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Variability in essential oil compositions of *Thymus pulegioides* L. and *Thymus serpyllum* L. growing in the Republic of Belarus

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Abstract
The present paper represents the study of chemical variability of the thyme herb (*Thymus pulegioides* L. and *Thymus serpyllum* L.) growing wild on the territory of the Republic of Belarus. As a result of GC/MS analysis it was found that the predominant components of the essential oil of *Thymus serpyllum* L. are caryophyllene oxide (2.0-33%), borneol (3.8-29%), camphor (4.2-28%), *β*-caryophyllene (1.1-23%), 1,8-cineol (0-23%). The content of thymol and carvacrol was very low (0-3.59% and 0-3.69%, respectively). In contrast, the predominant components of the essential oil of *Thymus pulegioides* L are carvacrol (15-78%), thymol (0.21-41%), p-cymene (0-26%), carvacrol methyl ether (0-22%), *β*-caryophyllene (0-20%). Using cluster analysis 5 chemotypes of *Thymus serpyllum* L. and *T. pulegioides* L were identified. Among *Thymus serpyllum* L. chemotypes no thymol/carvacrol chemotype was isolated. On the contrary, all *T. pulegioides* L. chemotypes contained carvacrol, as a predominant component in the essential oil composition, which makes *T. pulegioides* L. one of the most perspective species as a source of the crude drug among two species under the study.

Keywords: *Thymus pulegioides* L., *Thymus serpyllum* L., essential oil composition, chemotype, carvacrol, thymol, the Republic of Belarus

1. Introduction

The Thyme is one of the most abundant medicinal herbs growing on the territory of the Republic of Belarus and which is widely used in medical treatment of a broad spectrum of respiratory tracts illnesses [1]. In the Republic of Belarus the genus of thyme comprises three species: *Thymus pulegioides* L., *Thymus serpyllum* L., *Thymus Marschallianus Willd*. The first two are the most widely-spread. Historically and traditionally *Thymus serpyllum* L. is considered to be the source of *Serpylli herba*. Moreover, exactly this species of thyme is recommended by the State Pharmacopoeia of the Republic of Belarus as a crude drug [1].

The suppliers during the procurement of thyme crude drug, as a rule, do not distinguish various species of thyme, so crude drug can consist both of only one type of thyme (*Thymus serpyllum* L. or *Thymus pulegioides* L.*)* and their mixture in different proportions. As a result, the absence of dominant components such as thymol and carvacrol could be found.

What is more the existence of a number of chemotypes within one species cause serious difficulties during herb procurement. This assumption is based on the reports obtained through the modern Web-application Plant Chem DB (Plant Chemotype Data Base) on the basis of literature data [2]. In table 1 and table 2 there are pieces of such reports indicating a high variability of thyme essential oil composition.

Table 1: The chemical variability of *Thymus pulegioides* L.

Location	Chemical composition
Lithuania [3]	*α*-terpinyl acetate (49,5-70,4%), *β*-caryophyllene (6,2-11,5%), *β*-bisabolene (2,8-5,1%), carvacrol (0,2-5,6%), *α*-terpineol (2,3-3,4%)
	geraniol (16,3-29,2%), geranial (*E*-citral) (9,7-16,1%), *β*-caryophyllene (7,4-9,6%), linalool (0,4-13,7%), neral (*Z*-citral) (1,1-9,5%)
	carvacrol (16-25,5%), *β*-caryophyllene (11,4-15,8%), *β*-bisabolene (11,1-12,2%), *γ*-terpinene (5,9-14,5%), carvacrol methyl ether (5,9-8,9%)
Lithuania [4]	thymol (26,05-30,91%), p-cymene (10,21-11,36%), *β*-caryophyllene (10,13-10,27%), *γ*-terpinene (7,7-9,89%), thymol methyl ether (4,94-11,84%)
	carvacrol (5,85-32,76%), *γ*-terpinene (11,06-30,55%), p-cymene (7,52-27,43%), *β*-caryophyllene (5,21-11,95%), carvacrol methyl ether (3,74-8,61%)
	carvacrol (12,4-22,61%), p-cymene (13,4-16,03%), *γ*-terpinene (8,02-20,7%), thymol (11,77-14,2%), *β*-bisabolene (4,73-8,99%)
	geraniol (2,52-43,78%), geranial (*E*-citral) (11,39-29,36%), nerol (0,84-18,89%), *β*-caryophyllene (2,23-15,76%), neral (*Z*-citral)
	linalool (80,29%), *β*-caryophyllene (5,68 %), germacrene D (2,67%), *β*-bisabolene (1,37%), caryopyllene oxide (0,52%)
Romania [5]	carvacrol (50,5-62,6%), *γ*-terpinene (9,8-9,9%), p-cymene (5,8-7,1%), *β*caryophyllene (5,1-7,8%), thymol (1,6-6,6%)
Denmark [6]	(*Z*)-sabinene hydrate (62,1-63,2%), *β*-myrcene (3,9-8,7%), *γ*-terpinene (3,66,8%), *β*-bisabolene (2,3-6,5%), germacrene D-4*β*-ol (3,3-4,5%)
France [7]	*α*-terpinyl acetate (70-88%), *α*-terpineol (1,2-8,3%), geraniol (0-2,6%), 1-octen-3-yl acetate (0-2,5%), sabinene (0,9-1,3%)
	α-terpinyl acetate (64,8-70%), *α*-terpineol (6,9-20,2%), geraniol (0-6,3%), 1-octen-3-yl acetate (2,3-2,7%), nerol (0-2,7%)
	thymol (39,3-59,6%), *γ*-terpinene (13,1-14,1%), thymol methyl ether (3,2-15,5%), p-cymene (4,9-7,9%), *β*-caryophyllene (5,4-6,5%)
	carvacrol (41,7-55,3%), *γ*-terpinene (14-24,5%), p-cymene (6,6-9,6%), *β*bisabolene (3,7-7,2%), *β*-caryophyllene (2,5-7,5%)
Slovakia [8]	fenchone (25,4-41,4%), *β*-caryophyllene (4,1-16,7%), citral (3,7-8,7%), *γ*-terpinene (2,9-8,9%)
	linalool (49,4-60,2%), citral (7,5-11,1%), *β*-caryophyllene (5,8-11,2%), geraniol (2,2-5%), carvacrol (0-0,9%)
	citral (23-35,2%), geraniol (16,8-28%), *β*-caryophyllene (11,3-17,9%), linalool (2,6-5,6%), carvacrol (0,2-2,4%)
	thymol (16,8-24,8%), *β*-caryophyllene (10,5-19,7%), *γ*-terpinene (10,9-18,9%), carvacrol (3,9-17,7%), p-cymene (6,7-13,1%)
	carvacrol (28,9-36,9%), *β*-caryophyllene (14,4-18,8%), *γ*-terpinene (14,7-19,7%), p-cymene (7,3-10,7%), linalool (0,2-2,2%)
Norway [9]	carvacrol (35,24%), *γ*-terpinene (24,17%), p-cymene (10,18%), *β*-caryophyllene (6,83%), *β*-bisabolene (4,82%)
	thymol (37,24%), *γ*-terpinene (23,24%), p-cymene (9,25%), *β*-caryophyllene (5,05%), thymol methyl ether (3,62%)
Portugal [10]	thymol (26,0%), carvacrol (21,0%), *γ*-terpinene (8,8%), p-cymene (7,8%), octan-3-one (3,9%)
Croatia [11]	geraniol (18,4-31,9%), linalool (11,3-37,4%), thymol (2,0-14,8%), thymol methyl ether (4,0-9,3%), borneol (2,8-8,9%)

As there is no available information about the oil composition of thyme species growing on the territory of the Republic of Belarus, the study of the chemical variation of thyme essential oil is of a great importance nowadays. In this regard, the necessity to study the essential oil composition of thyme herb, growing on the territory of the Republic of Belarus has appeared.

The aim of this work is to study the chemical variability of essential oil composition of the thyme herb growing on the territory of the Republic of Belarus.

Table 2: The chemical variability of *Thymus serpyllum* L.

Location	Chemical composition
Lithuania [4]	1,8-cineole (29,5-30,32%), *β*-caryophyllene (4,52-16,09%), germacrene D (3,17-13,75%), *β*-myrcene (5,57-9,68%), caryophyllene oxide (2,31-6,06%)
	(*Z*)-carvyl acetate (21,58%), 1,8-cineole (13,87%), *β*-myrcene (12,15%), *β*-caryophyllene (5,43%), *τ*-cadinol (4,66%)
	(*Z*)-*β*-ocimene (34,78%), *α*-copaene (8,68%), *β*-myrcene (5,66%), borneol (4,1%), *β*-caryophyllene (2,35%)
	caryophyllene oxide (25,43-27,15%), borneol (4,67-27,05%), camphor (9,46-12,08%), *τ*-cadinol (2,24-14,13%), linalool (0,9-3,03%)
	β-caryophyllene (23,32-27,18%), germacrene D (13,5-20,38%), *β*-myrcene (9,1-15,85%), *β*-bisabolene (6,66-8,46%), camphor (2,35-6,23%)
	(*Z*)-p-menth-2-en-1-ol (24,06%), caryophyllene oxide (13,83%), camphene (9,5%), *β*-myrcene (7,3%), borneol (6,02%)
Estonia [12]	(*E*)-nerolidol (20,5-70,1%), caryophyllene oxide (4,2-25%), *β*-caryophyllene (1,8-13,3%), germacrene D (1,7-6,8%), thymol (0,2-2,9%)
	(*E*)-nerolidol (15,2-30,4%), *β*-myrcene (6,4-20,2%), germacrene D (3,1-10,2%), *β*-caryophyllene (2,2-9,4%), caryophyllene oxide (1,4-5,7%)
	β-myrcene (10,5-18,6%), *β*-caryophyllene (10,5-13%), caryophyllene oxide (8,2-10,8%), germacrene D (3,3-11,4%), (*E*)-nerolidol (2,2-6%)
	(*E*)-nerolidol (8,5-29,8%), (*Z*)-*β*-terpineol (7,2-20,2%), 1,8-cineole (2,7-14%), *β*-myrcene (4-10,3%), p-cymene (3,8-5,6%)
Finland [13]	1,8-cineol (19,7%), *β*-myrcene (14,3%), camphor (6,6%), camphene (3,6%), *α*-pinene (2,6%)
	germacrene D (9,5%), *β*-myrcene (9,1%), 1,8-cineol (6,6%), camphor (5,4%), *β*-caryophyllene (4,8%)
Poland [14]	carvacrol (37,49-44,93%), *γ*-terpinene (10,08-10,79%), p-cymene (6,06-7,39%), (*E*)-*β*-ocimene (0,12-4,63%), 3-octanone (0,18-6,19%)
Hungary [15]	carvacrol (25,8%), p-cymene (25,0%), carvacrol methyl ether (11,1%), terpinyl acetate (10,1%), 1,8-cineole (9,5%)
India [16]	thymol (58.8%), p-cymene (5.7%), thymol methyl ether (4.0%), borneol (3.8%), sabinene (3.4%)
Serbia [17]	*β*-caryophyllene (27.7%), *γ*-muurolene (10.5%), *α*-humulene (7.5%)
	(*E*)-nerolidol (24.2%), germacrene D (16.0%), thymol (7.3%), *δ*-cadinene (3.7%), *β*-bisabolene (3.3%)
Kazakhstan [18]	thymol (5,11-58,25%), carvacrol (1,22-55,85%), p-cymene (1,28-25,46%), *γ*-terpinene (1,37-16,52%), *α*-terpinene (0,84-1,94%)

2. Materials and methods

2.1 Plant material

The samples of *Thymus pulegioides* L. and *Thymus serpyllum* L. selected for the study were gathered in summer in 2011 in the places of their growth in the Republic of Belarus during the blooming period and were subjected to air-seasoning in the shade. Before the analysis, the samples were stored in paper bags. Pharmacognostic analysis was carried out according to the State Pharmacopoeia of the Republic of Belarus [1]. Before the analysis the samples were grilled to the particle size 0.5 cm approximately. Voucher specimens were deposited at the Herbarium of Vitebsk's State Medicinal University, Vitebsk, Belarus.

Table 3: Localization of samples of *T. pulegioides* L. and *T. serpyllum* L.

№	Latitude, ◦	Longitude, ◦	№	Latitude, ◦	Longitude, ◦	№	Latitude, ◦	Longitude, ◦
Thymus pulegioides L.								
1	55.258740	30.415030	10	55.254200	30.414540	18	53.106281	25.906309
2	55.243960	30.383060	11	55.288830	30.290610	19	55.043506	29.395332
3	55.315650	30.577240	12	53.222808	31.557368	20	52.198348	24.041805
4	55.255850	30.412430	13	53.885161	30.437774	21	52.779810	27.528281
5	55.241490	30.468330	14	54.486467	26.439116	22	54.853492	29.118044
6	53.232434	27.026158	15	55.641865	27.625676	23	55.416272	27.603396
7	55.249870	30.392500	16	54.292544	26.851611	24	51.816364	24.132875
8	55.247850	30.392600	17	54.888000	30.527148	25	53.594590	23.929330
9	55.289770	30.288770						
Thymus serpyllum L.								
1	54.889790	30.368290	4	55.429560	30.341520	7	55.439450	30.346730
2	54.883930	30.357890	5	55.432050	30.340250	8		
3	54.889780	30.356460	6	55.440640	30.357930	9		

2.2 Isolation of volatile oil

The samples of plant material (only aerial parts of the thyme herb) were exposed to extraction with diethyl ether (the mass ratio was 1:15) for 15 minutes in ultrasonic bath at 20 ◦C. The obtained extracts were filtered and exposed to solvent evaporation using rotary evaporator. The obtained fraction of lipophilic substances was analysed using GC/MS analysis.

2.3 GC/MS analysis

GC/MS analyses were performed with Agilent HP 5890SII model gas chromatograph-mass spectrometer equipped with 6890 autoinjector. Column: HP-INNOWAX 19091 N-205, 50 m × 0.25 mm ID × 0.25 µm film thickness. Temperature program: from 50 ◦C (3 min) to 140 ◦C at 3 ◦C/min, from 140 ◦C (10 min) to 200 ◦C at 7 ◦C /min, from 200 ◦C (10 min) to 255 ◦C (20 min) at 8 ◦C/min. Injection temperature: 250 ◦C. Injection volume: 3.0 µL. Inlet pressure: 130.0 kPa. Carrier gas: He, linear velocity (ū): 3 mL/min. Injection mode: split (50:1). MS interface temp.: 200 ◦C; MS mode: EI; detector voltage: 0.9 kV; mass range: 50-550 u. Data handling was made through Chem Station.

These optimal conditions were determined through the analysis of model mixtures, which contained all classes of substances found in the essential oil.

2.4 Identification of components

Quantitative analysis was based on the comparison of retention indexes and all mass-spectra with the appropriate dates of standard components of essential oil and pure substances (thymol, carvacrol and p-cymene), purchased from Fluka and Sigma-Aldrich, and with the data of the library of mass spectral library (NIST08 and Willey275). The mass spectrum of the substances under the study was considered to be identified if the similarity coefficient with library mass spectra was more than 80%.

The percentage composition of essential oils was calculated based on areas of gas-chromatographic picks without using correction indexes.

3. Results & Discussion

During the study of the composition of the essential oil of *Thymus serpyllum* L., growing on the territory of the Republic of Belarus, it was found that the predominant components of the essential oil in relation to the sum of identified substances are camphene (1.8-13%), *β*-myrcene (2.3-15%), 1,8-cineol (0-23%), camphor (4.2-28%), *β*-caryophyllene (1.1-23%), borneol (2.0-33%), caryophyllene oxide (3.8-29%) (Table 4). The quantity of thymol and carvacrol in the composition of the essential oil is only 0-3.59% and 0-3.69%, respectively.

Table 4: GC/MS data of *Thymus serpyllum* L. essential oil composition

№	Compound	Sample № of *Thymus serpyllum* L.								
		1	2	3	4	5	6	7	8	9
1	Camphene	1,8	11	12	11	9,8	4,0	9,7	13	5,4
2	*β*-Pinene	-	-	0,40	0,54	0,40	0,54	0,54	0,35	1,3
3	Sabinene	0,40	-	-	-	-	-	-	-	-
4	*β*-Myrcene	2,3	6,5	3,4	15	8,8	5,3	6,5	11	4,0
5	Limonene	-	-	-	0,51	0,31	0,32	-	0,42	0,22
6	1,8-Cineole	1,1	-	-	0,64	-	-	-	-	23
7	*β*-Ocimene	-	0,56	-	0,41	0,18	-	-	0,26	3,0
8	Δ-3-Carene	0,73	-	-	-	2,5	-	-	-	-
9	(*Z*)-Sabinene hydrate	-	-	-	0,88	0,95	0,81	2,7	0,51	1,3
10	Camphor	4,2	17	28	14	14	15	22	20	16
11	Bornyl acetate	-	-	5,14	4,33	2,63	5,97	4,44	8,61	-

12	*β*-Caryophyllene	19	23	12	7,3	11	9,4	8,5	1,1	12
13	Borneol	33	2,0	12	20	27	31	19	24	15
14	Germacrene D	2,2	3,0	3,5	0,34	0,18	8,3	3,3	8,2	4,1
15	*β*-Bisabolene	5,1	-	-	0,85	0,15	1,6	0,40	-	-
16	*α*-Farnesene	2,5	0,78	-	2,6	2,4	1,7	1,3	0,32	0,44
17	*β*-Sesquiphellendrene	1,2	5,0	1,4	1,5	-	-	0,76	0,86	-
18	Geraniol	-	0,67	-	-	-	-	-	-	-
19	(*E*)-Pinene	0,34	-	-	0,24	-	-	0,22	-	-
20	Caryophyllene oxide	11	29	12	3,8	16	14	14	11	9,8
21	Germacrene D-4-ol	1,8	-	7,6	6,5	-	0,38	3,46	-	-
22	Spathulenol	5,1	-	2,8	-	2,5	0,38	3,60	0,90	-
23	Thymol	0,68	2,3	0,43	0,68	-	0,27	-	0,22	3,6
24	Carvacrol	0,51	-	-	3,7	-	1,6	-	0,29	0,63
25	Geranyl acetate	3,2	-	-	-	-	-	-	-	-
26	(*E*)-Nerolidol	2,0	-	-	5,9	1,2	-	-	-	-

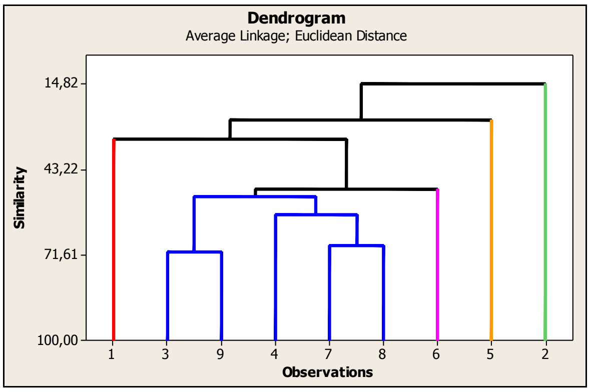

Fig 1: Cluster analysis dendrogramm of data matrix of *Thymus serpyllum* L.

We tried to classify the obtained data on the chemical composition of the essential oil of the crude drug of *Thymus serpyllum* L. For this purpose, the cluster analysis was applied using statistical package MINITAB 14.0. The Euclidean distance was taken as a criterion of distance. The obtained results are shown in the diagram (Figure 1).

The distribution of the aggregate examined samples of *Thymus serpyllum* L. can be represented in 5 groups or clusters respectively based on the results of cluster analysis. This allows distinguish the following chemotypes of the observed species of thyme according to the principle of the predominance of the main components in the composition of the essential oil (Table 5):

1. Borneol + *β*-Caryophyllene chemotype
2. Camphor + Borneol chemotype
3. Borneol + *β*-Myrcene chemotype
4. 1,8-Cineole + Camphor chemotype
5. *β*-Caryophyllene + Caryophyllene oxide chemotype

Table 5: Composition of isolated chemotypes of *Thymus serpyllum* L.

Sample №	Chemotype				
	I	II	III	IV	V
	1	3, 9, 4, 7, 8	6	5	2
Compound	Area, %				
Camphene	1,8	4-13	11	5,4	11
β-Myrcene	2,3	3,4-11	15	4	6,5
1,8-Cineole	1,1	0	0,64	23	0
Camphor	4,2	14-28	14	16	17
β-Caryophyllene	19	1,1-12	7,3	12	23
Borneol	33	12-31	20	15	2
Caryophyllene oxide	11	11-16	3,8	9,8	29

Because of so low content and even the absence of thymol and carvacrol, as the main components, which are responsible for the therapeutic effect of the thyme herb, the species *Thymus serpyllum* L. does not correspond to the requirements of medicinal crude drug according to the State Pharmacopeia of the Republic of Belarus. What is more, low content of phenols in the essential composition questions the suitability of *Thymus serpyllum* L. as a source of crude drug for *Serpylli herba* [1].

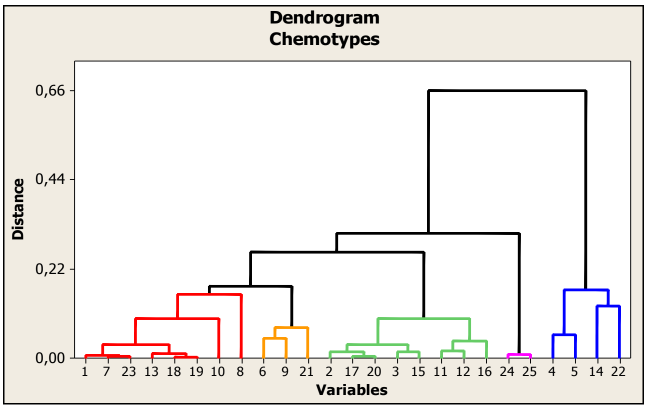

Fig 2: Cluster analysis dendrogramm of data matrix of *Thymus pulegioides* L.

What concerns extracts of *Thymus pulegioides* L. it was found that the predominant components of the essential oil relative to sum of identified substances of essential oil are *α*-terpinene (0-15%), *γ*-terpinene (0-20%), p-cymene (0-26%), thymol methyl ether (0-9%), *β*-caryophyllene (0-20%), carvacrol methyl ether (0-22%), *β*-bisabolene (0-14%), thymol (0.21-41%) and carvacrol (15-78%) (Table 6). High content of thymol and especially carvacrol in the essential oil composition of *Thymus pulegioides* L. should be noted. The results of cluster analysis are shown in figure 2.

Table 6: GC/MS data of *Thymus pulegioides* L. essential oil composition

№	**Compound**	**Sample № of *Thymus pulegioides L***												
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1	Camphene	1,0	2,1	-	-	0,1	-	-	0,8	-	0,4	0,6	-	-
2	*β*-Pinene	0,1	-	-	-	0,1	0,6	0,1	1,4	0,1	0,1	-	-	0,4
3	Sabinene	-	-	-	-	-	-	-	0,5	0,1	-	-	-	0,1
4	*β*-Myrcene	0,7	-	1,2	0,6	0,4	-	0,8	-	0,8	0,8	0,9	0,8	0,9
5	*α*-Terpinolene	-	-	-	-	-	0,2	-	-	-	-	0,1	-	0,3
6	*α*-Terpinene	0,9	-	0,5	0,2	0,5	0,3	0,2	14,9	2,4	1,4	1,3	1,4	0,2
7	*β*-Phellandrene	-	-	-	-	-	-	0,1	1,4	-	-	-	-	-
8	*γ*-Terpinene	8,5	-	3,4	1,8	5,6	15,0	9,3	6,0	9,7	2,2	15,6	9,4	3,2
9	p-Cymene	9,9	4,1	-	7,8	17,1	-	10,3	12,8	4,0	0,3	5,9	9,1	-
10	allo-Ocimene	-	-	-	-	-	-	-	-	-	-	-	-	0,6
11	(*E*)-Sabinene hydrate	0,5	-	0,9	0,6	1,3	-	0,8	0,5	1,3	-	0,8	0,8	0,9
12	*α*-Copaene	-	-	-	-	0,1	-	-	-	0,1	0,2	0,1	-	0,2
13	(*Z*)-Sabinene hydrate	-	-	-	0,1	0,3	-	0,2	-	-	1,0	0,2	0,2	-
14	Linalool	-	-	-	-	-	1,0	0,5	0,9	-	0,6	0,7	0,4	0,7
15	Bornyl acetate	-	-	-	-	-	-	-	1,7	-	-	-	-	-
16	Thymol methyl ether	2,5	-	-	2,7	4,2	0,5	3,4	1,0	1,1	8,6	-	1,4	1,1
17	*β*-Caryophyllene	-	-	-	12,3	11,7	16,2	-	2,8	19,7	0,2	-	-	-
18	Carvacrol methyl ether	-	16,4	17,4	-	-	-	-	1,6	-	-	17,5	17,0	2,2
19	(*E*)-*β*-Farnesene	-	-	0,3	-	0,1	-	0,2	-	0,1	0,2	0,1	0,2	-
20	*α*-Humelene	-	-	-	0,3	-	-	0,2	-	-	0,5	0,2	0,2	0,4
21	Borneol	4,6	4,3	-	-	-	-	-	0,3	-	2,8	3,1	-	-
22	Germacrene D	0,9	1,5	0,6	0,3	0,4	0,5	0,4	0,3	1,4	1,0	1,0	0,4	0,8
23	*β*-Bisabolene	11,6	7,1	11,3	7,1	7,3	9,3	11,8	6,1	13,1	11,8	7,8	11,4	13,5
24	*α*-Farnesene	-	-	-	0,3	-	-	0,3	-	0,2	0,1	-	-	0,3
25	Δ-Cadinene	0,4	-	0,2	0,3	0,2	0,2	0,2	0,2	0,3	0,3	0,8	0,3	0,4
26	*α*-Amorphene	0,2	-	-	0,2	0,1	-	-	0,1	0,2	0,3	-	0,2	0,3
27	*β*-Sesquiphellandrene	0,3	-	-	0,3	-	0,3	0,4	-	-	-	-	-	-
28	(*Z*)-*α*-Bisabolene	-	-	0,3	-	-	-	-	-	0,4	0,3	0,2	-	-
29	Geraniol	-	-	0,5	0,6	2,0	-	-	-	-	-	-	-	-
30	p-Cymen-8-ol	0,3	0,8	-	-	-	-	0,3	0,7	-	-	0,1	0,2	0,1
31	Carvacrol acetate	-	-	-	-	-	-	-	-	0,1	0,1	-	-	-
32	Caryophyllene oxide	1,9	5,8	-	2,1	2,7	0,7	1,6	2,6	1,0	1,4	0,9	0,9	1,8
33	Germacren D-4-ol	-	-	-	-	-	-	-	-	-	-	-	-	-
34	Thymol	0,5	2,4	0,8	37,9	30,3	12,6	1,1	5,2	0,7	18,7	0,2	1,5	8,3
35	Carvacrol	55,1	55,4	62,5	24,8	15,4	42,8	57,9	38,2	43,4	46,7	41,8	44,2	63,5

№	**Compound**	**Sample № of *Thymus pulegioides L.***											
		14	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**
1	Camphene	-	-	-	-	-	-	-	-	0,1	0,1	0,1	-
2	*β*-Pinene	-	-	-	-	0,1	0,1	0,2	0,3	-	0,1	0,2	0,2
3	Sabinene	-	-	-	-	-	-	-	-	-	-	-	-
4	*β*-Myrcene	-	0,6	0,2	-	-	-	0,9	0,1	-	-	-	-
5	*α*-Terpinolene	0,1	0,3	0,2	0,2	0,2	0,2	0,2	2,0	0,3	1,0	-	1,2
6	*α*-Terpinene	-	0,6	0,2	-	-	-	0,9	0,1	-	-	-	-
7	*β*-Phellandrene	-	-	-	0,2	-	-	-	-	0,1	0,1	0,3	-
8	*γ*-Terpinene	2,0	2,5	9,5	3,4	2,4	3,2	3,0	2-	8,5	9,3	6,9	6,5
9	p-Cymene	9,6	4,6	13,9	8,3	0,9	-	10,7	5,6	-	9,0	25,9	24,8
10	allo-Ocimene	-	0,2	-	-	0,5	-	-	0,1	-	0,7	-	-
11	(*E*)-Sabinene hydrate	0,6	0,5	0,3	-	1,0	0,7	0,5	1,0	-	0,8	0,8	1,3
12	*α*-Copaene	-	-	-	-	0,2	0,2	-	0,2	-	-	0,3	0,3
13	(*Z*)-Sabinene hydrate	-	-	-	-	0,2	-	-	0,2	-	-	0,2	0,5
14	Linalool	-	-	-	-	0,5	0,5	-	-	1,2	0,5	-	0,4
15	Bornyl acetate	-	-	-	-	-	-	-	-	-	-	-	-
16	Thymol methyl ether	2,8	0,2	0,7	0,2	0,6	0,4	0,2	2,7	1,8	0,9	1,0	0,9
17	*β*-Caryophyllene	-	-	-	-	-	-	-	4,5	16,3	-	3,3	-
18	Carvacrol methyl ether	2,4	22,2	21,0	13,4	2,4	2,6	15,3	-	-	1,9	2,6	2,9
19	(*E*)-*β*-Farnesene	-	-	0,4	-	0,2	0,2	-	0,2	-	0,1	0,1	-
20	*α*-Humelene	0,2	-	-	-	0,2	0,3	-	0,3	-	0,4	0,2	0,2
21	Borneol	-	-	-	-	-	-	-	-	-	-	-	-

22	Germacrene D	0,4	0,2	1,1	0,5	0,3	0,3	-	1,0	0,6	0,3	0,4	0,4
23	*β*-Bisabolene	-	12,5	11,6	5,4	11,7	9,2	7,3	11,5	8,3	11,3	13,3	13,7
24	*α*-Farnesene	0,6	-	-	-	0,2	0,2	-	0,3	-	0,2	-	-
25	Δ-Cadinene	0,4	0,2	0,5	0,4	0,3	1,1	0,2	1,3	0,3	0,5	0,6	0,8
26	*α*-Amorphene	0,6	-	-	0,2	0,2	-	-	-	0,1	0,2	0,4	0,3
27	*β*-Sesquiphellandrene	-	-	-	-	-	-	-	-	-	-	-	-
28	(*Z*)-*α*-Bisabolene	-	-	-	0,1	-	-	0,2	-	-	-	-	-
29	Geraniol	1,4	-	-	-	-	-	-	-	2,0	-	-	-
30	p-Cymen-8-ol	-	0,3	0,2	-	0,1	0,1	0,2	0,1	-	0,1	0,4	0,3
31	Carvacrol acetate	-	0,1	-	-	0,6	-	-	-	-	-	-	-
32	Caryophyllene oxide	2,4	2,6	2,5	0,9	-	0,6	1,0	0,4	1,3	1,0	1,2	1,4
33	Germacren D-4-ol	-	-	-	-	-	-	-	-	-	-	-	-
34	Thymol	40,6	1,0	1,4	1,0	3,6	1,4	2,6	1,0	27,6	4,4	11,9	10,1
35	Carvacrol	35,5	51,4	36,0	65,5	72,4	77,7	57,2	44,9	30,8	56,1	29,3	33,3

The distribution of the aggregate examined samples of *T. pulegioides L.* can be represented in 5 groups or clusters respectively based on the results of cluster analysis. This allows distinguish the following chemotypes of the observed species of thyme according to the principle of the predominance of the main components in the composition of the essential oil (Table 7):

1. Thymol + Carvacrol chemotype
2. Carvacrol + p-Cymene chemotype
3. Carvacrol + *γ*-Terpinene chemotype
4. Carvacrol + Carvacrol methyl ether chemotype
5. Carvacrol chemotype

Table 7. Composition of isolated chemotypes of *T. pulegioides* L.

Sample №	**Chemotype**				
	I	**II**	**III**	**IV**	**V**
	4,5,14,22	24,25	6,9,21	2,3,11,12, 15,16,17,20	1,7,8,10, 13,18,19,23
Compound	Area, %				
β-Myrcene	0,3-0,6	0,5-0,6	0-1,7	0-1,2	0-1,0
α-Terpinene	0,1-0,5	0-1,2	0-1,2	0-1,4	0,2-14,9
γ-Terpinene	1,8-8,5	6,5-6,9	9,7-20	0-15,6	2,2-9,3
p-Cymene	0-17,1	24,8-25,9	24,8-25,9	0-13,9	0-12,8
Thymol	27,6-40,6	10,1-11,9	0,7-12,6	0,2-2,6	0,5-18,7
Carvacrol	15,4-30,8	29,3-33,3	42,8-44,9	36-65,5	38,2-77,7
Carvacrol methyl ether	0-2,4	2,6-2,9	-	13,4-22,2	0-2,6
Thymol methyl ether	1,8-4,2	0,9-1	0,5-2,7	0-1,4	0,4-8,6
β-Caryophyllene	0-16,3	0-3,3	4,5-19,7	-	0-2,8
β-Bisabolene	0-8,3	13,3-13,7	9,3-11,5	5,4-12,5	9,2-13,5
Caryophyllene oxide	1,3-2,7	1,2-1,4	0,4-1,0	0-5,8	0-2,6

The common feature of all distinguished chemotypes is the presence of a considerable amount of *β*-bisabolene in its composition along with the substances mentioned above. In all cases, carvacrol predominates quantitatively in the composition of the essential oil, which makes *T. pulegioides* L. be one of the most perspective species as a source of the crude drug among the forms of the *Thyme* complex. Moreover, *T. pulegioides* L. is characterized by higher essential oil accumulation of (0,11-1,4%) [1] compared with *T. serpyllum* L. (0,12-0,27%) [1].

The binding of *Serpylli herba* as a source of the crude drug specifically to *Thymus pulegioides L.* instead of *Thymus serpyllum L.* is fully reasonable and desirable. Incorporation of new species of the crude drug such as *Thymi pulegioidis herba* instead of *Thymi pulegioidis herba* along with the introduction some specifying changes into the appropriate sections of Pharmacopoeia is a more rational decision, as this makes it possible to avoid confusion and misunderstanding, occurring in the procurement and analysis of the thyme herb.

4. Conclusions

As a result of the study of essential oil composition of the thyme herb on the territory of the Republic of Belarus it was found that both *Thymus serpyllum* L. and *Thymus pulegioides* L. are characterized by high variability in the essential oil composition on territory under the study.

The predominant components of the essential oil of *Thymus serpyllum* L., growing on the territory of the Republic of Belarus are camphene (1.75-12.62%), *β*-myrcene (2.26-14.61%), 1,8-cineol (0-23.12%), camphor (4.24-27.59%), *β*-caryophyllene (1.12-22.64%), borneol (2.02-33.39%), caryophyllene oxide (3.79-28.7%). The quantity of thymol (0-3.59%) and carvacrol (0-3.69%) is low.

The predominant components of the essential oil of *Thymus pulegioides* L., growing on the territory of the Republic of Belarus, are *α*-terpinene (0-14.93%), *γ*-terpinene (0-19.99%), p-cymene (0-25.91%), thymol methyl ether (0-8.64%), *β*-caryophyllene (0-19.69%), carvacrol methyl ether (0-22.17%), *β*-bisabolene (0-13.74%), thymol (0.21-40.60%) and carvacrol (15.44-77.71%).

Five chemotypes of each species *Thymus serpyllum* L. and *T. pulegioides* L. were isolated using cluster analysis. Among *Thymus serpyllum* L. chemotypes no thymol/carvacrol chemotype was isolated. On the contrary, all *T. pulegioides* L. chemotypes contained carvacrol, as a predominant component in the essential oil composition.

High variability of chemical composition of the thyme herb is of great interest in terms of the selection of definite chemotypes of these species with its subsequent introduction into the industrial culture.

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