

Comparative Study of the Biologically Active Substances Composition and Content in Meadowsweet (*Filipendula ulmaria* (L.) Maxim) Crude Herbal Drugs (Herb, Leafs, Flowers) of Russian Origin

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ABSTRACT

Filipendula ulmaria (L.) Maxim. (Meadowsweet) is known in traditional medicine as anti-inflammatory, wound-healing, astringent and antibacterial remedy. However recent studies show that it also has neurotropic activity. In Russia meadowsweet flowers are used as crude herbal drugs (temporary pharmacopoeial monograph 42-1777-87), also leaf and herb are used in the traditional medicine. Objective of the study was to carry out comparative investigation of composition and content of major biologically active compounds (BAC) in *Filipendula ulmaria* herb, flowers and leaf by thin-layer chromatography, differential spectrophotometry with aluminum chloride reagent (total flavonoids in terms of rutoside), gravimetry (total extractives, extracted by water), permanganometric titration (total tannins in terms of tannin). Rutoside, tannin, gallic acid and salicylic acid were identified in *Filipendula ulmaria* herb, flowers and leaf by TLC. Also we analyzed content of substances extracted by water, flavonoids and tannins. Total extractives, extracted by water in *F. ulmaria* herb is 13.12±0.10%, in leaf – 13.98±0.37%, in flowers – 18.09±0.17%. Total tannins in *F. ulmaria* herb is 11.87±0.47%, in leaf – 12.06±0.18%, in flowers – 12.26±0.29%. Total flavonoids in *F. ulmaria* herb 4.34±0.17%, in leaf – 6.98±0.23%, in flowers – 11.75±0.57%. The obtained data will be used for development of a pharmacopoeial monograph project "Filipendula ulmaria (L.) Maxim., herba" for inclusion in the State Pharmacopoeia of the Russian Federation.

Keywords: *Filipendula ulmaria*, thin-layer chromatography, spectrophotometry, flavonoids, tannins, extractives extracted by water

INTRODUCTION

Filipendula ulmaria (L.) Maxim. (Meadowsweet) is used in traditional medicine for the treatment of various diseases and possesses wound healing, antimicrobial, astringent, antioxidant, anti-inflammatory, gastroprotective and other types of action^{1,2}. *F. ulmaria* herb medicines have a beneficial effect on nervous system, this aspect makes relevant the study of the composition and content of the main groups of biologically active substances (BAS), in connection with the current prevalence of diseases of the nervous system²⁻⁵.

At present, *F. ulmaria* flowers are only one pharmacopoeial crude herbal drugs (CHD) listed in temporary pharmacopoeial monograph (TPhM) 42-1777-87 of Russian Federation, but herb is also used in traditional medicine^{2,6}.

According to the literature data, the main BAS groups in *F. ulmaria* CHD are salicylates in essential oil, content of salicylaldehyde is up to 70%⁷, flavonoids (3-4 in herb, up to 6% in flowers, quercetin-4'-glucoside, hyperoside,

kaempferol-4'-glucoside)^{8,9,10}, hydrolysable tannins (1-12%, the dimeric compound rugosin D)². The Russian authors have developed a TLC procedure to detect flavonoids in *F. ulmaria* herb and a spectrophotometric procedure to calculate total flavonoids in terms of quercetin in *F. ulmaria* CHD¹¹.

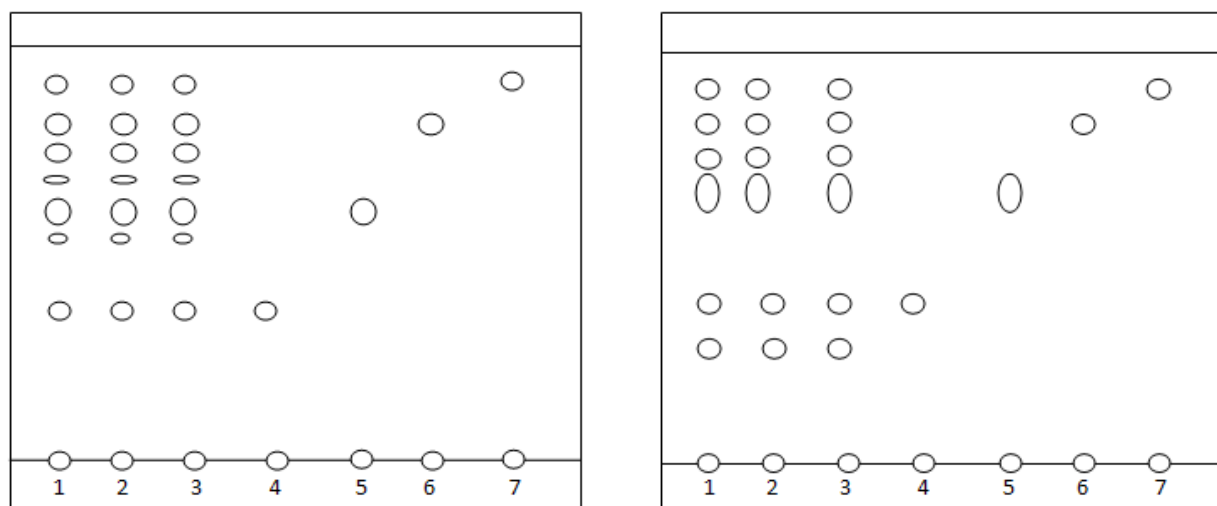
Also, simple and fast HPLC procedure for identification and assay of flavonoids in *F. ulmaria* flowers (40% alcohol CHD extract) was performed. Conditions were: a RP column (Zorbax SB C-18 250×4,6 mm, 5 μm); a mobile phase 0,01M potassium dihydrophosphate (pH=3,0) and acetonitrile in the ratio 80:20 (v/v) (isocratic elution); column temperature 30°C¹².

The aim of the study is a comparative investigation of the BAS composition and content in various *F. ulmaria* CHD (herb, leaf, flowers) of Russian origin.

MATERIALS AND METHODS

Chemicals

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Scheme 1

Scheme 2

1 – test solution 1; 2 – test solution 2; 3 – test solution 3; 4 – rutoside reference standard solution; 5 – tannic acid reference standard solution; 6 – gallic acid WS solution; 7 – salicylic acid WS solution

Figure 1: Schemes of chromatograms of *F. ulmaria* CHDs extractsTable 1: Chromatographic profiles of water-alcohol extracts (70% ethanol) of *F. ulmaria* CHD various types

Standard	Detection conditions		
	UV light (254 nm) (5 zones of adsorption)	3% solution of iron chloride (III) + heating (4 adsorption zones)	10% alcohol solution of sodium hydroxide + heating + diazo- reagent (6 adsorption zones)
not identified	–	–	Rf*=0,33; blue color
rutoside	Rf=0.49; green color	Rf = 0.49; dark green color	Rf = 0.46; green color
not identified	Rf=0.66; green color	Rf=0.66; green color	–
tannic acid	Rf=0.76; dark blue color	Rf= 0.76; dark blue color	Rf = 0.77; dark blue color
not identified	Rf=0.83; brown color	Rf=0.83; brown color	–
not identified	–	Rf=0.89; brown color	Rf=0.88; brown color
gallic acid	Rf = 0.95; brown color	Rf = 0.95; brown color	Rf = 0.94; brown color
salicylic acid	Rf=0.99; pale purple color	Rf=0.99; pale purple color	Rf=0.99; blue-violet color

*Rf – retention factor

Table 2: Total extractives extracted by water content in *F. ulmaria* CHD ($n = 5, f = 4, P = 95\%, T(f, P) = 2.78$)

CHD	\bar{X}	ΔX	s	E.%
Herb	13.12	0.10	0.036	0.76
Leafs	13.98	0.37	0.134	2.65
Flowers	18.09	0.17	0.061	0.94

Note. n – number of repeat tests, f – number of degrees of freedom, P % – confidence figure, $T(f, P)$ – Student's coefficient, \bar{X} – mean value, S – standard deviation, $E, \%$ – relative error.

Table 3: Total tannins content in *F. ulmaria* CHD ($n = 5, f = 4, P = 95\%, T(f, P) = 2.78$).

CHD	\bar{X}	ΔX	s	E.%
Herb	11.87	0.47	0.168	3.94
Leafs	12.06	0.18	0.063	1.45
Flowers	12.26	0.29	0.104	2.36

Note. n – number of repeat tests, f – number of degrees of freedom, P % – confidence figure, $T(f, P)$ – Student's coefficient, \bar{X} – mean value, S – standard deviation, $E, \%$ – relative error.

The stationary phase was a plates of "Sorbfil" STH-1A, 10x10 cm. The mobile phase was a system of organic solvents: water – formic acid – ethylacetate (5: 5: 40). Reference standards solutions – 0.125% rutoside ($\geq 97\%$, CN Acros organics, CAS 153-18-4), 1% tannic acid ($\geq 95\%$; Acros organics; CAS: 1401-55-4) and working

standard (WS) solutions – 0.025% gallic acid; 1% salicylic acid – were used to identify BAS.

Plant material collection

Air-dry *F. ulmaria* CHD (3 types: aerial parts (herb, leaves, flowers) was collected during the flowering period in the Tver region (Russian Federation) in 2015-2016.

Determination of BAS

Table 4: Total flavonoids content in *F. ulmaria* CHD ($n = 5, f = 4, P = 95\%, T(f, P) = 2.78$).

CHD	\bar{X}	ΔX	s	E.%
Herb	4.34	0.17	0.06	4.03
Leafs	6.98	0.31	0.11	4.4
Flowers	11.75	0.57	0.204	4.82

Note. n – number of repeat tests, f – number of degrees of freedom, P % – confidence figure, $T(f, P)$ – Student's coefficient, \bar{X} – mean value, S – standard deviation, $E, \%$ – relative error.

Studies were conducted at Pharmacognosy Department and Testing Laboratory for the Quality Assurance of Medicines of the Research Institute of Pharmacy.

At the first stage, the presence of the BAS groups in *F. ulmaria* CHD was confirmed by qualitative reactions. Then the composition of BAS was studied using the chromatography method in a thin layer sorbent (TLC).

TLC detection was carried out by two methods:

- 1) UV light at 254 nm followed by 3% solution of iron chloride (III) (Scheme 1)
- 2) 10% alcohol NaOH solution, followed by heating in an oven at a temperature of 100-110 ° C and treatment with a diazo-reagent (Scheme 2).

Water-alcohol (70% alcohol, 1:10) extracts of the *F. ulmaria* herb (test solution 1), *F. ulmaria* leaves (test solution 2), *F. ulmaria* flowers (test solution 3) were used for TLC analysis.

Test solutions (15 μ l) and reference standard solutions of rutoside (60 μ l), tannic acid (15 μ l), gallic acid WS (15 μ l) and salicylic acid WS (75 μ l) were applied to the chromatogram. Determination of the moisture content in CHD was carried out according to the methodology of State Pharmacopoeia of Russian Federation XIII edition, Vol. 2, p. 413 "Determination of the moisture content in medicinal plant material and medicinal herbal preparations" (General Pharmacopoeal Monograph – GPM 1.5.3.0007.15)¹³.

Determination of the content of extractives extracted by water was carried out according to the methodology of SP RF XIII ed., Vol. 2, p. 408 "Determination of the content of extractive substances in medicinal plant raw materials and herbal preparations" (GPM.1.5.3.0006.15)¹³.

Determination of total tannins was carried out according to the methodology of SP RF XIII ed., Vol. 2, p. 417 "Determination of the content of tannins in medicinal plant raw materials and herbal preparations" (GPM.1.5.3.0008.15) method 1 (permanganometric titration). The results of total tannins are expressed in terms of tannin (C₇₆H₅₂O₄₆; Mr=1434~1701.18; CAS 1401-55-4)¹³.

Determination of the flavonoids content was carried out according to the adapted methodology of SP RF XIII ed., Vol. 3, p. 418 "St. John's wort Herb" (PM 2.5.0015.15)¹³.

The spectrophotometric study was performed at Varian CARY 4000 UV-Vis Spectrophotometer. The wavelength range was from 600 to 200 nm, the cuvette was with a 10 mm layer thickness. CaryWin UV Scan software was used for data processing. Conversion of flavonoids amount was carried out in terms of rutoside. Since the maxima of the absorption spectra of the extracts studied after the reaction with the aluminum chloride solution coincided with the

maximum of the absorption spectrum of the rutoside complex with aluminum chloride ($\lambda=415$ nm).

Statistical processing of the results was carried out in accordance with the requirements of SP RF XIII GPM.1.1.0013.15 "Statistical processing of experimental results" using the program Microsoft Office Excel 2015¹³.

RESULTS AND DISCUSSION

The main BAS groups presence in the *F. ulmaria* CHD was confirmed by qualitative reactions:

- 1) ferric chloride (tannins). Black-blue staining was observed. The qualitative reaction was positive.
- 2) cyanidic reaction (flavonoids). Red staining was observed. The qualitative reaction was positive.

Further, a qualitative analysis of the BAS composition of *F. ulmaria* CHD various types was carried out by TLC.

The schemes of chromatograms 1 and 2 are shown at Fig. 1.

4 adsorption zones were detected in UV light at 254 nm. 7 adsorption zones were detected by further processing with a 3% solution of iron chloride (III) and heating in an oven at a temperature of 100-105 ° C. 6 adsorption zones were detected upon detection with an alkali alcoholic solution, followed by treatment with a diazo-reagent (Table 1).

Rutoside, tannic acid, gallic acid, salicylic acid were found in the studied extracts.

The content of the total extractives extracted by water, total tannins, total flavonoids was determined in *F. ulmaria* CHD. Since these BAS groups are hydrophilic, water extracts from CHD are planned to be used as medicines.

The results of determination of the total extractives extracted by water are presented in Table 2.

According to Table 2, the content of extractives extracted by water in the *F. ulmaria* herb is $13.12 \pm 0.10\%$, in leaves – $13.98 \pm 0.37\%$, in flowers – $18.09 \pm 0.17\%$.

The results of determination of the total tannins are presented in Table 3.

From the data given above, it follows that the content of total tannins in the *F. ulmaria* herb is $11.87 \pm 0.47\%$, in leafs – $12.06 \pm 0.18\%$, in flowers – $12.26 \pm 0.29 \%$.

The results of total flavonoids determination are shown in Table 4.

Thus, the maximum content of total flavonoids is determined in the *F. ulmaria* flowers ($11.75 \pm 0.57\%$).

The data obtained in the course of this study in *F. ulmaria* samples of Russian origin is completely consistent with the data of foreign researchers with the content of tannins and flavonoids^{2,3,11,12}. Further studies will be continued with samples from other different parts of Russia to develop normative documentation for "Filipendula ulmaria (L.) Maxim., herba" for inclusion in SP RF.

CONCLUSION

Rutoside, tannic acid, gallic acid and salicylic acid have been identified in *F. ulmaria* CHD of Russian origin by TLC. The BAS composition of the various types of *F. ulmaria* CHD (herb, leaf, flowers) is identical.

The results of quantitative determination of total tannins and flavonoids testify to the advisability of using the apical part of a plant, which mainly includes inflorescences, leaves and thin stems, as CHD.

Content of total extractives, extracted by water, total tannins, total flavonoids in *F. ulmaria* herb, leaf and flowers of Russian origin were determined. The obtained data will be used to create a pharmacopoeial monograph project "*Filipendula ulmaria* (L.) Maxim., herba" for inclusion in the State Pharmacopoeia of the Russian Federation.

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