The dominating component of the studied essential oil is α-terpyneol, its content makes 37,56%, and also nerolydol (4,75%) against a small quantity of thymol (0,18%) and absence of cymophenol.

Thus, essential oil of a Thymus pallasianus L. herb of flora of the Midland of Russia was investigated. The maintenance of connections of thymol typical of a Thymus genus in studied essential oil is insignificant (0,18%). The main components of oil are α-terpyneol (37,56%) and nerolydol (4,75%). As a result it is possible to claim that the studied sample of essential oil of a Thymus pallasianus L. belongs to a chemotype terpyneol. There were 29 connections identified, basic of which are α-terpyneol (37,56%), and nerolydol (4,75%).

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DEVELOPMENT OF METHODOLOGY FOR THE QUANTITATIVE DETERMINATION OF TANNINS IN THE GERANIUM SIBIRICUM L. HERB

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In the flora of the middle belt of the European part of Russia there are 17 species of the Geranium genus, among which the most common are the Geranium pratense L., Geranium sanquineum L., Geranium sylvaticum L., Geranium palustre L. and Geranium sibiricum L. [3, 6, 11]. The chemical composition of these species contains phenolic compounds, terpenes polysaccharides. Among the phenolic compounds can be identified phenol carboxylic acids, flavonoids, tannins [8, 9].

Plants of the Geranium genus is widely used in traditional medicine as an astringent, anti-inflammatory, hemostatic agents [9]. These types of activity are specific for tannins [8] and apparently cause the pharmacological action of the genus Geranium. From this point of view it would be acute to study tannin plants of the genus Geranium flora of the central European part of Russia.

The research problem was to develop a method for quantitation of tannins in the Geranium sibiricum L. herb and demonstration of its benefits.

The object of the study was a herb geranium sibiricum (Geranium sibiricum L.), harvested in the Kursk region in 2011–2012. during the period of mass flowering plants.

Research methods. For a qualitative determination of tannins the reaction with iron – ammonium alum, bromine water, formaldehyde and hydrochloric acid were carried out to identify their origin [4].

Over 100 different methods are used for quantitation of tannins, which are divided into several groups: gravimetric, which are based on the precipitation of tannins by gelatin, hide powder, salts of heavy metals; titrimetric, which are based on oxidative reaction with potassium permanganate, iodine; photocolorimetric, which are based onreactions with iron salts, phosphotungstic acid, spectrophotometric [5]. For quantitation of tannins there were also used pharmacopoeia – permanganometric and complexometric and spectrophotometric methods of analysis [1, 2, 8].

Pharmacopoeia titrimetric method is based on the ability of tannins to be oxidized with a potassium permanganate [1]. However, this method has several disadvantages: the accuracy of the results depends on the clearing coefficient, which is different for different groups of tannins and plants, the ability to oxidize potassium permanganate and other natural compounds, which belongs to different classes of biologically active substances in chemical structure, and a number of others – prolixity of the color while titration, extent of dilution of titrated solutions, etc. Accordingly, the method does not allow objective evaluating of the content of tannins in the medicinal plant material, particularly if there are less than 10% of them, because the inaccuracy vastly increased due to related substances.

The spectrophotometric determination of tannins is based on the method of determination of them in the aboveground part of the marsh cinquefoil [10]. Calculation of the content of tannins was based on tannin as we have determined that the dominant group is hydrolyzed tannins, as well as while the study of the absorption spectra of alcohol – water extract of Geranium sibiricum L. herb and tannin solutions with 70% ethyl alcohol is established that they are the same and at a wavelength of 280 nm.

To develop a methodology for quantitation of tannins in the Geranium sibiricum L. herb we used a complexometric method proposed for the determination of tannin in the leaves of sumac and smoke tree [2]. A complexometric method is based on the ability of tannins to be precipitated by salts of heavy metals. The method is in precipitation of tannins of zinc with ammonia solution, isolating the precipitate, centrifugation, destruction of complex zinc – tannin by acid with following titration of precipitated zinc cations by solution of Trilon B in the presence of indicator – xylene orange [2].

Research results For the detection of tannins an aqueous extract of the herb geranium sibiricum

in a ratio of 1:10 was made. While addition of a 2 ml of the extract solution 1% ferric alum dark appearance – blue staining was seen, which indicats that researched materials contain tannins of principally hydrolyzable group. In addition to extracting of the mixture of formaldehyde and hydrochloric acid, the small precipitate of condensed tannins forms, and there are hydrolysable tannins in the filtrate, which are detected by the iron – ammonium alum, which indicates that hydrolysable tannins are predominant group in the Geranium sibiricum L. herb [5].

Determination of optimal conditions for development of quantification techniques tannins was carried out by using a single sample of material.

In the first phase extraction conditions of tannins have been studied: the extent of grinding of raw materials, the extraction time, type of solvent, the ratio of raw materials – the solvent. The grinding of raw materials influence the process of extraction. [7]. The study of grinding of raw materials showed that the maximum extraction of tannins is achieved with the extent of grinding 1 mm (Table 1).

Table 1

Effect of extraction conditions on the content of tannins

Extraction conditions	Content of tannins, %		
	permanganometric methods	complexometric methods	spectrophotometric methods
the extent of grinding of raw materials, mm			
1,0	21,25	11,08	5,32
2,0	18,15	8,95	4,55
3,0	18,15	10,85	4,51
extractant: purified water ethyl alcohol, %			
30	16,25	10,89	4,78
40	20,55	12,98	5,40
50	21,25	11,08	5,32
70	22,90	13,20	6,00
	20,80	10,90	5,20
Extraction time, min			
20	18,51	11,09	4,89
30	20,14	12,12	5,92
45	22,63	13,34	6,02
60	22,39	13,13	5,94

According to State Pharmacopoeia XI [1] publishing as extractant for the quantitation of tannins boiling distilled water is used, however, in the literature there is evidence, tannins are extracted by 40–50% ethyl alcohol better [1]. Our studies have shown, that the maximum extraction of tannins from the Geranium sibiricum L. herb is achieved by using 50% ethyl alcohol (Table 1). Therefore, we used 50% ethyl alcohol as extractant. To extract tannins we used extraction before equilibrium, which in this case occurs within 45 minutes (Table 1) when the ratio of raw materials and solvent is 2:250.

Thus, were chosen the extraction conditions of tannins from raw geranium sibiricum: about 2,0 grams (accurately weighed) herb geranium sibiricum milled to a particle size of 1 mm were placed in a flask with ground glass with a capacity of 500 ml, is filled with 250,0 ml of 50% ethyl alcohol. The flask was weighed to an accuracy of ± 0,01 g, attached to a reflux condenser and heated in a boiling water bath within 45 minutes, shaking periodically to flush content from the walls of the feed particles. Then the flask was cooled to room temperature, weighed, and if necessary adjusted to the initial weight of 50% ethyl alcohol. The resulting extract was filtered through filter paper, discarding the first 10,0 ml of the filtrate.

Further extraction was used for the determination of tannins in three ways: permanganometric, spectrophotometric and complexometric.

During the complexometric determination of tannins in Geranium sibiricum L. raw material it was found out, that washing of the precipitate zinc – tannins with 0,25% ammonia solution does not provide a full release from the zinc ions. This is proved by repeated washing of the precipitate 96% ethyl alcohol and the formation of the red – violet color when adding to a mixture consisting of 10,0 ml of acetate buffer, 100,0 ml of purified water and 10,0 ml of xylene – orange. Thereby, the formed complex zinc- tannins, except washing 0,25% ammonia solution, was washed with 20,0 ml of 96% ethyl alcohol.

For complexometric determination of tannins 10,0 ml of obtained extract was placed in a tube for centrifugation with a capacity of 50,0 ml, then 10,0 ml of precipitation reagent (solution 1% zinc oxide in an ammonia buffer solution) was added, and the mixture was stirred with a glass rod. The rod was washed with 5,0 ml of purified water, which was added to the main mixture. After 30 minutes the mixture was centrifuged for 10 minutes at a rotation speed of 5–6 thousand revolutions per minute. The liquid was decanted from the precipitate, the

precipitate in the tube was suspended in 20,0 ml of 0,25 % ammonia solution, added to the centrifuged mixture. After 10 minutes centrifugation, the washing liquid was decanted and discarded. The precipitate was washed with 20,0 ml of 96 % ethyl alcohol and centrifuged for 10 minutes. The washing liquid was decanted and discarded, and the precipitate was dissolved in 3,0 ml of 30 % acetic acid. The resulting solution was quantitatively transferred to a 250 ml flask with 100,0 ml of purified water, the liquid was neutralized with 25,0 ml of 5 % sodium hydrogencarbonate, was added 0,5 ml of xylene and the orange solution was titrated with Trilon B 0,01 mol/l to turn red – purple color of the solution into yellow. Tannin content (X2) in percent based on the air – dry raw material was calculated by the formula:

$$X2 = \frac{V \cdot k \cdot 0{,}00151 \cdot 250 \cdot 100 \cdot 100}{m \cdot 10 \cdot (100 - W)},$$

where V – volume of solution trilon B 0,01 mol/ l, consumed for the titration of the extract, ml; k – Amendment to titrate of Trilon B 0,01 mol/l, g, 0,00151 – amount of tannins, corresponding to 1 ml of the solution Trilon B 0,01 mol/l, r; m – mass of raw material, g, 250 – total recovery ml, 10 – the volume of extract, taken for titration, mL; W – humidity, %.

Two batches of Geranium sibiricum L. raw material were analyzed with proposed methods. The results of quantification of tannin is shown in Table 2.

Table 2

The content of tannins in the Geranium sibiricum L. herb (n = 5, P = 95 %)

Place and time of procurement of raw materials	Metrological characterization of methods				
	X, %	S^2	S_x	E_α	$E_{отн}$
Spectrophotometric method					
Kursk region, 2012.	5,9	0,00625	0,07906	0,22	3,73
Orel region, 2013.	7,02	0,00485	0,06964	0,19	2,71
Permanganometric method					
Kursk region, 2012.	22,9	0,1	0,31623	0,88	3,88
Orel region, 2013.	29,63	0,23413	0,48387	1,35	4,56
Complexometric method					
Kursk region, 2012.	13,1	0,025	0,15811	0,44	3,36
Orel region, 2013.	15,34	0,02308	0,15192	0,42	2,74

The content of tannins, determined by different methods, showed, that the highest content of tannins was determined with a permanganometric method (17,86–29,63 %) and the lowest one was determined with a spectrophotometric method (5,7–7,02 %). Complexometric method showed increase of the content of tannins compared to spectrophotometric method that is apparently due to the fact that recalculation, according to this allocation method, based on tannin. Inaccuracy of methods for quantitation of tannins ranged from 3,88 to 4,56 % with the use of permanganometric method, from 2,71 to 3,73 % with the use of spectrophotometric determination and from 2,74 to 3,36 % with the use of complexometric method.

Conclusions: method of complexometric determination of tannins in the Geranium sibiricum L. herb was modified, optimal conditions for their extraction were defined. A comparative assessment of the content of tannins with the use of three methods was made. Among three proposed methods for standardization of raw materials of Geranium sibiricum L. herb the method of complexometric titration can be recommended as the most accurate.

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