonoids were detected in leaves, stems, blossom clusters and roots of the plants of 22 species; the highest content was detected in blossom clusters and leaves [15]. Substantial amount of flavonoids was detected in the plants of *Hedysarum* genus which are the subject of our investigation. For example, flavonoid content of the leaves, stems and roots of *H. flavescens* Regel et Schmalh. is 8.6–13.2 % [16], flavonoid content of the leaves, flowers, stems and seeds of the plants of *H. gmelini* Ledeb. and *H. neglectum* Ledeb. is 6.8–15.3 mg/g [17]. As a rule, flavonoids are represented by quercetin glycosides, hyperoside, polystachoside, avicularin, quercitrin, quercetin 3-α-*L*-ramnofuranoside, quercetin 3-α-*L*-ramnopyranoside [18–22].

In the present work we describe the results of the investigation of flavonoid content in the organs of the top parts of seven plant species belonging to *Hedysarum* L., in the natural cenopopulations of Siberia and Kazakhstan and the plants introduced into the forest-steppe zone of West Siberia (Novosibirsk).

MATERIALS AND METHODS

The objects of investigation were plant samples of the five *Hedysarum* species from natural sites: *H. austrosibiricum* B. Fedtsch., *H. neglectum* Ledeb., *H. theinum* Krasnob., *H. gmelini* Ledeb., *H. tschuense* A. I. Pjak *et* A. L. Ebel. In addition, we studied the plants of four *Hedysarum* species grown at the experimental ground of the Central Siberian Botanical Garden (Novosibirsk, the forest-steppe zone of West Siberia): *H. alpinum* L., *H. flavescens* Regel et Schmalh., *H. neglectum* and *H. theinum*. The plants were sampled during June–July 2008 in the phase of blossoming. Blossom clusters, leaves and stems were examined.

The quantitative determination of flavonoids was carried out using the procedure based on the method of V. V. Belikov and M. S. Shraiber [23] that involves the formation of complexes of flavonols with aluminium chloride. Exactly weighted portions of air-dry raw material (about 0.5 g), ground and sieved through a sieve with 1 mm mesh, were placed in a flask 100 mL in volume, and exhaustive extraction with 70 % ethanol was carried out. The completeness of extraction was checked using the reaction with a 5 % solution of NaOH (till the disappearance of yellow colour), and the volume of filtered united extract was measured. Then 0.1 mL of the extract was placed in a measuring tube, 0.2 mL of 2 % $AlCl_3$ solution in 96 % ethanol was added, and ethanol solution with the same concentration was added to the volume of 5 mL. In the reference version, 1–2 drops of 30 % acetic acid solution were added to 0.1 mL of the extract, and then the volume was driven to 5 mL. The solutions were mixed, kept for 40 min, and then the optical density of the solution with aluminium chloride was measured using a SF-26 spectrophotometer, at the wavelength of 415 nm in a cell with the layer thickness of 1 cm. The solution with acetic acid was used for comparison.

The total flavonoid content (in per cent of the mass of absolutely dry raw material) was determined as

$X = YV_1V_2 \cdot 100/(MV_3 \cdot 10^6)$

where Y is flavonoid content in 1 mL of solution under examination, which was determined using the calibration plot obtained for rutin, mg; V_1 is the volume of the extract, mL; V_2 is dilution volume, mL; V_3 is the extract volume taken for analysis, mL; M is the mass of raw material calculated for absolutely dry mass, g.

Determinations were carried out three times; average values are presented.

RESULTS AND DISCUSSION

Tick trefoil species studied by us belong to two sections: *Gamotion* (*H. alpinum, H. flavescens, H. austrosibiricum, H. neglectum* and *H. theinum*) and *Multicaulia* (*H. gmelini* and *H. tschuense*). Results of the measurement of flavonoid content in blossom clusters, leaves and stems of the plants are presented in Table 1. One can see that almost in all the samples the flavonoid content of leaves exceeds that of blossom clusters by a factor of 1.2–4.8, and that of stems by a factor of 1.5–10.0. All the plant species of *Gamotion* section are distinguished by the high flavonoid content: 6.2–15 % in leaves, 2.1–6.3 % in blossom clusters, 0.8–2.4 % in stems. Variations are due to different reasons, such as species affiliation, age, growing conditions *etc*. The highest flavonoid content was detected in the plants of *H. alpinum* and *H. flavescens* species.

H. alpinum is a perennial grassy plant 40–150 cm high. The stems are upright and almost always bare. The leaves are imparipinnate. Blos-

TABLE 1

Flavonoid content in the top organs of the plants belonging to the species of *Hedysarum* L. genus (collected in 2008, blossom phase), % calculated for absolutely dry raw material

Sampling site, origin of plants	Sampling date	Plant organs		
		Blossom clusters	Leaves	Stems
Hedysarum alpinum				
Novosibirsk, forest-steppe zone of West Siberia, experimental ground of CSBG, SB RAS. Origin of seeds: Omsk, Botanical Garden of the Agricultural Institute, Sowing date: 2001	14.07	5.0	13.2	1.3
Hedysarum flavescens				
The same. Origin of seeds: Upsala city, Botanical Garden. Sowing date: 1998.	14.07	4.6	15.0	1.5
Hedysarum austrosibiricum				
Northern Kazakhstan. Northern macro-slope of the Ivanovskiy Ridge. Alpine dianage-grass meadow	05.07	4.4	9.8	1.5
The same place, aromatic spicate meadow	06.07	5.4	8.2	2.1
Hedysarum neglectum				
Altai Republic. The foot of the Seminskiy pass. Herb-bunchgrass forest meadow with bushes	28.06	4.8	6.2	1.1
Novosibirsk, forest-steppe zone of West Siberia, experimental ground of CSBG, SB RAS. Origin of seeds: Altai Republic, Ust-Koksa district, side ridges of the Katunski Ridge, near Lake Multa. Siberian pine-larch forest with undergrowth (*Betula rotundifolia, Lonicera altaica*). Sowing date: 2001.	06.06	5.1	6.5	2.1
The same. Origin of seeds: Altai Republic, Yabogan pass. Siberian pine-larch light forest. Sowing date: 2000.	06.06	3.0	7.6	1.2
The same. Origin of seeds: Altai Republic, Kyrlykskiy pass, foot of the slope of western exposure. Larch light forest. Sowing date: 2001.	06.06	4.2	6.4	0.9
The same. Origin of plants: Khakassia, Shira district, the foothills of the Kuznetskiy Alatau. Grass-sedge-herb meadow at the side of the larch forest. Sowing date: 2000.	18.06	4.2	8.5	1.3
Hedysarum theinum				
Northern Kazakhstan, near the Maloulbinskoye water reservoir Tick trefoil-hellebore-grass sub-alpine meadow	12.07	4.2	8.0	1.6
Northern Kazakhstan. Prokhodnoy belok. Grass sub-alpine meadow	18.07	4.1	10.4	1.2
The same, grass alpine meadow	20.07	4.0	8.9	1.7
The same, aromatic spicate alpine meadow	21.07	3.6	9.0	2.4
The same, fescue tundra	20.07	2.3	11.1	1.5
The same, tick trefoil-geranium-grass sub-alpine meadow	08.07	3.7	9.8	1.3
Novosibirsk, forest-steppe zone of West Siberia, experimental ground of CSBG, SB RAS. Origin of seeds: Altai Republic, Ust-Koksa district, near the Krasnaya mountain, the slope of eastern exposure, 1700 m above the sea level. High-grass sub-alpine meadow. Sowing date: 2000.	18.06	3.1	7.5	0.9
The same, sowing date: 2004.	06.06	4.0	7.4	1.5
The same, sowing date: 2005.	06.06	3.3	8.0	1.3
Novosibirsk, forest-steppe zone of West Siberia, experimental ground of CSBG, SB RAS. Origin of seeds: Northern Kazakhstan, Prokhodnoy Belok, 1900 m above the sea level. Sub-alpine meadow in the light forest of Siberian pine and larch. Sowing date: 1998.	18.06	2.1	8.9	1.7
The same, origin of seeds: Northern Kazakhstan, Ivanovskiy Ridge, 1650 m above the sea level. A clearing in the light forest of Siberian pine and larch. Sowing date: 2000.	18.06	6.3	9.7	1.5
The same, sowing date: 2001.	18.06	3.2	8.7	1.3

TABLE 1 (End)

Sampling site, origin of plants	Sampling date	Plant organs		
		Blossom clusters	Leaves	Stems
Novosibirsk, forest-steppe zone of West Siberia, experimental ground of CSBG, SB RAS. Origin of seeds: Northern Kazakhstan, Ivanovskiy Ridge, 1700 m above the sea level, the slope of north-eastern exposure. Sub-alpine light forest of larch and Siberian pine. Sowing date: 2000.	18.06	3.1	8.1	1.2
The same. Origin of seeds: Northern Kazakhstan, Ivanovskiy Ridge, 1800 m above the sea level. Sub-alpine light forest of Siberian pine and larch. Sowing date: 2000.	18.06	4.4	7.9	0.8
The same. Origin of seeds: Northern Kazakhstan, Ivanovskiy Ridge, 1850 m above the sea level. Mountain valley with Siberian pine. Alpine meadow. Sowing date: 2001.	18.06	3.8	8.2	0.9
Hedysarum gmelinii				
Altai Republic. The foot of Chike-Taman pass. Grass meadow steppe.	28.06	5.0	2.6	1.1
Hedysarum tschuense				
Altai Republic. Ulagan region, near Chibit village, semi-matted debris stream	24.06	0.7	1.5	0.3
The same, the mouth of the Boka River. Grass steppe.	24.06	0.9	1.7	1.0
The same, near Erbalyk village. Grass-sedge steppe.	24.06	1.3	1.8	0.9
The same, carbonate debris stream with a steepness of 50 °, southern exposure, along the road (210 km from Tashanta)	24.06	2.1	1.8	0.6
The same place. Near Chuy-Oozy (the band of Chuya river). Stony carbonate slope of southern exposure, the slope of 25°	25.06	1.1	1.3	0.9

som clusters are long dense trusses with 20–30 flowers. The flowers are of papilionaceous type on short pedicles with linear bracts. Coronas are rose, lilac-rose. This species is widespread in the north of Mongolia, China and Korea, in Russia it occurs in Arctic, in the north of the European part, in West and East Siberia, in the Far East on forest, wet meadows, in rare forests, scrubs, along river banks [9]. The plants of *H. alpinum* species are decorative; they are good melifers, they are also used as medicinal and forage plants [24]. *H. alpinum* is applied in Tibetan medicine against chronic lung and cardiac diseases, and atherosclerosis [25]. In the folk medicine of Transbaikalia, this species is used to treat intestinal colic and pulmonary tuberculosis, the dry plant powder is used against epilepsia, decoction of the top part is used as analgesic agent [26].

A broad range of biologically active substances was detected in the leaves of *H. alpinum*, but the fact that the top organs of the plants of this species contain xanthones deserves special attention [10, 27]. It is *H. alpinum* from which xanthone mangiferin was extracted; antiviral preparation alpizarin was developed on its basis. In addition, this species is the source of flavonoids. According to our data, very high flavonoid content was detected in seven-year-old *H. alpinum* plants grown under the conditions of the forest-steppe zone of West Siberia (near Novosibirsk, at the experimental ground of the Central Siberian Botanical Garden): 13.2 % in leaves, 5.0 % in blossom clusters, 1.3 % in stems (see Table 1). When cultivated in West Siberia, *H. alpinum* plants reach the adult state and the second or third year after seeding and then for a long time (10 years and more) they blossom and give seeds, producing a large amount of the raw material (mainly due to leaves).

Along with *H. alpinum*, the plants of *H. flavescens* are characterized by the large amount of flavonoids. The plants of this species are perennial grassy plants up to 1.5 m high, with compound imparipinnate leaves, yellow flowers arranged in one-sided trusses at the tops of stems and branches. These plants grow in

Western Tien Shan, Pamiro-Alai, Afghanistan on high-altitude territories (2500–3400 m above the sea level), along the banks of mountain rivers, near glaciers and snowfields, along debris streams near the upper boundary of forests and among bushes [11]. Local population uses these plants for washing to treat skin diseases and as wound-healing means [24]. The flavonoid content in the leaves of blossoming 10-year-old *H. flavescens* plants introduced at the CSBG, SB RAS, is 15.0 %; blossom clusters contain 4.6 %, and stems 1.5 %.

The *H. austrosibiricum* plants studied by us were collected in meadow communities of Northern Kazakhstan, along the northern macro-slope of the Ivanovskiy Ridge. *H. austrosibiricum* is endemic of the mountains in the south of Siberia and the Altai, it grows in the high mountainous belt on forest, alpine and subalpine meadows, in the tundra, on stony fields, on rubbly lichen mountain tops [9]. Similarly to the two species described above, *H. austrosibiricum* is a grassy perennial plant 20–40 cm high. Its small leaves (4–9 pairs) are oblong elliptic. Flowers are lilac or lilac-violet, assembled in dense trusses. This is a good forage plant for deer, sheep and horses [28]. We detected 8.2–9.8 % flavonoids in the leaves of *H. austrosibiricumi*, which is less than the value for the two above-described species. The flavonoid content of blossom clusters and stems is close to that in *H. alpinum* and *H. flavescens* species.

Extremely variable species *H. neglectum* is a grassy perennial plant 25–30 cm high, with small oblong or elliptic leaves (4–10 pairs), accumbent downy on both sides. The flowers are lilac or magenta-lilac. The truss is loose. This species is widespread in the south of Siberia, in Eastern Kazakhstan, in the Tien Shan and Pamiro-Alai mountains. In Mountainous Altai, *H. neglectum* grows most frequently on the slopes of northern exposure where it usually occurs in forests with Siberian pine or larch Siberian pine, on forest, sub-alpine and rarely on alpine near-brook meadows, and also in sites with large stones (so-called kurumniks). The altitudinal range of the occurrence of this species in the Altai is 1400–2500 m above the sea level but most frequently it occurs at the altitude of 1500–1900 m above the sea level [9]. *H. neglectum* possesses antiviral and protistostatic activity [29], is considered as a good forage plant [28]. Sheep, horses, marals eat this plant [24].

The leaves of *H. neglectum* cultivated in Siberia, 7–8 years old, contain 6.4–8.5 % flavonoids, blossom clusters contain 3.0–5.1 %, stems contain 0.9–2.1 %.; the origin of seeds may be essential in this situation. The plants from the Shira cenopopulation (Khakassia) are characterized by the higher flavonoid content (in the leaves) than those from the Altai cenopopulations. Wild plants of the Altai cenopopulation (the foot of the Seminsky pass) only slightly differ from the introduced plants in flavonoid content of top organs.

H. theinum was accepted as a separate species from *H. neglectum* Ledeb. [30]. These species are very close to each other, however, they differ in structure, colour, chemical composition of roots and some morphological features of top organs. *H. theinum* is a perennial grassy plant with a large reddish-brown root. This high-mountain alpine species is rare; it grows in limited territories over Russia, where it occurs only in the high-mountain belt of the Altai [31]. It has a disjunctive central Asian – southern Siberian habitat (the Altai, Mongolia, the Jungarsky Alatau). It grown in the high-mountain area, in adjacent regions of the forest belt on alpine, sub-alpine meadows, stony slopes, along brooks, in forest meadows [9]. In the Kazakhstan Altai this species is spread in the sub-golets belt, enters the lower part of the golets belt, as well as the medium and upper parts of te forest belt. The threshold of the vertical distribution of this species is 1700–2100 m, sometimes it goes lower into the forest belt to 1300 m above sea level [32].

H. theinum is a valuable medicinal and food plant of the Altai flora. The underground organs of the plant, known as the red root, are widely used as a food additive possessing the tonic and immunomodulating action. The application of the red root in the cases of disorder in male sex system. However, most popular among the local population of the Mountainous Altai is the tea drink prepared from this root, possessing the tonic action, so the species was called theinum [33].

The flavonoid content of *H. theinum* has been practically non-investigated. The *H. theinum* plants of different ages grow at the experimental ground of the CSBG, SB RAS. After sowing the seeds of the same origin, at different ages the flavonoid content of three-, four- and eight-year-old plants was: leaves – 8.0, 7.4, 7.5 %, blossom clusters – 3.3, 4.0, 3.1 %, stems – 1.3, 1.5, 0.9 %, respectively. So, the maximal content was detected in three- (leaves) and four-year-old plants (blossom clusters and stems). Comparison between *H. theinum* plants 7–8 years old, grown from the seeds of different cenopopulations of northern Kazakhstan (the region of the Ivanovskiy Ridge) revealed that the plants from the seeds of one of five cenopopulations contained 9.7 % flavonoids in leaves and 6.3 % in blossom clusters, which is much higher than the content detected in the others. High content is also characteristic of the 10-year-old plants: leaves contain 8.9 % flavonoids, while blossom clusters contain 2.1 %. The age is likely to be not of prime importance for the ability of plants to synthesize flavonoids, though this factor cannot be neglected. The flavonoid content of leaves, blossom clusters and stems of wild *H. theinum* plants from different cenopopulations of northern Kazakhstan was 8.0–11.1, 2.3–4.2, and 1.2–2.4 %, respectively, which is comparable with, or slightly higher than, their content in the plants growing in culture. In general, the flavonoid content is likely to be determined to a higher extent by the systematic position of plants. The *H. theinum* species is closer in this respect to *H. austrosibiricum* than to *H. neglectum*.

H. gmelinii, the representative of *Multicaulia* section, is a perennial grassy plant 10–40 cm high. Stems are upright or ascending. The number of leave pairs reaches 11–14; the shape of the leaves is oblong or elliptic. Two fragments of the range of *H. gmelinii* are distinguished: the first one, with the larger area, embraces a part of Siberia and Central Asia, while the smaller one is situated in the region to the west of the Urals and the region on the left side of the Volga. At the territory of West Siberia, *H. gmelinii* grows in meadow and stony steppes, in mountain meadows, stony and rubbly slopes, it is widespread both in steppe and in meadow phytocenoses. *H. gmelinii* occupies the places with periodic or small permanent deficit of wetting; it is distinguished by polymorphism and high ductility [9].

The plants serve as good forage for sheep, horses, and as satisfactory forage for cattle [28]. It is possible to use this species as a melliferous, food and vitamin-bearing plant [34]. At the same time, *H. gmelinii* species is completely unknown in respect of its flavonoid content. This species from the Republic of Altai is distinguished by much lower flavonoid content than that of above-described species, the representatives of *Gamotion* section: leaves contain 2.6 %, flowers 5.0 %, and stems 1.1 %. The flavonoid content of the flowers is two times as high as that of leaves.

According to the data obtained in the investigation of samples from five cenopopulations of the Altai Republic, the flavonoid content of wild plants of the *H. tschuense* species is even lower: 1.3–1.8 % in leaves, 0.7–2.1 in blossom clusters and 0.3–1.0 in stems.

CONCLUSION

Flavonoid content in the top organs (blossom clusters, leaves and stems) of seven species of *Hedysarum* L. genus was studied: in *H. alpinum, H. flavescents* Regel et Schamh., *H. austrosibiricum* B. Fedtsch., *H. neglectum* Ledeb., *H. theinum* Krasnob., *H. gmelinii* Ledeb. and *H. tschuense* A. I. Pjak et A. L. Ebel) growing in the natural cenopopulations of the Republic of Altai and in northern Kazakhstan. and at the experimental ground of the Central Siberian Botanical Garden, SB RAS (Novosibirsk, the forest-steppe zone of West Siberia). The samples were collected during June–July 2008 during the blossom phase.

All the studied species of the *Gamotion* section are distinguished by exclusively high flavonoid content, independently of the age, even 10-year-old plants are able to synthesize up to 15.0 % flavonoids. The species specificity in flavonoid accumulation was observed: the highest content was detected in the leaves of *H. flavescens, H. alpinum* and *H. theinum* (up to 15.5, 13.2 and 11.1 %, respectively). The flavonoid content is slightly less in the plants of *H. austrosibiricum* and *H. neglectum* (up to 9.8 and 8.5 %, respectively). Blossom clusters contain 1.2–4.8 times smaller amounts of flavonoids than leaves and 1.5–10.0 times smaller than stems.

The representatives of *Multicaulia* section *H. gmelinii* and *H. tschuense* are distinguished by lower flavonoid content than the above-listed species: 2.6 and 1.8 USD, respectively).

The species of the *Gamotion* section should be recommended as the source of flavonoids because both introduced and wild plants are characterized by the exclusive capacity to synthesize large amounts of these compounds.

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