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Biologically Active Substances in *Filipendula ulmaria* (L.) Maxim. Growing in the Middle Urals

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Abstract

The plants of *Filipendula ulmaria* (L.) Maxim. growing in the Middle Urals can serve as a source of flavonols, catechins, tannins, saponins and carotenoids. A definite specificity in their accumulation in organs was established. The high carotenoid content in the leaves of *Filipendula ulmaria* was established by us for the first time.

Key words: *Filipendula ulmaria* (L.) Maxim., flavonols, catechins, tannins, pectic substances, saponins, carotenoids

INTRODUCTION

Much attention is attracted during the recent years to the development of the preparations of plant origin. Special interest is caused in this respect by the plants that are widely used in folk medicine in different countries. Meadowsweet *Filipendula ulmaria* (L.) Maxim. (Rosaceae fam.) is used in folk medicine in Russia and in some European countries as a corroborant, anti-inflammatory, wound-healing, analgesic, diuretic, antiulcerogenic, hypoglycemic, sedative, antihemorrhoid and astringent means [1, 2]. At present, enormous data are also available on other kinds of pharmacological activity (stress-protective, immunomodulating, antitumour). Special attention of researchers is paid to the possibility to use meadowsweet raw material to develop therapeutic and prophylactic preparations with high antioxidant activity [3–6]. The flowers of *Filipendula ulmaria* are used in Russian scientific medicine as anti-inflammatory and wound-healing medicine [7]. The *Filipendula ulmaria* raw material basis is rather well provided by natural resources [8].

Meadowsweet is used in folk medicine to treat various diseases; all parts of the plant are used (flowers, leaves, roots) in the form of decoctions, water extracts, tinctures, and ointments. It is often recommended to use separate organs or their combinations, which is undoubtedly due to the differences in the chemical composition of active substances present in different parts of plants [8]. Preparations of meadowsweet flowers have won the widest application in folk medicine [9]. Water extract of its flowers is used to treat rheumatism, asthma [10, 11], stomach and duodenal ulcer [12–14], water extract of leaves is used to treat trophic ulcer of lower extremities, wounds and burns [13, 15, 16]; decoction of roots is applied to treat nervous disorders, hypertension, as antihelminthic agent and spasmolytics [11, 13]. Herb in general also possesses valuable pharmacological properties [17, 18].

Wide application of *Filipendula ulmaria* to treat various pathologies is connected with the diverse chemical composition of the plant; it is very interesting to study its chemical composition. According to the data of [8, 19, 20], *Filipendula ulmaria* plants contain phenol compounds (flavonols, catechins and tannins). These compounds play important part in various physiological processes involving redox reactions. Preparations based on these compounds are used as bactericidal, antiseptic, anti-inflammatory, haemostatic means. They are able to inhibit chain free radical reactions, which determines their efficiency in chemotherapy of cancer.

Pectic substances present in meadowsweet leaves carry out detoxication of human organism by binding and removing toxins, poisons and radioactive isotopes, which is especially important [21]. Due to the presence of saponins, *Filipendula ulmaria* is efficient in treating vascular sclerosis, atherosclerosis combined with hypertension, and malignant neoplasms [20]. The importance of carotenoids which were detected by us in the leaves of *Filipendula ulmaria* for the first time cannot be overestimated. Undergoing transformation into vitamin A, they affect endocrine and reproductive system, ensure the stability of organism to infections and mycoses, slow down the growth of tumours and accelerate wound healing [22].

The goal of the work was to study the features of the accumulation of various groups of biologically active substances (BAS) in separate organs of *Filipendula ulmaria*.

MATERIALS AND METHODS

The object of investigation was meadowsweet *F. ulmaria* (L.) Maxim. (Rosaceae family) – a large (40–100 cm high) perennial herbaceous plant with short horizontal rhizome. The leaves are green from above and white-pannose from beneath. Inflorescences are paniculate and large. Flowers are 7–8 mm in diameter, their colour is yellowing-white. This is Eurasian species. Its areal embraces almost the whole territory of Russia and partially the territories of Central and Northern Europe. Meadowsweet grows on floodplain meadows, grass bogs, along river banks, in humid forests, cutover patches [23].

The raw material of meadowsweet was collected in 2010–2012 in different natural populations at the territory of Sverdlovsk Region (the Middle Urals). We also analyzed the raw

TABLE 1

Characterization of the samples of raw material of *Filipendula ulmaria* (L.) Maxim.

Sample No.	Description of sample collection site	Year of sample collection
	Natural populations	
1	Kamyshlov District, near Obukhovo village; the valley of the Pyshma River, along the field path. Relatively dry, well illuminated place	2010
2	Achit District, near Yalym settlement; brook bank, in a low place near the motorway. Wet, well iluminated place	2011
3	To the south from Revda torn, the bank of the Bolshaya Puzanikha River. Well illuminated wetland.	2012
4	Territory related to Polevskiy town, near Kurganovo village, low place near forest road. Rather wet, weakly illuminated place	2012
5	Pyshma District, near Cheremysh settlement, forest edge not far from brook bank. Relatively dry, well illuminated place	2012
6	Alapayevsk District, near Aromashevo village, in a low place near the motorway. Rather wet well illuminated place	2012
7	Territory related to Berezovskoye town, near Klyuchevsk settlement, forest edge, along motorway. Wet, well illuminated place	2012
	Culture conditions	
8	Botanical Garden, Ural Branch of the RAS, Yekaterinburg. Dry, well illuminated place	2011

material of *F. ulmaria* cultivated in the Botanical Garden, Ural Branch of the RAS, Yekaterinburg (Table 1). Flowers, leaves and stems were stores in July during mass blossoming of the plants. The sample for biochemical investigation was composed of several ten individuals from each population.

To determine the concentrations of flavonols, catechins, tannins, pectic substances, saponins, carotenoids, we used raw material dried in shadow in aired rooms. All biochemical indices were calculated per mass of absolutely dry raw material. The result was averaged over three parallel determinations for each parameter.

Quantitative determination of flavonols was based on the method proposed by V. V. Belikov and M. S. Shraiber [24]. It involves the formation of the complex of flavonols with aluminium chloride. The precise weighted portion of ground raw material (about 0.5 g) was placed in a flask 100 mL in volume, and complete extraction with 70 % ethanol on the boiling water bath was carried out. The completeness of extraction was monitored with the help of the reaction with 5 % NaOH solution. Then 0.1 mL was placed in a volumetric tube, 0.2 mL of 2 % $AlCl_3$ solution in 96 % ethanol was added, and the volume was brought up to 5 ml using ethanol of the same concentration. In the reference version, 1–2 drops of 30 % acetic acid were added to 0.1 mL of the extract and then the volume was brought up to 5 mL. The solutions were mixed; optical density of the solution with aluminium chloride was measured after 40 min with a SF-26 spectrophotometer at 415 nm in a cell with the layer thickness 1 cm using a cell with the acid as a reference.

Total content of flavonols (in per cent of the mass of absolutely dry raw material) was determined as

$$X = YV_1V_2100/MV_3 \cdot 10^6$$

where Y is flavonol content in 1 mL of solution under test, determined from the calibration curve plotted for rutin, mg; V_1 is the volume of extract, mL; V_2 is dilution volume, mL; V_3 is extract volume taken for analysis, mL; M is the mass of absolutely dry raw material, g.

Catechins were determined by means of spectrophotometry; the samples of 0.8 mL of 80 % ethanol extract were placed in test tubes, 4 mL of 1 % vanillin solution in concentrated hydrochloric acid was added to each sample; 4 mL of concentrated hydrochloric acid were added to reference tubes. The volume was brought up to 5 mL in each tube. After 5 min, in the case of the presence of catechins, the colour became rose. The concentration of catechins in the samples was determined from the calibration curve plotted for (±)-catechin Sigma [25].

The content of tannins was determined by means of titration [26]. A precise weighted portion of air-dry raw material (0.5–1 g) was extracted by water on boiling water bath for 45 min. The resulting extract 5–10 mL in volume was titrated with a 0.1 N $KMnO_4$ solution in a beaker with 400 mL of water in the presence of indigo carmine under permanent mixing till golden-yellow colour. In parallel, reference titration without the extract under study was carried out. Calculated for tannin, 1 mL of 0.1 N $KMnO_4$ solution is equivalent to 4.157 mg of tanning substances.

Pectic substances (protopectins and pectins) were determined using the carbazole method based on obtaining specific violet-rose colour of uronic acids with carbazole in sulphuric medium. The density of coloured solutions was measured with the help of a FEK-56M photoelectric colorimeter at the wavelength of 535 nm in a cell with the working length of 5 mm. The content of pectic substances was determined using the calibration curve plotted with galacturonic acid [27].

Saponin content was determined using the gravimetric method. Approximately 2 g of the air-dry material was extracted with chloroform in Soxhlet extractor till complete decolouring to remove lipids and gum which hinder saponin determination. Then the raw material was extracted sequentially with 50, 60, 96 % ethanol, twice in each concentration for 30 min at 70 °C. The united extract was evaporated to 5 mL; a seven-fold excess volume of acetone was added. After 18 h, the formed precipitate was separated by filtering, dried at 70 °C, weighted, and the content of "raw" saponin was calculated [28].

To determine carotenoids, a weighted portion of the plant raw material (0.1 g) was attrited in a mortar to obtain homogeneous mass, then 0.1 g of calcium carbonate was added, followed sequentially by 1 mL of dimethyl-

formamide and 2 mL of anhydrous sodium sulphate to neutralize acids and to fix enzymes. Extraction of carotenoids was carried out at fist with acetone (40 mL × once, 10 mL × twice), then 96 % ethanol 5 mL × three times) because lycopin is better soluble in it than in acetone. Then thorough extraction (till the disappearance of colour) with acetone was carried out; the volume of united extract was measured. Total carotenoid content was determined in the acetone-ethanol extract by means of spectrophotometry at the wavelengths of 450 and 550 nm [29]. Carotenoid content C_{cr} was determined as

$$C_{cr} = DVK/LH$$

where D is the optical density of the extract, V is its volume, mL; H is weighted portion, g; K is the coefficient of recalculation for β-carotene, which is equal to 0.4; L is the working length of the cell, cm.

RESULTS AND DISCUSSION

It was established that *F. ulmaria* growing in the Middle Urals contains valuable BAS: flavonols, catechins, tannins, pectic substances (pectins and protopectins), saponins, carotenoids (Table 2).

Flavonol content in the flowers of these plants is very high: 8.3–12.9 %. We demonstrated previously [30] that total flavonoid content of *F. ulmaria* flowers from some natural populations varies from 6.6 to 13.8 %. Leaves contain almost two times smaller amount of flavonols: 3.3–5.5 %. However, these data also deserve attention of researchers. The following quality parameters are adopted for medicinal

TABLE 2

Concentrations of the major groups of biologically active substances in the organs of the top part of *Filipendula ulmaria* (L.) Maxim. growing in the Middle Urals

Sample No.	Organs	Flavonols, %	Catechins, mg%	Tannins, %	Pectins, %	Protopectins, %	Saponins, %	Carotenoids, mg%
1	F	11.8±0.4	170.7±0.9	48.1±0.2	0.3±0.0	2.8±0.0	15.7±0.1	20.6±0.1
	L	4.5±0.1	592.4±1.0	22.0±0.2	0.3±0.0	4.5±0.0	4.8±0.0	134.3±0.6
2	F	11.7±0.1	104.3±0.9	53.7±0.2	0.8±0.0	4.1±0.0	5.9±0.0	17.8±0.1
	L	4.1±0.1	631.4±1.3	23.3±0.1	0.4±0.0	3.9±0.0	4.2±0.0	140.1±0.7
3	F	12.9±0.1	149.6±1.9	56.8±0.2	n/d	3.8±0.0	30.7±0.2	15.2±0.1
	L	5.5±0.1	1399.9±2.8	28.1±0.2	n/d	5.0±0.0	12.7±0.1	121.2±0.6
	S	0.9±0.0	234.2±1.1	5.3±0.1	n/d	2.7±0.0	0.8±0.0	18.9±0.1
4	F	8.7±0.1	129.8±0.9	43.0±0.2	n/d	2.8±0.0	23.6±0.1	23.4±0.2
	L	4.3±0.1	1631.6±2.9	24.2±0.1	n/d	2.5±0.0	12.4±0.1	130.0±0.5
	S	0.5±0.0	170.4±1.4	3.4±0.0	n/d	1.4±0.0	10.6±0.1	15.7±0.1
5	F	8.5±0.1	161.2±1.6	43.2±0.2	n/d	2.8±0.0	11.6±0.1	13.9±0.1
	L	3.9±0.0	1329.7±3.1	23.8±0.1	n/d	3.0±0.0	8.7±0.1	75.4±0.6
	S	0.7±0.0	170.0±1.1	4.4±0.0	n/d	1.7±0.0	12.4±0.1	13.5±0.1
6	F	9.6±0.1	141.2±0.9	46.7±0.2	n/d	2.9±0.0	22.6±0.1	16.6±0.1
	L	4.9±0.0	1498.5±4.9	23.5±0.1	n/d	4.0±0.0	14.6±0.1	129.9±0.6
	S	0.9±0.0	120.3±0.7	4.6±0.0	n/d	1.6±0.0	3.2±0.0	12.1±0.1
7	F	9.9±0.1	139.9±1.0	51.2±0.3	n/d	2.1±0.0	4.0±0.0	18.9±0.1
	L	3.6±0.0	1951.1±5.8	21.7±0.2	n/d	3.3±0.0	0.6±0.0	123.0±0.1
	S	0.5±0.0	199.8±1.0	3.3±0.1	n/d	1.3±0.0	8.9±0.1	11.1±0.0
8	F	8.3±0.0	70.5±0.7	38.3±0.2	0.4±0.0	2.5±0.0	15.6±0.1	17.8±0.1
	L	3.3±0.0	442.4±1.1	19.4±0.1	0.3±0.0	5.3±0.0	10.3±0.1	177.8±0.8

Notes. 1. F – flowers, L – leaves, S – stems. 2. n/d – not detected.

raw material: the flowers of sandy everlasting, (*Helichrysum arenarium*) should contain not less than 6 % flavonoids, tansy flowers – not less than 2.5 %, hypericum grass – not less than 1.5 %, the leaves of *Menyanthes trifoliate* – not less than 1.0 %, door-weed grass – not less than 0.5 % [31]. Taking into account substantial resources of *F. ulmaria* raw, it is evident that this plant is promising for the needs of medical industry. High flavonol content of *F. ulmaria* seems to be independent of wetting conditions: it is rather high both in relatively dry regions of its growing and in excessively wetted ones.

Ultraviolet radiation (UV) is a powerful stressing factor for all living systems including plants [32]. Protecting themselves from the negative action of large doses of natural UV radiation, plants work out a series of adaptive mechanisms. One of them is the synthesis of phenol compounds that are able to absorb the short-wavelength part of solar radiation [19]. We discovered that flavonoid content of *F. ulmaria* increases with an increase in illumination of the places where these plants grow: for example, the maximal value (11.7–12.9 % in flowers and 4.5–5.5 % in leaves) is characteristic of the populations with the highest illumination (see Table 2, Nos. 1–3), slightly lower (8.7 % in flowers, 4.3 % in leaves) – for populations with decreased illumination (see Table 2, No. 4). Exclusion is population No. 5 with not very high flavonol concentration in spite of good illumination.

Catechins are highly reactive phenol compounds. They occupy the extreme position in the oxidation-reduction sequence of phenol compounds, as opposed to flavonols. A kind of the "difference in potentials" arises between these two groups of compounds, which to a substantial extent provides metabolism in plants. Inflorescences of *F. ulmaria* contain 70.5–170.7 mg% catechins; leaves contain much more – 442.4–1951.1 mg%; substantial amounts of catechins are accumulated in well illuminated places with increased wetting. Catechin content in stems is comparable with that in flowers and is equal to 120.3–234.2 mg%.

Along with flavonols and catechins, *F. ulmaria* contains large amounts of tannins. Their content in inflorescences is 38.3–56.3 %, which is twice as high as in leaves (19.4–28.1 %); in stems it is insignificant (below 5.3 %).

Pectic substances are present in *F. ulmaria* plants in two major forms: protopectins and pectins. Pectin is formed through protopectin splitting, which means that protopectin serves as an additional source of pectin [21]. Top parts of *F. ulmaria* contain very small amounts of pectin (0–0.8 %), while protopectins are present in substantial amounts in inflorescences (2.1–4.1 %), in leaves (2.5–5.3 %) and in stems (1.3–2.7 %). Pectins were not detected in plant samples collected in 2012. It is possible that pectins were formed during storage in the samples collected in 2010 and 2011.

The entire top part of *F. ulmaria* can serve as a source of triterpene saponins; especially large amounts of these compounds are accumulated in flowers (4.0–30.7 %). Leaves and stems contain smaller amounts of saponins – up to 14.6 and 12.4 %, respectively. We did not reveal any connection between saponin accumulation and humidity. It should be noted that among all the studies BAS groups saponins are characterized by the highest variability from one population to another: the studied samples differ in saponin content of flowers by a factor of 7.7, in leaves by a factor of 24.7, in stems by a factor of 16.6. This group of BAS seems to be most sensitive to the action of various environmental factors.

It was established that the leaves of *F. ulmaria* are unique accumulators of carotenoids (75.4–177.8 mg%), while inflorescences and stems contain smaller amounts (13.9–23.4 and 11.1–18.9 mg%, respectively). It is known that *F. ulmaria* often occupies open space with high illumination. Under the conditions of excessive insolation carotenoids are likely to function as light-absorbing agents sharing the key part in energy metabolism with chlorophyll. They are involved in various protective mechanisms: they inhibit the formation of free radicals, provide protection from UV radiation transforming the energy of UV light into visible light, act as antioxidants protecting sensitive tissues and labile compounds from oxidation [22].

The plants of sample No. 2 are the richest in BAS. They contain the largest amounts of flavonols, tannins, protopectins in all organs, and saponins in flowers. Their locality is characterized by the highest wetting degree (up to waterlogged state) and high illumination; this may also affect the intensity of BAS accumulation.

CONCLUSION

In view of the diversity of climatic and ecological-geographic conditions of *F. ulmaria* growth in the Middle Urals, the amount of BAS in inflorescences, leaves and stems differs substantially. At the same time, definite organ specificity in BAS accumulation is exhibited. For example, inflorescences of *F. ulmaria* contain larger amounts of flavonols, tannins, pectins and saponins in comparison with leaves, while catechins, protopectins and carotenoids prevail in leaves.

The results obtained provide evidence that the multipurpose use of all the organs of *F. ulmaria,* not only inflorescences as it happens today, is promising. Even the stems of *F. ulmaria* are valuable from the viewpoint of BAS content: they contain much flavonols (about 1 %) and tannins (up to 5 %), while the amount of catechins and carotenoids is comparable with their content in flowers.

So, clearly pronounced curative properties of *F. ulmaria* (L.) Maxim. are due to the rich complex of BAS. The plants of this species are a promising source of flavonols, catechins, tannins, saponins and carotenoids; they are of substantial interest for the development of novel medical preparations.

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