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VARIABILITY OF THE CONTENT OF FLAVONOIDS IN THE FLOWERS OF THE *FILIPENDULA ULMARIA* (L.) MAXIM *

ABSTRACT. Meadowsweet (*Filipendula ulmaria* (L.) Maxim., Rosaceae) is widely used in traditional and alternative medicine in Russia. The influence of various factors on the accumulation of flavonoids in the flowers of the plants of this species in natural populations in the Middle Ural as well as in the conditions of the Botanical Garden of Ural Branch of RAS was studied. High content of flavonoids in the Ural populations of meadowsweet was revealed. No differences in the content of flavonoids between *F. ulmaria* s. str. and *F. denudata* (J. t C. Presl) Fritsch were detected. Interpopulation variability in the content of flavonoids is high enough. The relationship between the content of flavonoids in the flowers and the geographic location of the studied populations is not traced. The influence of the conditions of the vegetative season on the accumulation of flavonoids in the flowers of *F. ulmaria* was observed. The content of flavonoids in the Botanical garden was lower than in most natural populations. The flowers of the plants growing in wet soils contain the same amount of flavonoids as those in the drier soils.

KEY WORDS. Meadowsweet, *Filipendula ulmaria*, *Filipendula denudata*, content of flavonoids.

Filipendula ulmaria (L.) Maxim. is a large perennial grassy plant which is widely used in the traditional and alternative medicine in Russia and a number of European countries [1]. In our country the flowers of the plant of this species are used as an anti-inflammatory and wound healing means [2]. Nowadays, it is proved that the flowers of *F. ulmaria* display various types of pharmacological activity – anticoagulant, gastroprotective, antidiabetic, anticarcinogenic, immune-modulating, antioxidant, etc [3] which explains a great interest in this species and the medicinal raw material it produces.

F. ulmaria is a polymorphic species with a complicated population structure and a possible intra-species subdivision of a vague taxonomic rank. Often scientists divide *F. ulmaria* into two species: *F. ulmaria* s.str. and *F. denudata* (J. et C. Presl) Fritsch which differentiate in the intensity of their leaf downiness [4]. Other botanists either tend to consider them as subspecies of *ulmaria* and *denudata* (J. et C. Presl) Hayek [5] or do not see any intra-species differences [6, 7] explaining the above mentioned

* The research has been sponsored by the Ural Branch of RAS: project No. 12-C-4-1028 «Adaptation mechanisms in the natural and introduced populations of plants in Siberia and the Urals».

features by purely ecological factors. The habitat of *F. ulmaria s.str.* encompasses the whole Northern Europe. *F. denudata* is found only in Europe. The Urals mark the eastern boundary of the habitat of the latter taxon. We find it interesting to reveal the differences between the given taxons according to their chemical composition, in particular, content of flavonoids, that condition the pharmacological effect of the meadowsweet flowers. The goal of the paper is to study the specificity of flavonoid accumulation in the meadowsweet flowers of different populations on the territory of the Middle Urals as well as in the Botanical garden of the Ural Academy of Sciences and the influence of various factors on the accumulation of these biologically active substances.

Material and methods of research. Flower samples for the research were collected in the southern part of Sverdlovsk region in 2009-2011; one population was gathered in the far south-east of Kirov region; and some samples were garnered in the Botanical garden: 2010 in the moist area and in 2011 both in the moist and drier areas.

We picked the flowers in July during the phase of the active flowering of the plants. We dried them outside in the shadow.

According to the current normative documentation standardization of the meadowsweet flowers is carried out judging by the sum of flavonoids calculated as quercetin glycosides [2]. To define the content of flavonoids in the flowers we employed the method of differential spectrophotometry with the use of the complexing reaction with aluminum chloride. As a comparison solution we used extraction without aluminum chloride which allowed us to exclude the influence on the results of the analysis of the other groups of compounds having optical density around maximum regarding consumption of extraction from the raw material. Rutin was used as a standard sample as it is close in its spectral characteristics to other quercetin glycosides which are present in the flowers of *F. ulmaria*. Under the condition of complex formation with $AlCl_3$ the extraction specter from the meadowsweet flowers has maximum consumption for wave length 420 nm, as for rutin solution it is equal to 410 nm.

Flavonoid extraction from the raw material was carried out according to the methodology developed by E. Yu. Avdeeva et al. for *F. ulmaria* [8, 9]. Each sample was analyzed three times.

Findings of the research and their discussion. Results of the analysis revealed high content of flavonoids (calculated as rutin) in *F. ulmaria* flowers in the Ural populations and the population from Kirov region (table 1). According to the data from other researchers, flavonoid content (calculated as rutin) in the flowers gathered in Moscow region is 4.5% [10] and in Novosibirsk region it ranges from 5.2% [11] to 9.8% [12].

Table 1

Flavonoid content in *F. ulmaria* s. str. and *F. denudata* flowers from various populations

Population No.	Sample No.	Year of collection	Taxon	Flavonoid content
Sverdlovsk region				
1	1	2009	<i>F. ulmaria</i> s. str.	10.8 ± 0.04
1	2	2010	- " -	11.6 ± 0.1
2	3	2010	- " -	12.8 ± 0.2
3	4	2010	- " -	11.8 ± 0.4
3	5	2011	- " -	9.1 ± 0.1
4	6	2010	- " -	11.5 ± 0.3
5	7	2010	- " -	8.4 ± 0.1
6	8	2010	Combined sample <i>F. ulmaria</i> s. str. and <i>F. denudata</i>	13.2 ± 0.2
7	9	2010	- " -	12.4 ± 0.2
8	10	2010	- " -	13.0 ± 0.2
9	11	2010	- " -	9.9 ± 0.2
9	12	2011	<i>F. ulmaria</i> s. str.	6.4 ± 0.01
9	13	2011	<i>F. denudata</i>	8.0 ± 0.3
10	14	2010	Combined sample <i>F. ulmaria</i> s. str. and <i>F. denudata</i>	12.2 ± 0.7
10	15	2011	<i>F. ulmaria</i> s. str.	10.1 ± 0.2
10	16	2011	<i>F. denudata</i>	9.9 ± 0.3
11	17	2011	<i>F. ulmaria</i> s. str.	9.7 ± 0.1
11	18	2011	<i>F. denudata</i>	9.5 ± 0.4
Kirov region				
12	19	2011	<i>F. ulmaria</i> s. str.	9.9 ± 0.2
12	20	2011	<i>F. denudata</i>	9.5 ± 0.1

Analysis of the samples collected in 2010 revealed that in the “mixed” (i.e. containing simultaneously both *F. ulmaria* s. str. and *F. denudata*) natural populations (No. 6-10) flavonoid content in the flowers in the majority of the cases was a little increased in comparison with the populations containing only *F. ulmaria* s. str. (No. 1-5): correspondingly 9.9 (13.2%) and 8.4 (12.8%) (table 1). On average flavonoid content in the “mixed” populations was equal to 12.1 ± 0.4, in the “pure” populations it was 11.2 ± 0.4.

In 2011 we organized separate gathering of flowers *F. ulmaria* s. str. and *F. denudata* in a number of “mixed” natural habitats (No. 9-12). Data analysis has shown that in populations 10-12 the difference between the taxons under study is insignificant; only in population 9 flowers *F. denudata* contained significantly more flavonoids than flowers *F. ulmaria* s. str. (fig. 1).

We carried out a two-factor dispersion analysis of the data for the “mixed” populations where taxonomic belongingness was one of the factors and belongingness to a certain population was the second factor. Absence of significant differences between *F. ulmaria s. str.* and *F. denudata* was revealed; flavonoid content in the first taxon equaled on average to 9.0 ± 0.11 , in the second 9.2 ± 0.1 . Yet, however, within each taxon there were significant differences between the populations ($p < 10^{-10}$) (fig.1).

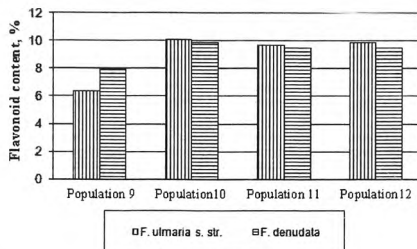


Fig. 1. Flavonoid content in flowers *F. ulmaria s. str.* and *F. denudata* from mixed populations No. 9-12 in 2011

We have also established significant interaction between the studied factors ($p = 1.8 \cdot 10^{-3}$). In population 9 flavonoid content as average in the population and in each taxon is truly lower than in the other populations; at that, in this population flavonoid content in *F. denudata* is significantly higher than in *F. ulmaria s. str.* In populations 10-12 differences in the content of flavonoids in the taxons in question are not proved. We can suppose that flavonoid content in *F. ulmaria s. str.* is characterized by a higher interpopulation variability than in *F. denudata*; the number of flavonoids fluctuates from 6.4% to 10.1% in *F. ulmaria s. str.* and from 8.0% to 9.9% in *F. denudata*.

There is no interconnection between the content of flavonoids in the flowers and the geographical location of the populations in question. In populations 9 and 10 located at a distance of about 10 km from each other flavonoid content was different in 2010 for the combined sample by 1.2; in 2011 for *F. ulmaria s. str.* – 1.6, for *F. denudata* – 1.2. At the same time population 10 almost did not differ in flavonoid content from population 7 located 70 km away from it to the north-east (in 2010) and from population 12 located 500 km to the west outside the Urals (in 2011) (table 1).

In 2010 the minimum flavonoid content (7.8%) was registered in the inflorescence of *F. ulmaria s. str.* grown in the UAS Botanical garden (sample 21; table 2). In 2011 the content of flavonoids in the flowers of the cultivated plants (samples 22 and 23) was also lower than in the natural populations (correspondingly 6.7% and 6.5%). Only in population 9 the amount of flavonoids in 2011 was almost the same as in the Botanical garden – 6.4% for *F. ulmaria s. str.* (tables 1, 2). Thus, cultivation may negatively influence on flavonoid accumulation in *F. ulmaria s. str.* flowers. However, according to N. Yu. Gudkova [10] transportation of the plants of this species from the

natural environment to the test field of the Botanical garden did not produce any negative effect on the content of the substances in question.

Table 2

Flavonoid content in cultivated flowers *F. ulmaria s. str*

Growth environment, Botanical garden	Sample No.	Year of gathering	Flavonoid content, %
Moist land	21	2010	7.8 ± 0.1
“-“	22	2011	6.7 ± 0.1
Dry land	23	2011	6.5 ± 0.4

In the UAS Botanical garden the plants were grown on two different plots: under high moisture conditions (samples 21 and 22) and on a drier plot (sample 23). Comparison of the flowers from these two plots has shown no distinct differences in the content of flavonoids in the flowers (table 2). However the plants on these plots significantly differed in the speed of seasonal development: in 2011 on the dry plot the plants definitely earlier entered the phenophases of aftergrowth (4 days earlier), budding (6 days earlier), and seed ripening (15 days earlier) than on the moist plot (results of the phenological observations have been processed according to the methodology by G. N. Zaytseva[13]).

We have analyzed some of the populations in question twice – in 2009 and 2010 (population 1- samples 1 and 2), 2010 and 2011 (population 3 – samples 4 and 5, as well as samples 21 and 22 as cultivated plants). It was stated that in 2010 flavonoid accumulation was higher than in 2011 both in the natural and cultivated environments (tables 1, 2). In population 9 in 2010 flavonoid content in the combined flower samples was higher than in 2011 separately in each of the studied taxa (*F. ulmaria s. str.* and *F. denudata*). In population 10 in 2010 the amount of flavonoids in the combined sample was also higher than in 2011, for *F. ulmaria s. str.* (fig. 2).

Thus, in 2010 flavonoid accumulation was significantly higher than in 2011. In population 1 in 2010 flavonoid accumulation was also higher than in 2009 (table 1). This fact can be connected with the conditions of the vegetative period which in 2010 was much warmer and drier than usual, with a large number of sunny days.

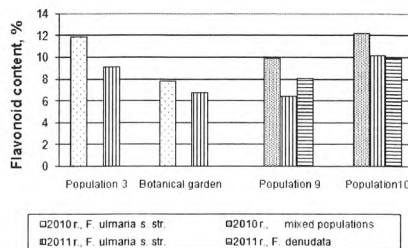


Fig. 2 Flavonoid accumulation in different vegetation seasons in the meadowsweet natural and cultivated populations

Conclusions.

Studies of the peculiarities of flavonoid accumulation in the meadowsweet flowers of various natural populations revealed high content of these substances (calculated as rutin) in the Ural populations (on the territory of Sverdlovsk region) and in the population of Kirov region: ranging from 6.4% to 13.2%.

In the populations comprising both *F. ulmaria s. str.* and *F. denudata* (J. et C. Presl) Fritsch plants there are generally no differences between the taxons in flavonoids content; on the whole their amount in 2011 ranged from 6.4% to 10.1% for *F. ulmaria s. str.* and from 8.0% to 9.9% for *F. denudata*.

Interpopulation variability of flavonoid content in the flowers is rather high, especially for *F. ulmaria s. str.* There is no interconnection between the content of flavonoids in the flowers and their geographical location.

It has been noted that conditions of the vegetation season influence on the accumulation of flavonoids: in 2011 during an unusually warm and dry vegetation season there were significantly more flavonoids than in 2009 and 2011 (by 1.1 and 1.3).

As a cultivated species in the Ural Botanical garden flavonoid content is lower than in the majority of the natural populations. Probably cultivation negatively influences on flavonoid accumulation. At that the flowers which grew on a moist plot of land have the same amount of flavonoids as those growing on a dry plot: correspondingly 6.7% and 6.5% (in 2011).

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