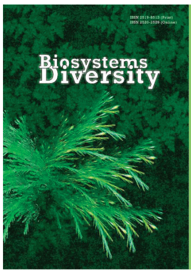

Biosystems Diversity

ISSN 2519-8513 (Print)
ISSN 2520-2529 (Online)
Biosyst. Divers., 2019,
27(3), 270–275
doi: 10.15421/011936

Antimicrobial, antibiofilm and biochemichal properties of *Thymus vulgaris* essential oil against clinical isolates of opportunistic infections

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Article info

Received 12.06.2019
Received in revised form 17.07.2019
Accepted 19.07.2019

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Kryvtsova, M. V., Salamon, I., Koscova, J., Bucko, D., & Spivak, M. (2019). Antimicrobial, antibiofilm and biochemichal properties of Thymus vulgaris essential oil against clinical isolates of opportunistic infections. Biosystems Diversity, 27(3), 270–275. doi:10.15421/011936

Thyme belongs to a genus encompassing over 215 species of hardy perennial herbaceous plants and sub-shrubs, which are native to Europe, particularly around the Mediterranean. *Thymus vulgaris* L., or garden thyme, with narrow small leaves and clusters of tubular mauve flowers, is used mainly in cookery. Dried herb yields 1% and more essential oil, which is a pale yellowish-red liquid with a sweet, very aromatic odour. Thyme is widely used in the pharmaceutical industry and is a source of substances of antimicrobial effect upon antibiotic-resistant strains of microorganisms. The purpose of our work was to identify the biochemical and antimicrobial peculiarities of *Th. vulgaris* essential oil against clinical isolates of opportunistic microorganisms. The analysis of thyme essential oil was carried out using GC/MS analysis. The clinical isolates were isolated with the use of differentially diagnostic nutrient media. The antibiotic susceptibility was identified with the help of the disc-diffusion test. The sensitivity of microorganisms to plant extracts was determined by the agar diffusion test. The antibiofilm activity of the extracts was tested in standard 96-well microtitration plates. The GC/MS results confirm the earlier reports that the major volatile constituents obtained from the aerial parts of thyme species were thymol, γ-terpinene, p-cymene, 3-carene and carvacrol. After subjecting the selected essential oil to effective steam distillation, substantial contents of phenolic monoterpenoids were obtained – thymol (67.7%) and γ-terpinene (8.2%). The European Pharmacopoeia set quality standards for thyme essential oil, which dealt mainly with the % content (w/w) of the volatile phenols (expressed as thymol: 36.0–55.0%). Garden thyme essential oil has been found to show a high antimicrobial activity against antibiotic-resistant microorganism strains. The obtained results proved the wide spectrum of antibiotic activity of thyme essential oil. The highest antimicrobial activity was registered against the typical and clinic strains of *S. aureus* and microscopic *Candida* genus fungi. Garden thyme essential oil was ascertained to show high antibiofilm-forming activity against *S. aureus*. The antimicrobial and antibiofilm-forming activities of thyme essential oil against both bacterial pathogens of opportunistic infections and microscopic fungi have proven the good prospects for development of a broad-spectrum agent against opportunistic microbial associations based on this oil.

Keywords: antimicrobial effect; antibiofilm formation; essential oil; garden thyme, large-scale distillation.

Introduction

The rapid development of antibiotic resistance of microorganisms is a difficult problem for contemporary biology and medical science. Considerable attention is paid to the mechanisms and causes of development of bacterial resistance to antimicrobial preparations as well as the possible ways to overcome it. The development of antibiotic resistance is one of the causes for formation of chronic persistent inflammatory processes. Besides, there is a possibility for bacterial existence in the form of biofilm – an elaborate association present in the environment and in the human organism. The formation of biofilm adds to the aggravation of the infection process, because the bacteria in the biofilm structure tend to become more resistant to such environmental factors as temperature, pH values, etc., on one hand, and to the antibiotics used to treat infections, on the other hand. The discovery of bacteria in biofilm structure qualitatively changed the views on the peculiarities of the course, therapy and prevention of infectious diseases (Marshall et al., 1998; Sidashenko et al., 2015). The ability to form biofilms is considered to be an additional pathogenic factor. Among the opportunistic bacteria, staphylococci remain among the most widely spread infectious agents. Staphylococci are known to be the agents of a significant part of community-acquired and nosocomial infections. The main affections are caused by *S. aureus* and *S. epidermidis*, which may colonize and affect human organs and tissues demonstrating at the same time a broad scope of adaptive opportunities. Say, Voronkova et al. (2015) showed that among the 122 clinical strains of staphylococci isolated from the inflammatory nidus, 37 cultures (30.3%) belonged to the species *Staphylococcus epidermidis*, of which 54% formed biofilm.

The study of biofilms is an extremely promising direction of microbiological research, due to their high resistance to antibiotics and disinfectants. The ways of resistance of biofilm bacteria to antimicrobial materials include reduction of antibiotic diffusion through biofilm matrix, synthesis of new specific molecules, and change in the metabolic activity. Thereby, the concentration of the antibiotic substance on the surface differs from that in the depth of the biofilm. Affected by a sufficient amount of the antibiotic substance, the outer layer perishes quickly; among the bacteria located in the lower layers of the biofilm, the antibiotic concentration is considerably lower, which is why resistance is formed when affected by low concentrations of an antibiotic material (Clarke et al., 1986; Liamin et al., 2012; Kiranasari et al., 2018).

A considerable number of publications are devoted to the development of approaches, methods and techniques meant to overcome resistance by using newly synthesized or alternative means with antimicrobial activity. Medicinal plants containing a broad spectrum of biologically active substances with total additive antimicrobial activity are a mighty source of antimicrobial substances. Although the antimicrobial activity of medicinal plants has for a long time been actively studied and used by folk and conventional medicine, it is crucially necessary to study the impact of components based on medicinal plants upon antibiotic-resistant strains of microorganisms. The number of publications devoted to the study of their antibiofilm-forming activity is low, not withstanding the fact that it is the overcoming of biofilm's resistance to antimicrobial materials and disintegration of biofilm which is a promising field of development of new antimicrobial means and approaches. With this in mind, it is worth noting that among the newest approaches to the development of antimicrobial preparations, significant attention should be paid to their ability to destroy biofilm, i.e. their impact not only upon the plankton forms but also microbial associations.

Plant products as a source of antimicrobial substances have a series of advantages from the viewpoint of low probability of side effects and high antioxidant activities that contribute to the improvement of the body's resistance and serve a source of biologically active substances, vitamins, micro- and macronutrients. Essential oils, including thyme oil, can be a promising source from which to derive such substances.

Garden thyme or common thyme, *Thymus vulgaris* L., a small shrubby plant with a strong, spicy taste and odour, is extensively cultivated in Europe and the U.S. for culinary use. The numerous quadrangular, procumbent, woody stems grow 0.10–0.25 m high and are finely hairy. Slightly downy on top and very downy underneath, the opposite, sessile leaves are ovate to lanceolate in shape and have slightly rolled edges. The small bluish-purple, two-lipped flowers are whorled in dense, head-like clusters, blooming from May to September.

The *Thymus* L. genus has for a long while been claiming attention as a source of primary materials for the pharmaceutical industry and development of biologically active additives, beauty and medicinal preparations. Mostly, common thyme (*Th. vulgaris* L.) and creeping thyme (*Th. serpyllium* L.) are used in medical practice in this role. The components of essential oil extracted from *Thymus* L. genus plants are characterized by polymorphism on both intraspecific and interspecific levels. The antimicrobial activity of the essential oil also depends upon the percentage composition of its ingredients (Zazharskyi et al., 2019). The difference in the composition of essential oil may be caused by genetic peculiarities and environmental conditions. Thus, the antimicrobial activities of the herbal substances will to a considerable extent depend upon the plant chemotype as well as the environmental conditions of its growing (Ghasemi Pirbalouti et al., 2013).

The antimicrobial activity of plant essential oils has long since been used in traditional and folk medicine, cosmetology and the food industry (Ryman, 1991; Tadele et al., 2009; Quesada et al., 2016; Jain & Sharma, 2017; Ulukanli et al., 2018), alternative medicine and natural therapies (Zhiani et al., 2016; Özdemir et al., 2018). Over the past decade, the antimicrobial activities of essential oils, including thyme EO (Shree, 2019), have been widely used in food industry and cosmetology (Bueno et al., 2017), which indicates good prospects of their application as an antibacterial remedy. A number of researchers have noted the ability of essential oils to affect the biofilm-producing properties of microorganisms.

The purpose of our work was to identify the biochemical and antimicrobial peculiarities of *Thymus vulgaris* L. essential oil against clinical isolates of opportunistic microorganisms.

Material and methods

The research was performed on the basis of the Microbiological Laboratory of the Department of Genetics, Plant Physiology and Microbiology of Uzhhorod State University, Ukraine; University of Veterinary Medicine and Pharmacy in Košice, Slovakia; and the Bacteriological Laboratory of Svaliava Central Rayon Hospital, Ukraine. To isolate clinical isolates, the bacteriological analysis of the material taken from patients suffering from inflammatory diseases of the upper respiratory tract was performed using generally known microbiological methods by plating on differentially diagnostic and universal nutrient media. The identification was performed according to the morphological, tinctorial and biochemical properties with the use of Entero-test, Strepto-test, and Staphylo-test, made by Erba Lachema. The sensitivity of the reviewed strains to antibiotics was determined using the disc-diffusion test, according to Order No. 167 "On approval of the recommended practices 'Identification of sensitivity of microorganisms to antibacterial materials'" of the Ministry of Public Health of Ukraine of 05/04/2007, with the use of standard discs.

The antimicrobial activity of Thyme EO was determined using the agar diffusion test (Rhos & Reico, 2005). The bacterium inocula 100 μL in the physiological solution were adjusted to the equivalent of 0.5 McFarland standard, and evenly spread on the surface of Muller-Hinton agar (incubated at 37 ± 2 ºC for 24 hours); yeasts – on SDA agar (incubated at 35 ± 2 ºC for 48 hours). The extracts 20 μL were introduced into wells 6 mm in diameter. The diameters of the inhibition zones were measured in millimetres including the diameter of the well. Each antimicrobial assay was performed at least three times.

As test cultures, the following bacteria and yeasts from the American Type Culture Collection were used: *Candida albicans* ATCC 885-653, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Streptococcus pyogenes* ATCC 19615, *S. aureus* CCM 4223 biofilm-forming strain. We also used clinical strains of bacteria and yeasts (*S. aureus*, *E. coli*, *S. pyogenes*, *E. faecalis*, *C. albicans*) isolated from the nidus of the pathological process of patients suffering from inflammatory diseases of the upper respiratory tract.

We chose the clinical strains with multiple resistance at least to two classes of antibiotics. As a positive control we used: gentamicin (10 mg/disk) for Gram-negative bacteria, ampicilin (10 mg/disk) for Gram-positive bacteria, nystatin (100 UI) for *Candida*. As negative control we used DMSO. The antibiofilm activity of the EO was tested in standard 96-well microtitration plates (Greiner-BioOne, Austria) using a modified staining method according to O' Toole (2011).

With the purpose of study of the antibiofilm-forming activity, an 18-hour culture of the reference *S. aureus* CCM 3953 biofilm-forming strain grown at 37 ºC was used. Into the wells, 180 μL of bacterial suspension, Mc Farland in broth (TSB, Himedia, India) was introduced. The *Thymus vulgaris* EO dissolved to the concentrations of 1%, 5% and 10% in dimetylsulfoxide (DMSO; Sigma-Aldrich, USA) was introduced into the wells in the amount of 20 μL. Following the addition of the bacterial suspension, the concentration of plant extracts in the broth equalled 0.10%, 0.05% and 0.01%, respectively. The wells with only 180 μL of broth and 20 μL of 10% DMSO served as the control.

Following a 24-hour-long incubation in the thermostat at 37 ºC, the supernatant was withdrawn and washed 3 to 5 times with distilled water. Following a 30-minute-long incubation, it was dyed with 200 μL of 0.1% solution of crystal violet; then the dye was withdrawn, and the supernatant washed 3 to 5 times with distilled water. Into every well, 200 μL of 30% acetic acid was added and incubated for 10 minutes. The optical density was measured on the Synergy HT (Biotek, USA) spectrophotometer at 550 nm.

More than 50% reduction in absorbance of CV was considered as significant inhibition (Raut et al., 2014).

The raw material of garden thyme (*Th. vulgaris* L.) was imported with cooperation of companies in Italy (Agrilatina-Biodinamica, Latina; Agronatura Societa' Cooperativa Agricola, Bergagiolo Alexandria; Ersat e Laore, Cagliari and ESAC Lamezia Terme) in 2016 and 2017.

Normally, a thyme plant is grown from a division. Thyme is easy to divide. In the spring or fall, find a mature thyme plant. Use a spade to gently lift the clump of thyme up from the ground. Tear or cut a smaller clump of thyme from the main plant, making sure there is a root ball intact on the division. Thyme monoculture prefers light, well-drained soils with a pH 5.0–8.0. Thyme species do best in coarse, rough soils that would be unsuitable for many other plants. Although thyme grows easily, especially in calcareous light, dry, stony soils, it can be cultivated in heavy wet soils, but it becomes less aromatic. For essential oil, thyme is harvested once per annum, during late summer when flowering begins. Yields of *Th. vulgaris* for fresh herb production can be 5 to 6 t/ha and for dry herb production can be 2 t/ha (Haban et al., 2008). The raw stock is composed of dry plant leaves and flower tops, which are chopped before the distillation process. Garden thyme essential oil (EO) was obtained by the large-scale distillation apparatus (Oravec et al., 1988) specifically designed for aromatic and medicinal plants.

The GC/MS analysis of garden thyme EO were carried out on a Varian 450-GC connected with a Varian 220-MS. The separation was achieved using the capillary column: BPX-5MS (50 m × 0.25 mm i.d., 0.25 µm film thickness). Injector type 1177 was heated on temperature 220 °C. Injection mode split less (1 µL of a 1:1,000 n-hexane/diethyl ether solution). Helium (He_2) was used as a carrier gas at a constant column flow rate of 1.2 mL/min. Column temperature was programmed: initial temperature 50 °C for 10 minutes, then to 100 °C at 3 °C/min; isothermal for 5 minutes and then continued to 150 °C at 10 °C/min. Total time for analysis of one sample was 54.97 minutes. Identification of components was made by comparison of their mass spectra with those stored in NIST 14 (software library) or with mass spectra from the literature (Hudaib et al., 2002). The NIST 14 (2014 version) mass spectral library was used. It is a fully evaluated collection of electron ionization (EI) and MS/MS mass spectra, with chemical and GC data, plus search software to identify own unknown spectra. Kovat's samples (C-5 to C-22 alkane mixture) were injected during sample analysis and Kovat's indices were calculated from retention time using a third order polynomial. 40 reference authentic compounds (Extrasynthese, Merck, Fulka, Sigma and Roth) were purchased. Many more authenticated from MS data or retention indices were compared by literature (Adams, 2007). Compounds' concentrations (as % content) were calculated by integrating their corresponding chromatographic peak areas assuming a unity response by all.

Data obtained were expressed as mean ± standard deviation (SD) of three measurements. The Tukey test was applied for comparisons of means; differences were considered significant at $P < 0.05$.

Results

We isolated 210 clinical isolates from the pathological material of patients suffering from inflammatory diseases of the upper respiratory tract (tonsillitis, pharyngitis, etc.). By the level of isolation of microorganisms during the process of inflammation, Staphylococcaceae genus bacteria dominated – these bacteria were isolated in 64.7% cases (Table 1). Within that genus, the most frequently isolated species was *S. aureus* (49.0%); much more rarely *S. epidermidis* (11.9%), and *S. haemolyticus* (3.3%). The mean priority level belonged to microscopic fungi of *Candida* spp. genus (15.8%) (of which, 27 isolates were *C. albicans*, 2 isolates were *C. tropicalis*, and 1 isolate was *C. crusei*). *S. pyogenes* was isolated from thirty (out of 206) patients. *E. faecalis* and *P. aeruginosa* occurred sporadically.

It is worth noting that in certain cases we observed isolation of microorganism associations of the Staphylococcaceae genus and microscopic fungi of *Candida* spp. (11.0% cases). This trend proved aggravation of the inflammatory process and required application of several antimicrobial preparations. Thus, representatives of the Staphylococcaceae genus were the dominant agent of inflammatory diseases of the upper respiratory tract. For further research, we chose clinical strains of microorganisms characterized by antibiotic resistance at least to 10 antibiotic substances, which belonged to different groups.

Table 1
Microorganisms isolated from the nidus of the pathological process of patients suffering from inflammatory diseases of the upper respiratory tract

Year	*S. aureus*		*S. haemolyticus*		*S. epidermidis*		*S. pyogenes*		*Candida spp.*		*E. faecalis*		*P. aeruginosa*	
	%	abs. value	%	abs. value	%	abs. value	%	abs. value	%	abs. value	%	abs. value	%	abs. value
2016	50.7	36	2.8	2	10.0	7	14.0	10	16.9	12	4.2	3	1.4	1
2017	50.6	42	2.4	2	9.6	8	13.3	11	19.3	16	3.6	3	1.2	1
2018	45.6	26	5.3	3	17.5	10	15.8	9	8.8	5	3.5	2	3.5	2
Total	49.5	104	3.3	7	11.9	25	14.3	30	15.8	33	3.3	7	1.9	4

Th. vulgaris EO was established to manifest a high antimicrobial effect against typical and clinic isolates of microorganisms (Table 2). This EO was shown to exert the most expressive effect upon various isolates of *S. aureus* – both typical and clinical, including *S. aureus* MRSA isolated from the mouth cavity of patients suffering from inflammatory diseases of the periodontium and pharynx. *Th. vulgaris* EO was established to have a high antifungal activity. Its simultaneous high anti-staphylococcus activity and antifungal activity are of great importance. The lowest activity of *Th. vulgaris* EO was observed for *Streptococcus pyogenes* and *Escherichia coli*.

Table 2
Antimicrobial activities of the *Thymus vulgaris* essential oil against typical and clinical opportunistic infectious agents (mm, n = 3, x ± SD)

Test culture	Zone inhibition, mm
Staphylococcus aureus ATCC 25923	65.83 ± 0.76^{b}
S. aureus (clinic)	59.67 ± 0.58^{c}
S. aureus MRSA (clinic)	49.67 ± 0.58^{f}
S. aureus (clinic), pharynx isolate	52.33 ± 0.58^{e}
S. aureus (clinic), pharynx isolate	44.67 ± 0.58^{f}
S. aureus CCM 4223 (biofilm creation)	55.33 ± 0.58^{d}
Streptococcus pyogenes ATCC 19615	15.50 ± 0.50^{k}
S. pyogenes	14.67 ± 0.58^{k}
Escherichia coli ATCC 25922	18.67 ± 0.58^{j}
E. coli	17.33 ± 0.58^{j}
Enterococcus faecalis ATCC 29212	30.67 ± 0.58^{h}
E. faecalis	27.22 ± 1.00^{i}
Candida albicans ATCC 885-653	70.00 ± 1.00^{a}
C. albicans	37.33 ± 0.58^{g}

Note: * – diameter of well 6 mm.

We have shown antibiofilm-forming activity of thymus essential oil (Fig. 1). It has been established that biofilm destruction by 53% was registered even when exposed to the lowest possible concentration of thyme essential oil (0.01%). The introduction of a higher concentration of essential oil (0.05%) caused biofilm destruction by 76.0%; of a 0.01% concentration – by 81.5%.

The chemical profile of the tested EO, the identity and the percenttage content of the individual components are summarized in Table 3.

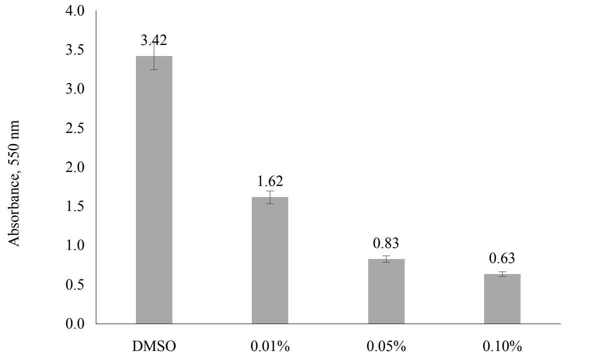

Fig. 1. Antibiofilm activity of different concentrations of *Thymus vulgaris* EO on *S. aureus* 4223

The separation of 27 EO components was conducted. Generally, the plant oil was characterized by the highest percentage of the monoterpene phenols, chiefly thymol. Neumann was the first chemist to isolate and discover thymol in thyme EO in 1719; later Cadeac and Meunier isolated carvacrol and pinene (Ryman, 1991). The analysis of EO detected five major compounds, thymol (67.7%), γ-terpinene (8.2%), p-cymene (4.5%), 3-carene (2.5%) and carvacrol (2.5%). This EO composition is characteristic for the thymol chemo type growing in Italy (Piccaglia &

Marotti, 1991). The genus *Thymus* has about 215 species and numerous hybrids as well. Three principal varieties are usually grown for use, the broad-leaved, the narrow-leaved and the variegated (DPP-Division Industrial Crop, 2012). The narrow-leaved type, with small, grey-green leaves, is more aromatic than the broad-leaved, and is also known as winter or German thyme. The fragrant lemon thyme, has a lemon flavour and rather broader leaves than the ordinary garden thyme, is not curved at the margins, and ranks as a variety of *Th. serpyllum*, the wild thyme. The silver thyme is the hardiest of all and has the strongest flavour. At present, the cultivar 'Varico' is used for the large scale cultivation. It is a robust cultivar, has an upright growth form with greyish-blue foliage and excellent herbage yield. It produces thymol levels of 50% and higher as well as more than 3% essential oil yield. It also has a good resistance to frost. It can be propagated from seed. Other promising new cultivars are currently developed in various countries (Small, 1997).

Table 3
Composition of essential oil from *Th. vulgaris* after steam distillation

Compound	Rt, min	Kovat's Index	Content, %
α-Thujone	5.390	1056	1.06
α-Pinene	6.890	1062	1.07
Camphene	11.578	1077	0.28
Sabinene	17.829	1097	0.17
β-Pinene	17.989	1105	0.29
β-Myrcene	22.519	1107	1.21
α-Phellandrene	23.216	1121	0.37
3-Carene	27.000	1197	2.47
Terpinen-4-ol	27.816	1208	0.25
α-Terpineol	27.972	1217	0.31
p-Cymene	30.676	1271	4.47
Limonene	30.916	1276	0.78
1,8-Cineol	31.014	1278	0.96
γ-Terpinene	32.692	1313	8.21
Terpinolene	32.941	1317	0.14
Linalool	32.948	1319	2.19
Borneol	32.979	1320	0.42
Carvacol methyl ether	33.032	1321	1.64
Thymol	33.191	1323	67.68
Carvacrol	35.743	1331	2.51
β-Caryophyllene	38.864	1443	1.25
α-Humulene	39.642	1460	0.15
Germacrene D	41.934	1513	0.17
α-Muurolene	42.384	1521	0.32
γ-Cadinene	42.832	1531	0.47
δ-Cadinene	43.057	1536	1.13
Caryophyllene oxide	43.271	1547	0.03
–	–	Total	100.00

The results of EO qualitative-quantitative characteristics with a wide range of biodiversity (chemo types) confirm earlier reports (Lisi et al., 2011; Pirbalouti et al., 2013; Aljabeili et al., 2018) that major volatile constituents are obtained from the aerial parts of thyme. Besides the major influence of genetic factors, the environment has an important effect on essential oil accumulation and composition of the aromatic plant species including garden thyme. In regard to environment of aromatic crops, it is very important to investigate abiotic factors (light intensity, day length, temperature, nutrition, irrigation, plant growth regulators, tissue cultures and their genetic transformation) and biotic factors (intraspecific interactions, population dynamics, parasites, diseases, pest control, interspecific competition and harvest management) (Salamon, 2019). According to Table 4, the thyme EO after large-scale steam distillation presented high levels of the precursor monoterpenes – p-cymene and γ-terpinene (12.7%) and monoterpene phenols – thymol and carvacrol (more 70%). The levels of total sesquiterpenes and diterpenoids were observed to be very low (≥ 4%).

The European Pharmacopeia (the 7th edition, 2011) presented the characters of thyme EO (Thymi aetheroleum). The required appearance is mobile liquid, clear, yellow or very dark reddish-brown with aromatic, spice odour reminiscent of thymol. In regard to the content of components, the limits are within the following ranges: β-myrcene from 1.0 to 3.0%, γ-terpinene from 5.0 to 10.0%, p-cymene from 15.0 to 28.0%, terpinen-4-ol from 0.2 to 2.5 %, thymol from 36.0 to 55% and carvacrol from 1.0 to 4.0% (Table 5). These standards dealt mainly with the % contents of volatile phenols, expressed a thymol, in EO. Our tested EO after large-scale steam distillation was found to be in substantial agreement with these content ranges of components. However, the higher level was observed for thymol (Table 5), the biologically most active natural substance of thyme EO (Marchese et al., 2016).

Table 4
Class-composition of *Th. vulgaris* oil after steam distillation

Class and individual components	Content, %
Monoterpenes (mainly *p*-Cymene and γ-Terpinene)	12.68
Monoterpene alcohols	4.13
Monoterpene phenols:	
Thymol	67.68
Carvacrol	2.51
Monoterpene phenol derivates (thymol and carvacrol ethers)	1.64
Sesquiterpenes	3.49
Oxygenated sequiterpenes	0.03

Table 5
Percent contents of principal thyme oil components compared to official European Pharmacopeia (7th edition, 2011) chromatographic profile

Component	Percentage range by the European Pharmacopeia, % content	% content in the test essential oil
β-Myrcene	1.0–3.0	1.21
γ-Terpinene	5.0–10.0	8.21
p-Cymene	15.0–28.0	4.47
Terpinen-4-ol	0.2–2.5	0.25
Thymol	36.0–55.00	67.68
Carvacrol	1.0–4.0	2.51

Discussion

The antibiofilm activity of *Th. vulgaris* EO and compounds (eugenol) at 2xMIC values indicated their potential therapeutic application alone or in combination with antibiotics for treating biofilm associated clinical problems caused by *Staphylococcus aureus* (Jafri et al., 2014). The thesis also notes that *Th. vulgaris* essential oil could be an alternative to classical antibiotics against bacterial biofilms, which show increased tolerance to antibiotics and host defence systems and contribute to the persistence of chronic bacterial infections (Perez, 2019).

Research shows (Kalemba & Kunicka, 2003; Bueno et al., 2017) the effect of essential oils, including of that of thyme, upon biofilms. Carvacrol and thymol, two biocidal compounds present in *Th. vulgaris* oil have an important antimicrobial effect on biofilms formed by *S. aureus*, *S. epidermidis* and *Salmonella enterica* serovar *typhimurium*. Also, *Candida albicans*, *C. glabrata* and *C. parapsilosis* biofilms were treated with carvacrol, geraniol and thymol producing inhibition in biofilm formation of > 75%.

The established antibiofilm-forming antimicrobial activity of thyme essential oil is of great importance (Huma et al., 2014; Piegerová et al., 2019), for mouth cavity microorganisms are known to exist mostly in the form of biofilm, which causes a significant problem for treatment of inflammatory diseases of the mouth cavity. The determined antibiofilm-forming ability of *Th. vulgaris* EO suggests the long-term viability of the development of mouth cavity care products based on this essential oil. The development of such preparations seems especially important due to the wide circulation of antibiotic-resistant microorganism strains in the mouth cavity in the form of biofilm, leading to a persistent inflammatory process.

The results obtained are well coordinated with those of other authors who indicated to the antimicrobial activity of essential oils of *Th. serpyllum* L., *Th. algeriensis* Boiss. and Reut and *Th. vulgaris* L. against pathogens isolated from the mouth cavity (Fani & Kohanteb, 2017).

Scientific studies have evidenced that thymol (or thyme) may exert beneficial effects for the treatment of several disorders affecting the respiratory, cardiovascular, and nervous systems. This compound also exhibits antimicrobial, antioxidant, immunomodulatory, anti-inflammatory, and antispasmodic properties. Some of these bioactivities of thymol may provide the basis of the formulation of new pharmacologically active ingredients to be used in pharmaceutical and cosmetic products, functionnal foods, for food control, and animal production (Salehi et al., 2018).

Thereby, we have established the highly pronounced antimycotic effect of essential oils of *Th. vulgaris* L. (Kryvtsova et al., 2017; Salamon et al., 2018). According to other authors, the essential oil of this plant is also efficient against typical strains of *C. albicans* (Borugă et al., 2014) and *Clostridium* genus bacteria isolated from different places (Kačániová et al., 2013).

However, *Thymus* essential oil showed no significant antimicrobial activity against *Acinetobacter baumannii* and *Klebsiella pneumoniae* (Fatma et al., 2018). Our previous research (Kryvtsova et al., 2018; Kryvtsova, 2019) established a significant level of combination of fungal flora with other pathogens, such as *S. aureus*, *S. epidermidis*, and *S. pyogenes*. It was noted that mixed infections are usually characterised by an aggravated and longer clinical course. Experimental research proves possible mutual potentiation of the pathogenic properties of *Staphylococcus* genus bacteria and *Candida* genus microscopic fungi. In this case, the staphylococci's sensitivity to antibiotics decreases.

Lately, the search for antibiofilm-forming substances of plant origin has become especially intensive. In particular, the antibiofilm-forming activity of substances isolated from *Rosopis laevigata*, *Opuntia ficus-indica*, *Gutierrezia microcephala* (Sánchez et al., 2016); seed extract of *Ammi majus* (Nijampatnam et al., 2014) and extract of *Carum copticum* (Mohammadi et al., 2019) has been shown.

It has also been shown that products based on medicinal plants are able to potentiate the activity of the antibiotic substance, i.e. to amplify the sensitivity of microorganisms to antibiotic preparations due to different mechanisms of impact upon bacterial cells.

Therefore, plant-based products, including essential oils, reveal antimicrobial, antibiofilm-forming and antioxidant properties and thus they are a promising basis for production of antimicrobial remedies.

Conclusion

Garden thyme is a native to the Mediterranean (Italy), but is introduced into many countries and extensively cultivated in large fields. The important elements for optimal technology of its cultivation are: selection of biological material, soil cultivation, seeding and planting, nutrition and fertilization, control of harmful factors, harvest, processing and conservation. It blossoms in the summer, when it should be collected and carefully dried. It has a strong pungent spice and odour. The EO of thyme was steam-distilled from the fresh or dry leaves and flower tops. Its qualitative compositions were determined by GC-MS (Calendula Co., Nova Lubovna, Slovakia). The qualitative-quantitative characteristics showed five principal constituents – thymol (67.7%), γ-terpinene (8.2%), p-cymene (4.5%), 3-carene (2.5%) and carvacrol (2.5%). In regard to the standards of the European Pharmacopeia, our tested EO after large-scale steam distillation was found to be in substantial agreement with these content ranges of components. However, the highest level was observed for thymol, the biologically most active natural substance. *Th. vulgaris* L. EO was tested for antimicrobial activities using the agar diffusion method. Thyme EO shows a wide spectrum of antimicrobial activity, which proves especially promising in terms of combatting microbial associations of agents of opportunistic infections. However, the highest antimicrobial effect was observed against antibiotic-resistant isolates of *S. aureus* and *C. albicans*.

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