

*Materials of Conferences***THE INVESTIGATION OF ESSENTIAL OIL OF THE THYMUS PALLASIANUS L. HERB**

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In Russian scientific medicine there are official plants from the *Thymus* genus: *Thymus serpyllum* L. and *Thymus vulgaris* L., which are used as expectorant means. The *Thymus vulgaris* L. grows in the Mediterranean countries. The main area of a *Thymus serpyllum* L. is the European part of Russia, but its resources are strongly exhausted. Now in the area of the Central Chernozem region the thyme is rarely spread, but along with it there grows about 7–8 close types in the territory of the specified areas: a *Thymus marschallianus* L., a *Thymus pulegioides* L., a *Thymus calcareus* L. and *Thymus pallasianus* L.

As a rule during preparation of raw materials of a thyme suppliers don't distinguish types therefore there can be other types of raw materials along with a thyme and instead of it, widespread in this region and their different combinations. However, the species of a thyme widespread in the midland of the European part of Russia belong to various sections and subsections and naturally differ by morphological features and by chemical structure, according to the contents and qualitative structure of essential oil, as one of the main classes of biologically active agents of plants of a *Thymus* genus.

The thyme herb contains essential oil which includes aromatic terpenes: thymol; cymophenol; monoterpenes, sesquiterpenes, the content of which strongly varies depending on a place of growth, a habitat, height above a sea level, phases of development of a plant, etc.

There are more than 8 chemotypes of essential oil of *Thymus* genus plants, one of them has prevailing components such as thymol and cymophenol, while the other has in α -terpyneol, the third has geraniol, etc.

Thus, it was interesting to study a qualitative and quantitative composition of essential oil of a *Thymus pallasianus* L. belonged to the flora of the Midland of Russia.

Materials and methods. The object of research was the dry crushed herb of a *Thymus pallasianus* L. collected in the period of mass flowering in 2012 in the Voronezh region (area of Liski town).

For receiving and quantitative definition of essential oil a hinge plate of dry raw materials of a *Thymus pallasianus* L. (20 g) was placed in a flask, filled in 300 ml of water, closed with a stopper with the refrigerator and the receiver and brought to boiling. Distillation time from the boiling moment is

2 hours. After cooling the volume of essential oil in the receiver was determined.

For the analysis of essential oil 0,5–5,0 g of the crushed dried-up *Thymus pallasianus* L. herb was placed into «Agilent» vial for 20 ml, added the internal standard (tridecane), at the rate of 50 mkg on a hinge plate, then added 10 ml of cleared water, fastened a cover with the refrigerator with air cooling and placed in a small sandy bath with adjustable heating and heated within 2 hours. In the process of distillation flying substances were adsorbed on an inner surface of the return refrigerator. The adsorbed substances after cooling of system washed away 3 ml of especially pure pentane into dry vial for 10 ml. The washout was concentrated by a purge (100 ml/min.) especially pure nitrogen up to the residual volume of extract 10 mkl which was completely selected with a chromatographic syringe. A further concoction of test was carried out in the syringe up to the capacity of 2 mkl.

The test input into a chromatographic column was carried out in the splitless mode, i.e. without stream division that allows to enter test without loss on division and it increases sensitivity of a chromatographic method.

The research of a component composition of essential oil was conducted with a method of a gas-liquid chromatography on the Agilent Technology 6890 N chromatograph with the mass and spectrometer detector 5973 N. Analysis conditions: the chromatographic column capillary DV-5, length of a column is 30 m, internal diameter is 0,25 mm; gas carrier – helium, speed of gas carrier is 1 ml/min.; test volume – 2 mkl, the speed of test input is 1,2 ml/min. within 0,2 minutes; temperature of the thermostat is 50°C with programming 3°/min to 220°C; temperature of the detector and evaporator of 250°C.

The components of essential oil were identified as a result of comparison of mass spectrums of the substances entering into studied essential oil, received in the course of a chromatography with library data of mass spectrums of NIST05 and WILEY 2007 with total of ranges more than 470000 in a combination with programs for AMDIS and NIST identification.

The quantitative maintenance of components was counted with the use of a method of the internal standard.

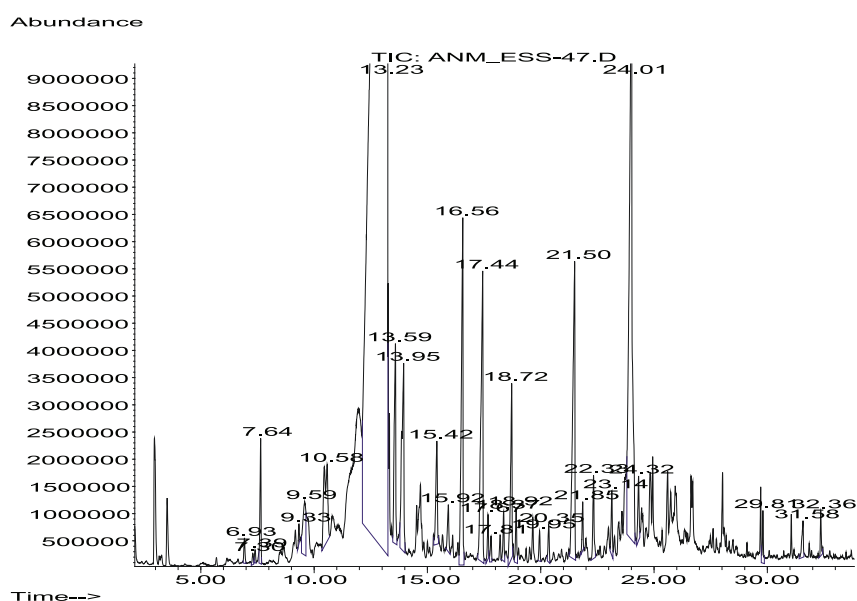
Results and discussion. At the first stage we carried out a quantitative definition of essential oil by a volume method at the basis of which distillation with water vapor lies.

As a result it was established that the content of essential oil in a *Thymus pallasianus* L. herb fluctuates from 0,22 to 0,58%. Essential oil represents easily mobile liquid of yellowish color with a pleasant specific smell.

The analysis of essential oil of a *Thymus pallasianus* L. herb showed on the chromatogram not less than 40 substances from which 29 were identified (Figure, Table)

Composition of essential oil of a *Thymus pallasianus* L.

Number	Keeping time	Name of a Component	Maintenance of Components in samples, %
1	6,92	1,3,8-p-menthatrien	0,08
2	7,29	α -terpynen	0,04
3	7,39	p-cimen	0,04
4	7,64	Limonene	0,37
5	9,33	Terpynolen	0,05
6	9,58	Linalool	0,43
7	10,57	Camfora	0,86
8	13,23	α -terpynenol	37,56
9	13,59	Trans-carveol	0,88
10	13,95	Carvon	1,14
11	15,41	Bornylacetate	0,46
12	15,92	Thymol	0,18
13	17,44	α -terpynilacetate	1,67
14	17,67	Trans-detsenal	0,13
15	17,80	Nerilacetate	0,08
16	18,36	Geranilacetate	0,15
17	18,71	β -burbonen	0,77
18	18,91	β -elemen	0,18
19	19,95	β -cubeben	0,09
20	20,35	β -caryophillen	0,12
21	21,50	Germakren D	1,82
22	21,85	Bicyclogermakren	0,14
23	22,33	α -farnesen	0,30
24	23,14	Burbonanol	0,22
25	24,00	Nerolydol	4,75
26	24,32	Salvial-4(14)-en-1-on	0,23
27	29,81	Hechsahydrofarnesilacetone	0,14
28	31,57	Palmitic acid	0,16
29	32,35	Farnesol	0,12



The scheme of chromatogram chromatomass and spectral analysis of essential oil of a *Thymus pallasianus* L. herb

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 sanguineum* 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; photocolometric, which are based on reactions 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*