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Comparison of the Chemical Composition and Antimicrobial Activity of *Thymus serpyllum* Essential Oils

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Abstract

The chemical composition of the essential oils obtained by hydrodistillation from the aerial parts of *Thymus serpyllum* and *Thymus serpyllum* 'Aureus' has been investigated by gas chromatography-mass spectrometry (GC-MS). Forty-seven compounds (99.67% of the total oil) were identified in the essential oil of *T. serpyllum*. The main components found in the oil were carvacrol (37.49%), γ-terpinene (10.79%), β-caryophyllene (6.51%), p-cymene (6.06%), (E)-β-ocimene (4.63%) and β-bisabolene (4.51%). Similarly, carvacrol (44.93%), γ-terpinene (10.08%), p-cymene (7.39%) and β-caryophyllene (6.77%) dominated in the oil of *T. serpyllum* 'Aureus'. A total of forty three compounds were identified in this oil, representing 99.49% of the total oil content. On the basis of the obtained data it was proved that the content of 1-octen-3-ol, eucalyptol, (Z)-β-ocimene, (E)-β-ocimene, γ-terpinene, carvacrol methyl ether, germacrene D and β-bisabolene was significantly higher for *T. serpyllum* while *T. serpyllum* 'Aureus' was characterized by a significantly higher content of 3-octanone, 3-octanol, p-cymene, borneol and carvacrol. The isolated essential oils were evaluated for their antimicrobial activity against nine reference strains (*Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus agalactiae, Enterococcus faecalis, Bacillus cereus, Micrococcus luteus, Proteus vulgaris* and *Candida albicans*) by the microdilution technique. Based on this test, the minimum inhibitory concentrations (MIC) of essential oil were calculated. The volatile oil obtained from *T. serpyllum* showed the highest antimicrobial activity relative to the strain of *E. coli* (MIC=0.025 μL/mL) and to the yeast *C. albicans* (MIC=0.05 μL/mL). Similarly, a significant antimicrobial activity exhibited *T. serpyllum* 'Aureus' essential oil, although the MIC values obtained in that case for *E. coli* and *C. albicans* strains were twice as high and were respectively 0.05 μL/mL and 0.1 μL/mL.

Keywords: antimicrobial activity, essential oil composition, hydrodistillation, wild thyme

Introduction

Thymus serpyllum L. (wild thyme, mother of thyme) belongs to the genus *Thymus,* which comprises about 350 species worldwide (Maksimovic *et* al., 2008). The aerial parts of *Thymus* possess antimicrobial (Ismaili *et al.*, 2002), antioxidant (Jukic and Milos, 2005), anti-inflammatory (Broucke and Lemli, 1983), antiviral and expectorant (Nabavi *et al.*, 2015) activities. These valuable properties are attributed with the presence of thymol, carvacrol, p-cymene and γ-terpinene in the essential oil (Dorman and Deans, 2000; Rasooli and Mirmostafa, 2002).

Thymus oils and extracts have found wide applications in cosmetic and perfume industry as well as flavourings and preservative agents for different food products (Guseinov *et al.*, 1987). Due to their antiseptic, antispasmodic and antimicrobial properties, they are also used for medicinal purposes (Jirovetz *et al.*, 2007).

In traditional medicine, the flowering parts and leaves of *Thymus* species plants are mainly used as herbal tea, flavouring agents (condiment and spice), for treating colds, coughs, sore throat and indigestion (Zargari, 1990; Morales 2002; Amin, 2005).

Wild thyme is well-known for its cough-suppressant, antiseptic and spasmolytic properties (Zarzuelo and Crespo, 2002). Especially, a strong decoction, sweetened with honey is recommended for easing spasms of whooping cough (Aziz and Rehman, 2008). The plant can be applied for preparation of herbal tea, herbal baths and herbal pillows (Zarzuelo and Crespo, 2002).

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T. serpyllum is also an important source of substances with antioxidant, antimicrobial and antitumor properties (Jaric *et al*., 2015). Recent studies have shown that *Serpylli aetheroleum* strongly act on fungi and bacteria (Farrukh *et al*., 2012; Sokolic-Mihalak *et al*., 2012; Nikolic *et al*., 2014). The oil is used medicinally as well as in the manufacturing of toothpastes, mouthwashes and gargles (Ahmad *et al*., 2006). According to Aziz and Rehman (2008), it can relieve rheumatism and may be used in hair loss treatments.

The high quality of essential oil is one of the main requirements of pharmaceutical and food industry. The essential oils derived from various *Thymus* species may differ in chemical composition and biological properties. Their market value may also be different.

Our previous studies concerned the influence of distillation time and distillation apparatus on the composition and quality of wild thyme oil (Wesołowska *et al*., 2012; Wesołowska *et al*., 2014).

The medicinal importance and biological activity of *T. serpyllum* volatile oil prompted us to investigate its cultivar 'Aureus'. The present study aimed to compare the chemical composition and antimicrobial activity of the oils extracted from *T. serpyllum* and *T. serpyllum* 'Aureus'. To the best of our knowledge, there are no scientific reports concerning this topic. Similarly, there is lack of publications on the composition and biological activity of oil obtained from *T. serpyllum* 'Aureus'.

Materials and Methods

Plant material

The studied biological material used in the current research consisted of wild thyme (*Thymus serpyllum* L.) and wild thyme 'Aureus' (*Thymus serpyllum* 'Aureus') from the *Lamiaceae* family. All the plants were grown in experimental plots with an area of 1.44 m^2, in four replications at the Horticultural Experimental Station near Szczecin (North-Western Poland), which belongs to the West Pomeranian University of Technology Szczecin.

The seedlings, obtained from older plants, after rooting in horticultural substrate, were planted into the open field in the second half of May 2012, with spacing of 20 x 20 cm. For laboratory analyses, an herb from two-year-old plants was harvested at the flowering stage (harvest date: July 8, 2014). The field was prepared according to agrotechnique proper for thyme cultivation. Mineral fertilization was quantified according to the results of the chemical analysis of the soil samples and supplemented to those recommended for thyme level. In the first year of the experiment only nitrogen (60 kg N ha^{-1}) and potassium (60 kg K_2O ha^{-1}) fertilization was applied, while in the second year – potassium (60 kg K_2O ha^{-1}) and phosphorous (60 kg P_2O_5 ha^{-1}) fertilization.

The experiment was performed on sandy clay soil, which is characterized by low water-holding capacity. During the growing season manual weeding and irrigation were performed.

After the harvest, plant material was dried in a shady and well ventilated place at room temperature (drying room). Dry herb was cut into small pieces and stored (in paper bags in a dry and cool place) until chemical analyses were performed.

Essential oil extraction

Twenty grams of the whole dried aerial parts of *T. serpyllum* and *T. serpyllum* 'Aureus' (separately) in a 1000 mL round-bottomed flask along with 500 mL distilled water was subjected to hydrodistillation for 2 hours using Clevenger apparatus according to the method recommended by European Pharmacopoeia (2010). The obtained essential oils were separated from water, then dried over anhydrous sodium sulphate, filtered and stored in dark sealed vial at low temperature (4 °C) prior to GC-MS analysis.

Gas Chromatography/Mass Spectrometry (GC/MS) analyses of essential oils

The qualitative analysis was conducted using HP 6890 gas chromatograph coupled with HP 5973 Mass Selective Detector operating at 70 eV mode. The essential oil samples (30 mg) were dissolved in dichloromethane (1.5 mL) and 2 μL of each solution were injected in a split mode at a ratio of 5:1. Compounds were separated on 30 m long capillary column (HP-5MS), 0.25 mm in diameter and with 0.25 μm thick stationary phase film ((5% phenyl)-methylpolysiloxane).

The flow rate of helium through the column was kept at 1.2 mL min^{-1}. The initial temperature of the column was 40 °C for 5 minutes, then increased to 60 °C at a rate of 30 °C min^{-1}, next to 230 °C at a rate of 6 °C min^{-1} (kept constant for 10 min), and then increased to a final temperature of 280 °C at a rate of 30 °C min^{-1}. The oven was held at this temperature for 5 minutes. The injector and the transfer line were kept at 280 °C. The ion source temperature was 230 °C. The solvent delay was 4 min. The scan range of the MSD was set from 40 to 550 m/z. The total running time for a sample was about 51 minutes.

The relative percentage of the essential oil constituents was evaluated from the total peak area (TIC) by apparatus software.

Essential oil constituents were identified by comparison of their retention indices (relative to n-alkanes C_7-C_{40} on HP-5MS column) with those reported in NIST Chemistry WebBook (http://webbook.nist.gov/chemistry) and the literature (Adams, 2007).

Further identification was made by comparison of their mass spectra with those stored in the Wiley NBS75K.L and NIST/EPA/NIH (2002 version) mass spectral libraries using different search engines (PBM, Nist02) or with mass spectra of authentic compounds available in our laboratory (thymol, carvacrol and p-cymene), purchased from Fluka and Sigma-Aldrich.

Antimicrobial activity

Nine reference strains were tested: six Gram-positive bacteria *Staphylococcus aureus* (FRI 913), *Staphylococcus epidermidis* (ATCC 49461), *Streptococcus agalactiae* (ATCC 12386), *Enterococcus faecalis* (ATCC 29212), *Bacillus cereus* (ATCC 14579) and *Micrococcus luteus* (ATCC 10240); two Gram-negative bacteria *Escherichia coli* (MG1655) and *Proteus vulgaris* (ATCC 6380) and yeast *Candida albicans* (ATCC 10231).

The bacteria species were maintained in Brain Heart Infusion Agar (BHIA, Emapol, Poland) and *C. albicans* was maintained on Sabouraud Dextrose Agar (SDA, Emapol, Poland).

Antimicrobial screening

Minimum inhibitory (MIC) and minimum bactericidal/fungicidal (MBC/MFC) concentrations were determined by microdilution method in 96-well microtitre plates described by Wiegand *et al*. (2008) as well as Levic *et al*.

(2011) with slight modifications (in case of yeast, the time of SDA plates incubation at 37 °C was prolonged to 48 h). The bacterial/fungal inoculates were prepared using fresh overnight cultures and suspensions was adjusted to 0.5 McFarland standard turbidity using turbidimeter (Biosan). Essential oils were diluted in propylene-glycol (2-(2-hydroxypropoxy)-1-propanol) to the test concentration ranging from 500 to 2 μL/mL. The optical density of tested microorganisms cells (OD, λ=600 nm) under exposure to tested oils were used to quantify of results. The optical density of tested microorganisms cells was measured at the wavelength of 600 nm in 96-well microtitre plates with 200 μL of each sample using Infinite 200 PRO NanoQuant microplate reader (Tecan, Männedorf, Switzerland).

All tests were performed in Mueller Hinton Broth (MHB, Emapol, Poland), in a volume of 200 μL. The aliquots 20 μL bacteria (or yeast) suspensions and 20 μL essential oil in geometric dilutions (ranging from 500 μL to 0.125 μL) were added into each well of 96-well microtitre plate. Then, aliquots of 160 μL of MHB were added. The final essential oils concentrations were 50 to 0.0125 μL/mL. Simultaneously sterility control (MHB + tested oils) and control of toxicity of propylene glycol (MHB + tested microorganisms + propylene glycol) were performed. As the controls of all tests, the same tested microorganisms, incubated under the same conditions but without exposure to tested oils were used.

The microplates were incubated for 24 h at 37 °C for bacteria and 48 h at 37 °C for yeast. The MIC was defined as the lowest concentration of essential oil at which microorganism shows no visible growth. Then, the 5 μL solution from each well was transferred to BHIA plates (Emapol) and incubated for 24 h at 37 °C for bacteria. For yeast SDA plates (Emapol) were used and incubated for 48 h at 37 °C. The lowest concentration of the essential oil at which 99.5% inoculated microorganisms were killed was defined as MBC for bacteria and MFC for yeast. All tests with the controls were repeated three times.

Statistical analysis

In order to observe the differences in the composition of *T. serpyllum* and *T. serpyllum* 'Aureus' oils, seventeen constituents of the content greater than 1% of the oil were selected for statistical analysis. An analysis of variance was performed using AWAR software made by Department of Applied Informatics, Institute of Soil Science and Plant Cultivation in Puławy. The means were separated by the Tukey's test at p=0.05.

The data gathered in Table 4 are presented as the means ± standard deviations (mean ± SD) calculated for the three repetitions of the experiment using Statistica 9.0 (StatSoft, Poland).

Results

Chemical composition of essential oils

The essential oils isolated by hydrodistillation from the aerial parts of *T. serpyllum* and *T. serpyllum* 'Aureus' were found to be yellow liquids and were obtained with a yield of 1.50% (v/w) and 1.45% (v/w), based on the dry weight of plant material. The present results are in conformity with the European Pharmacopoeia (2010) standard for *T. serpyllum* herb (a yield of at least 0.3%).

According to literature data, the content of essential oil in *T. serpyllum* is variable and depends mainly on the origin of the plants, but usually is between 0.1 and 1% (Raal *et al*., 2004). In Poland, the amounts of essential oil among wild growing populations vary from 0.21 to 0.60% (Pióro-Jabrucka and Osińska, 2003). However, plants grown in different regions of Jordan contain from 2.5 to 5.6% of oil (Abu-Darwish *et al*., 2009).

The relative percentage composition of essential oils and retention indices calculated for individual components of the oils are given in Table 1. All the constituents are listed in order of their elution from HP-5MS column.

Table 1. Relative percentage composition of wild thyme (*Thymus serpyllum* L.) essential oils

Compounds	RI	*T. serpyllum*	*T. serpyllum* 'Aureus'
α-Thujene	927	1.59	1.43
α-Pinene	933	0.73	0.98
Camphene	948	0.07	0.60
Sabinene	973	0.11	-
β-Pinene	976	0.47	0.31
1-Octen-3-ol	980	2.15	0.38
3-Octanone	987	0.18	6.19
β-Myrcene	991	1.75	1.79
3-Octanol	996	0.28	1.39
α-Phellandrene	1004	0.33	0.49
δ-3-Carene	1010	0.13	0.24
α-Terpinene	1016	2.09	2.15
p-Cymene	1025	6.06	7.39
β-Phellandrene	1029	0.24	0.69
Eucalyptol	1031	3.71	0.90
(Z)-β-Ocimene	1038	1.14	0.25
(E)-β-Ocimene	1049	4.63	0.12
γ-Terpinene	1061	10.79	10.08
cis-Sabinene hydrate	1068	0.95	0.72
α-Terpinolene	1089	0.16	0.18
Linalool	1101	0.54	0.90
Borneol	1169	0.18	2.10
Terpinen-4-ol	1181	0.56	0.67
α-Terpineol	1196	0.56	0.11
Thymol methyl ether	1236	0.06	-
Carvacrol methyl ether	1245	4.40	2.58
Thymol	1293	0.22	0.27
Carvacrol	1311	37.49	44.93
p-Thymol	1338	0.24	0.29
α-Copaene	1380	0.09	0.11
β-Bourbonene	1390	0.16	0.31
β-Caryophyllene	1427	6.51	6.77
β-Cubebene	1435	0.17	0.17
Aromadendrene	1445	-	0.05
α-Bergamotene	1439	0.07	-
α-Caryophyllene	1460	0.39	0.34
Alloaromadendrene	1468	-	0.13
γ-Muurolene	1482	0.41	0.55
Germacrene D	1488	1.33	0.31
Bicyclogermacrene	1501	0.19	0.32
α-Muurolene	1505	0.12	0.20
β-Bisabolene	1513	4.51	0.07
γ-Cadinene	1520	0.63	0.38
δ-Cadinene	1528	0.71	0.82
Caryophyllene oxide	1592	0.83	0.71
τ-Cadinol	1652	-	0.12
Octadecanal	2042	0.28	-
2-Methyleicosane	2055	0.45	-
3-Methyleicosane	2071	0.34	-
Nonadecanal	2119	0.67	-
Total identified		99.67	99.49

Table 2. Statistical analysis of the content of main constituents of investigated essential oils

Essential oil constituent (*factor I*)	Cultivar (*factor II*)		Mean
	T. serpyllum	*T. serpyllum* 'Aureus'	
α-Thujene	1.59 ± 0.03	1.43 ± 0.03	1.51
1-Octen-3-ol	2.15 ± 0.20	0.38 ± 0.03	1.27
3-Octanone	0.18 ± 0.01	6.19 ± 0.03	3.19
β-Myrcene	1.75 ± 0.04	1.79 ± 0.01	1.77
3-Octanol	0.28 ± 0.02	1.39 ± 0.02	0.84
α-Terpinene	2.09 ± 0.03	2.15 ± 0.05	2.12
p-Cymene	6.06 ± 0.04	7.39 ± 0.19	6.73
Eucalyptol	3.71 ± 0.16	0.90 ± 0.02	2.31
(Z)-β-Ocimene	1.14 ± 0.01	0.25 ± 0.00	0.70
(E)-β-Ocimene	4.63 ± 0.03	0.12 ± 0.02	2.38
γ-Terpinene	10.79 ± 0.06	10.08 ± 0.01	10.44
Borneol	0.18 ± 0.04	2.10 ± 0.08	1.14
Carvacrol methyl ether	4.40 ± 0.16	2.58 ± 0.09	3.49
Carvacrol	37.49 ± 1.09	44.93 ± 0.58	41.21
β-Caryophyllene	6.51 ± 0.18	6.77 ± 0.17	6.64
Germacrene D	1.33 ± 0.14	0.31 ± 0.05	0.82
β-Bisabolene	4.51 ± 0.53	0.07 ± 0.01	2.29
Mean	5.22	5.23	5.22
$LSD_{\alpha=0.05}$ for factor I	1.389		
$LSD_{\alpha=0.05}$ for factor II	n.s.		
$LSD_{\alpha=0.05}$ for interaction I x II	0.509		

± standard deviation (n=3)

Table 3. Main chemical classes of compounds identified in analysed essential oils (in %)

	T. serpyllum	*T. serpyllum* 'Aureus'
Monoterpene hydrocarbons	30.29	26.70
Oxygenated monoterpenes	6.50	5.40
Sesquiterpene hydrocarbons	15.29	10.53
Oxygenated sesquiterpenes	0.83	0.83
Phenolic compounds	42.41	48.07
Total	95.32	91.53

Table 4. Antimicrobial activity of *Thymus* essential oils (μL/mL)

Strains	*T. serpyllum* L. oil		*T. serpyllum* 'Aureus' oil	
	MIC	MBC/MFC	MIC	MBC/MFC
S. aureus	0.1±0.00	0.78±0.00	0.1±0.00	0.78±0.26
S. epidermidis	0.78±0.26	1.56±0.00	0.2±0.11	1.56±0.45
S. agalactiae	0.1±0.03	0.78±0.23	0.1±0.03	0.39±00.0
E. faecalis	0.1±0.03	0.78±0.45	0.1±0.03	1.56±0.45
B. cereus	0.1±0.00	0.39±0.26	0.2±0.11	0.39±0.00
M. luteus	0.1±0.00	1.56±0.45	0.2±0.06	0.78±0.00
E. coli	0.025±0.00	0.1±0.03	0.05±0.00	0.1±0.03
P. vulgaris	0.1±0.08	0.78±0.26	0.39±0.00	0.78±0.26
C. albicans	0.05±0.00	0.1±0.03	0.1±0.06	0.1±0.03

Values are given as mean ± SD. MIC: minimum inhibitory concentrations. MBC: minimum bactericidal concentrations. MFC: minimum fungicidal concentrations.

A total of 47 compounds were identified in the oil of *T. serpyllum* representing 99.67% of the total oil, and 43 compounds were identified in the oil of *Thymus serpyllum* 'Aureus', representing 99.49% of the total oil (Table 1).

The major constituents of *T. serpyllum* oil were carvacrol (37.49%), γ-terpinene (10.79%), β-caryophyllene (6.51%), p-cymene (6.06%), (E)-β-ocimene (4.63%) and β-bisabolene (4.51%). Among other constituents found in significant amounts were carvacrol methyl ether (4.40%) and eucalyptol (3.71%). Similarly, carvacrol (44.93%), γ-terpinene (10.08%), p-cymene (7.39%) and β-caryophyllene (6.77%) were the most abundant constituents in the essential oil of *T. serpyllum* 'Aureus'. The oil contained also high amount of 3-octanone (6.19%).

Significant differences in the chemical composition of both essential oils can clearly be observed from the results of statistical analysis presented in Table 2.

Carvacrol was found to be the main essential oil constituent for both of the wild thyme cultivars. Its amount was on average 41.21%. Lower amounts were detected as follows: γ-terpinene (on average 10.44%) < p-cymene and β-caryophyllene (on average 6.69%) < carvacrol methyl ether, 3-octanone, (E)-β-ocimene, eucalyptol, β-bisabolene and α-terpinene (on average 2.63%) < β-myrcene, α-thujene, 1-octen-3-ol, borneol, 3-octanol, germacrene D and (Z)-β-ocimene (on average 1.15%).

For most of the analysed essential oil constituents there were significant differences found between their content in *Thymus*

serpyllum L. and *Thymus serphyllum* 'Aureus'. The content of 1-octen-3-ol, eucalyptol, (Z)-β-ocimene, (E)-β-ocimene, γ-terpinene, carvacrol methyl ether, germacrene D and β-bisabolene was significantly higher for *T. serpyllum* while *T. serpyllum* 'Aureus' was characterized by a significantly higher content of 3-octanone, 3-octanol, p-cymene, borneol and carvacrol.

The investigated essential oils were composed mainly of three different chemical classes of compounds: phenolic compounds (42.41% *T. serpyllum;* 48.07% *T. serpyllum* 'Aureus'), monoterpene hydrocarbons (30.29% *T. serpyllum;* 26.70% *T. serpyllum* 'Aureus') and sesquiterpene hydrocarbons (15.29% *T. serpyllum;* 10.53% *T. serpyllum* 'Aureus') (Table 3). Moreover, *T. serpyllum* oil contained higher amount of monoterpene and sesquiterpene hydrocarbons, while *T. serpyllum* 'Aureus' oil was richer in phenolic compounds.

Antimicrobial activity

The antimicrobial activity of *T. serpyllum* oils was evaluated against Gram-positive and Gram-negative bacterial strains as well as for yeast *Candida albicans* using the microdilution method. The obtained results are summarized in Table 4. It was demonstrated a significant, but varied for each of the analysed test strains the antimicrobial activity of essential oils. At the same time, based on conducted scientific controls, the sterility of tested essential oils was confirmed and also the toxicity of propylene glycol was excluded with respect to all of the tested microorganisms. Reported values of MIC and MBC/MFC were respectively in the case of the *T. serpyllum* L. essential oil 0.025-0.78 μL/mL and 0.1-1.56 μL/mL, whereas for the *T. serpyllum* 'Aureus' essential oil 0.05-0.39 μL/mL and 0.1-1.56 μL/mL.

The *T. serpyllum* L. essential oil showed the highest antimicrobial activity relative to the strain of *E. coli* (MIC=0.025 μL/mL and MBC=0.1 μL/mL) and to *C. albicans* (MIC=0.05 μL/mL and MFC=0.1 μL/mL), however the lowest to the strain of *S. epidermidis* (MIC=0.78 μL/mL and MBC=1.56 μL/mL), and to *M. luteus* (MBC=1.56 μL/mL). Similarly, a significant antimicrobial activity exhibited the *T. serpyllum* 'Aureus' essential oil, although the MIC values obtained in that case for *E. coli* and *C. albicans* strains were twice as high and were respectively (0.05 μL/mL and 0.1 μL/mL). On the other hand, the lowest antimicrobial activity of the *T. serpyllum* 'Aureus' essential oil showed relative to the strain of *P. vulgaris* (MIC=0.39 μL/mL and MBC=0.78 μL/mL), and strains of *S. epidermidis* and *E. faecalis*, for which the observed for that oil MBC value was as high as 1.56 μL/mL.

Discussion

The present results are in agreement with the data provided by other authors who also showed significant antimicrobial effect after the application of *O. vulgare*, *T. vulgaris*, T. *serpyllum*, as well as *T. algerientis* essential oils (Lambert *et al*., 2001; Levic *et al*., 2011; Nikolic *et al*., 2014). Similarly to Hammer *et al*. (1999), the lowest values of minimum inhibitory concentrations (MIC) were shown for tested strains of *E. coli* and *C. albicans*. This may prove the particular sensitivity of these microorganisms to the essential oils. Significant antimicrobial activity of these oils is mainly connected with the destruction of the integrity and function of biological membranes of microbial cells exposed to their operation (Lambert *et al*., 2001). An essential role in this mechanism may be especially performed by carvacrol – an organic compound that constituted a dominant ingredient of both analysed essential oils (Tables 1 and 2). Soković *et al*. (2010) have proved that this compound had the greatest antimicrobial properties among all tested ingredients, including: camphor, 1,8-cineole, linalool, linalyl acetate, limonene, menthol, α- pinene, β-pinene and thymol. The similar activity of essential oils containing large amount of carvacrol has been pointed out by other researchers (Rasooli and Mirmostafa, 2002; Abed *et al*. 2014). Varga *et al*. (2015) additionally emphasize that essential oils of *Thymus* species, whose one of the most important ingredients is carvacrol, show a very wide spectrum of activity both against Gram-positive and Gram-negative bacteria and against yeast. Thus, these biologically active substances may play a significant role in the prevention of serious bacterial and fungal infections as pharmaceutical formulations or natural food preservatives (Hammer *et al*., 1999; Lambert *et al*., 2001).

The biological activity of essential oils depends mainly on the chemical structure of their components and their concentrations. Generally, phenolic compounds, terpenes, aliphatic alcohols, aldehydes, ketones, acids, and flavonoids are recognized as major components with antimicrobial activity, which can be found in plants. Among them, thymol, carvacrol, p-cymene and γ-terpinene are considered as the most potent (Shelef, 1983; Farag *et al*., 1989; Davidson, 1993; Santoyo *et al*., 2006). Sikkema *et al*. (1995) reported that these compounds act on microbial cells and cause structural and functional damage of their membranes, resulting in increased permeability.

The content of these active compounds in the essential oil of *T. serpyllum* varies depending on the origin of the raw material. Thymol (53.33%), carvacrol (10.40%) and p-cymene (8.80%) dominated in the oil from plants collected in Pakistan (Ahmad *et al*., 2006). Thymol (34.61-49.53%), γ-terpinene (8.26-9.30%) and p-cymene (7.43-11.45%) were the most abundant constituents of oil from Uttarakhand, India (Chauhan *et al*., 2011). Similarly, *T. serpyllum* cultivated in Kumaon region of Western Himalaya (India), contained thymol (19.40-60.10%), γ-terpinene (0.30-13.80%) and p-cymene (3.50-10.40%) as major oil components (Verma *et al*., 2011). Moreover, thymol (46.24-74.92%) and carvacrol (4.69-7.19%) rich volatile oils were also obtained from plants grown in Western Nepal and North India (Thakuri *et al*., 2009). In Libia (Eweis *et al*., 2012), thymol (64.20%) and β-phellandrene (13.50%) accounted for the most abundant components of wild thyme oil. In Southern Italy (De Lisi *et al*., 2011), thymol (32.57%), α-terpinene (22.83%) and γ-terpinene (9.15%) were found as the main essential oil constituents.

In contrast, the major constituents noted in the oil from *T. serpyllum* growing wild in Estonia, were (E)-nerolidol (1.70-70.10%), caryophyllene oxide (1.40-45.00%), β-myrcene (tr.-20.20%), β-caryophyllene (1.80-13.30%) and germacrene D (1.70-12.50%) (Raal *et al*., 2004). However, plants grown in Lithuania, contained mainly (E)-β-ocimene (0.70-34.80%), 1,8-cineole (0,70-30.30%), borneol (0.10-27.10%), (Z)-β-ocimene (0.10-20.00%) and β-myrcene (0.10-16.90%) (Loziene and Venskutonis, 2006). Similarly, wild populations of *T. serpyllum* in Central Poland, were characterized by the presence of camphene (8.07-13.91%), β-myrcene (6.53-17.97%), 1,8-cineole (1.41-11.64%), β-caryophyllene (1.06-9.02%) and borneol (0.15-16.91%) in the essential oils (Osińska *et al*., 2002).

Interestingly, linalool (40.35-88.18%), *trans*-geraniol (0.49-30.01%), geraniol acetate (0.56-7.04%) and nerol acetate (0.22-3.11%) dominated in the essential oils of wild thyme collected

from the territory of European North-East Russia and Ural (Alekseeva and Gruzdev, 2012). Uncharacteristic compounds, such as: 2,4,6-trimethylanisol (73.41%), 3,5-dimethyl benzoic acid (5.38%) and β-bisabolene (3.67%), were present in the essential oil obtained from plants growing in Turkey (Topal *et al*., 2008).

In this study, volatile oils isolated from *T. serpyllum* and *T. serpyllum* 'Aureus' were composed mainly from carvacrol (37.49 and 44.93%), γ-terpinene (10.08 and 10.79%), p-cymene (6.06 and 7.39%) and β-caryophyllene (6.51 and 6.77%). The content of thymol in the all analysed oil samples was lower as compared to the results obtained by researchers from Pakistan (Ahmad *et al*., 2006), India (Chauhan *et al*., 2011), Libia (Eweis *et al*., 2012) and Southern Italy (De Lisi *et al*., 2011). However, the amount of carvacrol was almost identical to that reported by Oszagyan *et al.* (1996) for wild thyme growing in Hungary (39.50-45.90%).

Comparing the content of active ingredients in both essential oils, it appears that *T. serpullum* 'Aureus' oil should be more effective against tested microbial strains. Surprisingly, slightly higher activity against *E. coli* and *C. albicans* was noted for *T. serpyllum* oil. Probably less abundant constituents of the oil are also responsible for the antimicrobial activity. They may be involved in some type of synergism with the other active compounds.

Conclusion

In all two *Thymus* essential oils, carvacrol, γ-terpinene, p-cymene and β-caryophyllene were identified as the major oil constituents. The investigated oils were the most active against *Escherichia coli* and yeast *Candida albicans*, and less potent against *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Micrococcus luteus* and *Proteus vulgaris*. The current study suggest the potential use of *T. serpyllum* and *T. serpyllum* 'Aureus' oils as antimicrobials for food preservation as well as in pharmaceutical formulations for prevention of bacterial and fungal infections.

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