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Antibacterial Activity of Thyme and Lavender Essential Oils

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Abstract: Strong antiseptic activity of essential oils has been known for a long time. The antibacterial activity of oils was tested against clinical bacterial strains of *Staphylococcus*, *Enterococcus*, *Escherichia* and *Pseudomonas* genera. The agar diffusion method was used for microbial growth inhibition at various concentrations of the oils from *T. vulgaris* and *L. angustifolia*. Susceptibility testing to antibiotics and chemotherapeutics was carried out using disc-diffusion method. 120 strains of bacteria isolated from patients with infections of oral cavity, respiratory, genitourinary tracts and from hospital environment were investigated. The results of experiments showed that the oil from *T. vulgaris* exhibited extremely strong activity against all of the clinical strains. Thyme oil demonstrated a good efficacy against antibiotics resistant strains of the tested bacteria. Lavender oil has been less activity against clinical strains of *Staphylococcus*, *Enterococcus* and *Escherichia* genus. The worst results have been observed against all strains of *Pseudomonas aeruginosa*.

Keywords: antibacterial activity, lavender oil, multiresistant strains, thyme oil.

1. INTRODUCTION

Effective fighting against many severe bacterial infections has been possible due to introduction of antibiotic in medicine. The fight continues because of growing resistance to antibiotics commonly used in clinical practice [1]. Opportunistic infections caused by Gram-negative bacteria, mainly of the *Klebsiella* sp., *Enterobacter* sp., *Serratia* sp., *Proteus* sp., *Escherichia* sp. and *Pseudomonas* sp. present problems [2]. There has been a significant increase in the number of infections caused by Gram-positive cocci belonging to *Staphylococcus* sp. and *Enterococcus* sp. [3-5]. Thus the search for effective and safe medicines that could be used to treat particularly persistent bacterial infections is on. Essential oils, a diverse group of plant metabolites, seem to be interesting. They have long been used in aromatherapy, dermatology and cosmetics. Nowadays experimental research confirms different pharmaceutical activity of oils. Various essential oils produce pharmacological effects, demonstrating anti-inflammatory, antioxidant and anticancerogenic properties [6, 7]. In the past years, a large number of essential oils and their constituents have been investigated for their antimicrobial properties against some bacteria, fungi, viruses and protozoa. Essential oils of thyme, oregano, mint, cinnamon, cumin, salvia, clove and eucalyptus have been found to possess the strongest antimicrobial properties among many tested. Many of them appeared to have a wide spectrum of antibiotic activity against microflora which provoke intrahospital infections. Their broad and complex activity, synergy of action, also in combination with antibiotic therapy and the lack of reports about emergence of resistance mechanisms of bacteria to their constituents makes them a valued complement to infection therapy in human diseases [8-11]. Essential oils may be an excellent alternative for other drugs and that the reason for an extensive assessment of their antimicrobial activity.

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2. THE AIM OF THE STUDY

The aim of this work was:

2. 1. to investigate the antimicrobial properties of thyme and lavender essential oils obtained from thyme (*Thymus vulgaris* L.) and lavender (*Lavandula angustifolia* Mill.) against standard and clinical strains isolated from patients, clinical staff, as well as from the hospital environment.

3. MATERIALS

3. 1. Bacterial Strains

3.1.1. Standard Strains

Staphylococcus aureus ATCC 433000, *Enterococcus faecalis* Van B ATCC 51299, *Enterococcus faecalis*, vancomicin-sensitive ATCC 29212, *Enterococcus faecium*, vancomicin-sensitive ATCC 35667, *Enterococcus durans*, vancomicin-sensitive ATCC 6656, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853.

The standard bacterial strains used in the agar dilution method came from collection of Medical and Sanitary Microbiology Department, Medical University in Lodz.

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3.1.2. Clinical Strains

Staphylococcus sp, *Enterococcus* sp, *Escherichia* sp, *Pseudomonas* sp. collected from different materials from patients, medical staff, hospital environment in the wards of internal diseases, surgery, urology, and intensive care unit of two hospitals in Lodz. Bacterial strains were isolated from abdominal cavity exudates (n=4), bronchical secretions (n=5), nose (n=9), ear (n=3), pharynx (n=6), hands (n=2), ulcers (n=14), wounds (n=22), bedsores (n=12), abscesses (n=1), groins (n=5), bile (n=1), toe (n=1), anus (n=6), sputum (n=1), blood (n=2), urine (n=14), drains (n=2), hospital staff (n=2), disinfecting dispencers (n=2), hospital beds (n=2) and cupboard swabs (n=4).

3.2. Bacteriological Media

Several microbiological media were used including Columbia Agar (bioMerieux, France), Mannitol Salt Agar (bioMerieux, France), Mc Conkey Agar (bioMerieux, France), D-Coccosel Agar (bioMerieux, France), Muller Hinton Agar II (bioMerieux, France).

3.3. Essential Oils

Essential oils from thyme - *Thymus vulgaris* L. and from lavender - *Lavandula angustifolia* Mill. (*Lamiaceae*).

3.4. Antibiotics and Chemotherapeutics (*Becton Dickinson*)

The following antibiotics and chemiotherapeutics (*Becton Dickinson*) were used for susceptibility testing of *Staphylococcus aureus* strains: FOX – cefoxitin (30 μg), E – erythromycin (15 μg), CC – clindamycin (2 μg), F / M – nitrofurantoin (300 μg) (for isolates from urine), VA – vancomycin (30 μg), TEC – teicoplanin (30 μg), TE – tetracycline (30 μg), C – chloramphenicol (30 μg), CIP – ciprofloxacin (5 μg), SXT - trimethoprim / sulfamethoxazole (1.25 μg /23.75 μg), FA – fusidic acid (10 μg), LZD – linezolid (30 μg); of Enterococcus genus:

AM – ampicillin (10 μg), C – chloramphenicol (30 μg), CIP – ciprofloxacin (5 μg), E – erythromycin (15 μg), FOS - fosfomycin (200 μg) (only for *E. faecalis*, the isolates from urine), FM - nitrofurantoin (300 μg) (for isolates from urine), GM – gentamicin (120 μg), LNZ - linezolid (30 μg), IPM – imipenem (10 μg), P – penicillin (10 μg), S – streptomycin (300 μg), SYN - synercid (4.5 μg /10.5 μg) (only for *E. faecium*), TE – tetracycline (30 μg), TEC – teicoplanin (30 μg), VA –vancomycin (30 μg),

of *Escherichia coli* strains: AMC - amoxicillin / clavulanic acid (20 μg /10 μg), CF – cefalotin (30 μg), CZ – cefazolin (30 μg), CXM – cefuroxime (30 μg), GM – gentamicin (10 μg), AM - ampicillin (10 μg) (only for the isolates from urine), NOR - norfloxacin (10 μg) (as above), F / M - nitrofurantoin (300 μg) (as above), FOX – cefoxitin (30 μg), CTX – cefatoxim (30 μg), CAZ – ceftazidime (30 μg), ATM – aztreonam (30 μg), IPM – imipenem (10 μg), CIP – ciprofloxacin (5 μg), NET – netilmicin (30 μg), NN - tobramycin (10 μg), C – chloramphenicol (30 μg), TE – tetracycline (30 μg), SXT - trimethoprim / sulfamethoxazole (1.25 μg /23.75 μg)

and of *Pseudomonas aeruginosa* strains: MZ – mezlocillin (75 μg), PIP – piperacillin (100 μg), CAZ – ceftazidime (30 μg), GM – gentamicin (10 μg), NN – tobramycin (10 μg), AMC - amoxicillin / clavulanic acid (20 μg /10 μg), TZP - piperacillin / tazobactam (100 μg /10 μg), CTX – cefotatoxime (30 μg), ATM – aztreonam (30 μg), IPM – imipenem (10 μg), MEM – meropenem (10 μg), NET – netilmicin (30 μg), CIP – ciprofloxacin (5 μg), SXT - trimethoprim / sulfamethoxazole (1.25 μg /23.75 μg), C – chloramphenicol (30 μg), CL – colistin (50 μg).

4. METHODS

4.1. Suspensions of the Tested Bacterial Strains

The standard and clinical strains were cultivated in Columbia agar medium and incubated at 37 ° C for 48 hours in aerobic conditions. Bacterial suspensions with an optical density of 0.5 on a Mc Farland scale were prepared. Bio Merieux densitometer was used.

4.2. Essential Oils Analysis

Commercial essential oil were purchased from the manufacturer and analyzed by GC-FID-MS in the Institute of General Food Chemistry, Technical University of Lodz, using a Trace GC Ultra apparatus (Thermo Electron Corporation) MS DSQ II detectors and FID-MS splitter (SGE). Operating conditions: apolar capillary column Rtx-1ms (Restek), 60 m x 0.25 mm i.d., film thickness 0.25 μm; temperature program, 50-300°C at 4°C/min; SSL injector temperature 280°C; FID temperature 300°C; split ratio 1:20; carrier gas helium at a regular pressure 200 kPa.; FID temperature 260°C; carrier gas, helium; 0.5 ml/min; split ratio 1:20. Mass spectra were acquired over the mass range 30-400 Da, ionization voltage 70 eV; ion source temperature 200°C.

Identification of components was based on the comparison of their MS spectra with those of laboratory-made MS library, commercial libraries (NIST 98.1, Wiley Registry of Mass Spectral Data, 8th Ed. and MassFinder 3.1) and with literature data [12, 13] along with the retention indices on apolar column (Rtx-1, MassFinder 3.1) associated with a series of alkanes with linear interpolation (C_8-C_{26}). A quantitative analysis (expressed as percentages of each component) was carried out by peak area normalization measurements without correction factors.

4.3. Antibacterial Analysis Using Agar Dilution Method

The essential oils were diluted in ethanol. These solutions were mixed with a nutrient broth to obtain concentrations from 0.03125 to 2.5 μl / ml for thyme oil and from 0.125 to 18.5 μl / ml for lavender oil and poured into petri dishes. Inoculum containing $1.5 \cdot 10^8$ CFU (0.1 ml) per spot was seeded upon the surface of agar with various oil concentrations, as well as upon that with no oil added (strains growth control). Minimal Inhibitory Concentration – MIC was determined after 24 h of incubation at 37 ° C in aerobic conditions. Antibacterial analysis of oil activity was performed three times independently

4.4. Tested Strains Susceptibility to Antibiotics and Chemotherapeutics

Susceptibility testing was carried out using disc-diffusion method, Mueller-Hinton II. Cultures were incubated at 37° for 16-18 h, vancomycin for 24 h. The results were interpreted according to *Clinical and Laboratory Standard Institute* (CLSI) [14].

5. STATISTICAL ANALYSIS

Statistical significance was evaluated by one-way non-parametric analysis of variance (ANOVA) (Kruskal – Wallis) and Sheffe test. The differences were considered significant when the probability of the zero hypothesis was less than 5% ($p < 0.05$).

6. RESULTS

6.1. Chemical Composition of the Tested Oils

The analysis of the tested essential oils derived from *T. vulgaris* and *L. angustifolia* revealed that its composition meets the requirements of the Polish Farmacopoeia VIII and the European Farmacopoeia. The content of thymol amounts to 38.1%, and carvacrol to 2.3% from thyme oil. The content of linalool amounts to 34.1%, and linalil acetate to 33.3% from lavender oil. The chemical composition of the tested oils are shown in Table **1** and Table **2**.

6.2. Susceptibility Testing

The tested strains of *Staphylococcus aureus* were resistant to 50% of β-lactams and macrolides and 40% of chemo-

Table 1. Components of the essential oil obtained from thyme - *Thymus vulgaris* L. (*Lamiaceae*)

No.	Compound	Total oil %	RI
1	α-Thujene	0.6	932
2	α-Pinene	1.9	936
3	Camphene	1.2	950
4	Oct-1-en-3-ol	1.0	962
5	β-Pinene	0.3	978
6	Myrecene	1.1	987
7	**p-Cymene**	**29.1**	**1015**
8	1.8-Cineole	2.1	1024
9	Limonene	0.2	1025
10	**γ-Terpinene**	**5.2**	**1051**
11	p-Cymenene	0.1	1075
12	Terpinolene	0.1	1082
13	**Linalool**	**3.7**	**1086**
14	Camphor	0.5	1123
15	Borneol	1.9	1150
16	Terpinene-4-ol	1.3	1164
17	α-Terpineol	0.3	1176
18	Thymol methyl ether	1.3	1215
19	Carvacrol methyl ether	1.0	1226
20	Borneol acetate	0.3	1270
21	**Thymol**	**38.1**	**1267**
22	**Carvacrol**	**2.3**	**1278**
23	Thymol acetate	0.2	1329
24	African-1-en	0.1	1356
25	α-Copaene	0.2	1379
26	β-Burbonene	0.1	1386

Table 1. contd…

No.	Compound	Total oil %	RI
27	**β-Caryopyllene**	**3.1**	**1421**
28	Thymohydroquinone	0.1	1509
29	α-Humulene	0.1	1455
30	γ-Muurolene	0.3	1474
31	cis-β Guaiene	0.1	1488
32	Cuparene	0.1	1498
33	γ-Cadinene	0.6	1507
34	Calamenene B	0.2	1517
35	δ-Cadinene	0.3	1520
36	α-Cadinene	0.1	1534
37	Caryophyllene oxide	0.5	1578
38	γ-Eudesmol	0.1	1618
39	Eudesm-3-en-7-ol	0.1	1650
40	Cadalene	0.1	1659

Table 2. Components of the essential oil obtained from lavender - ***Lavandula angustifolia*** **Mill. (*Lamiaceae*)**

No.	Compound	Total oil %	RI
1	α-Thujene	traces	932
2	α-Pinene	0.1	936
3	Camphene	0.1	950
4	Octan-3-one	1.3	969
5	Sabinene	traces	973
6	Myrcene	2.4	987
7	p-Cymene	0.2	1015
8	1,8-Cyneole	2.5	1024
9	Limonene	0.6	1025
10	(Z)-β-ocymene	3.2	1029
11	(E)-β-ocymene	2.7	1041
12	γ-Terpinene	0.1	1051
13	Terpinolene	0.2	1082
14	Linalool	34.1	1086
15	Octan -1-ene-3-yl	0.6	1093
16	Camfor	1.2	1123
17	Izoborneol	0.2	1142
18	Borneol	1.4	1150

Table 2. contd…

No	Compound	Total oil %	RI
19	Lavandulol	1.1	1150
20	Terpinene-4-ol	2.5	1164
21	α-Terpineol	1.8	1176
22	cis-Dihydrocarvone	0.2	1172
23	Linalil acetate	33.3	1239
24	Lavandulil acetate	3.2	1275
25	Neril acetate	0.8	1342
26	Geranil acetate	1.3	1362
27	β-Caryophyllene	2.7	1421
28	Aromadendrene	0.1	1443
29	α-Humulene	traces	1455
30	(E)-β-Farnezene	0.4	1446
31	Bicyclosesciphelandrene	0.1	1487
32	δ-Cadinene	traces	1520
33	Caryophyllene oxide	0.1	1578
34	T-Cadinol	traces	1633

therapeutics recommended for susceptibility testing. Clinical strains of the *Enterococcus* genus showed resistance to macrolides and chemotherapeutics in almost 70%, to β-lactams and aminoglycosides in 60% and to carbapenems in 40%. The examined *E. coli* strains were resistant to β-lactams and chemotherapeutics in 40% and 20% to aminoglycosides. The tested *P. aeruginosa* strains were resistant to β-lactams and chemotherapeutics in over 50%, to carbapenems in 30% and to aminoglycosides and monobactams in 20%. The results are presented in Tables **3, 4, 5** and **6**.

6.3. The Activity of Thyme and Lavender Oils Against

6.3.1. Staphylococcus aureus *strains*

The values of the MIC for *Staphylococcus aureus* were between 0.25 and 1.0 µl / ml thyme oil concentration. MIC was 0.25 µl / ml for the standard strain of *Staphylococcus aureus* ATCC 433000 and 6 isolated strains from the clinical material. The growth inhibition factors MIC to lavender oil were between 1.0 and 4.0 µl / ml. Oil at a concentration of 2.5 µl / ml inhibited the growth of *Staphylococcus aureus* ATCC 433000 standard strain. The results are shown in Fig. (**1**).

Most *Staphylococcus* sp. strains were sensitive to 0.5 µl / ml thyme oil concentration. Growth inhibition was obtained in 17 out of the 30 clinical bacterial strains. They were mainly isolated from nose, hand, wound and ulcer swabs.

There was a linear relationship between thyme oil concentration and degree of bacterial growth inhibition. Concentrations ranging from 0.5 to 1.0 µl / ml differed significantly in their ability to inhibit growth of *Staphylococcus aureus* strains from the 0.25 µl/ml concentration ($\chi^2(3)=12.72$, $p<0.01$). Lavender oil showed less activity against clinical strains of *Staphylococcus aureus*. The most sensitive to lavender oil was clinical strain isolated from the pancreas drain – MIC (1.0 ml / ml). The values of the MIC were 1.5; 2.0; 2.5 µl / ml for the 21 strains from clinical materials, 7 strains of these concentrations. Lavender oil concentration of 3,5 µl/ml inhibited the growth of 6 clinical strains of *S. aureus*. The results are presented in Fig. (**2**).

There were no statistically significant differences in growth inhibition of *Staphylococcus aureus* treated with lavender oil of 1.5; 2.0; 2.5 and 3.0 µl/ml concentrations ($\chi^2(3)=3.09$, $p=0.39$).

The tested clinical strains of *Staphylococcus aureus* resistant to many antibiotics were sensitive to thyme and lavender oils in low concentrations. *Staphylococcus aureus* strain isolated from abscess was resistant to 7 out of 11 tested antibiotics (sensitive to: VA, TEC, C, LZD); the MIC value for the thyme oil was 0.25 µl / ml and for lavender oil was 1.5 µl/ml. The MIC for highly multiresistant bacterial strains from the hand wound, ear, foot ulceration, exudates from episiotomy wounds, stump ulcers and urine were from 0.5 µl / ml to 0.75 µl / ml thyme oil concentration and for lavender oil were between 1.5 µl/ml and 3.0 µl/ml. Thyme oil at 0.5 µl / ml concentration and lavender oil at 1.5 µl/ml concentration inhibited the growth of over 45% of the resistant strains.

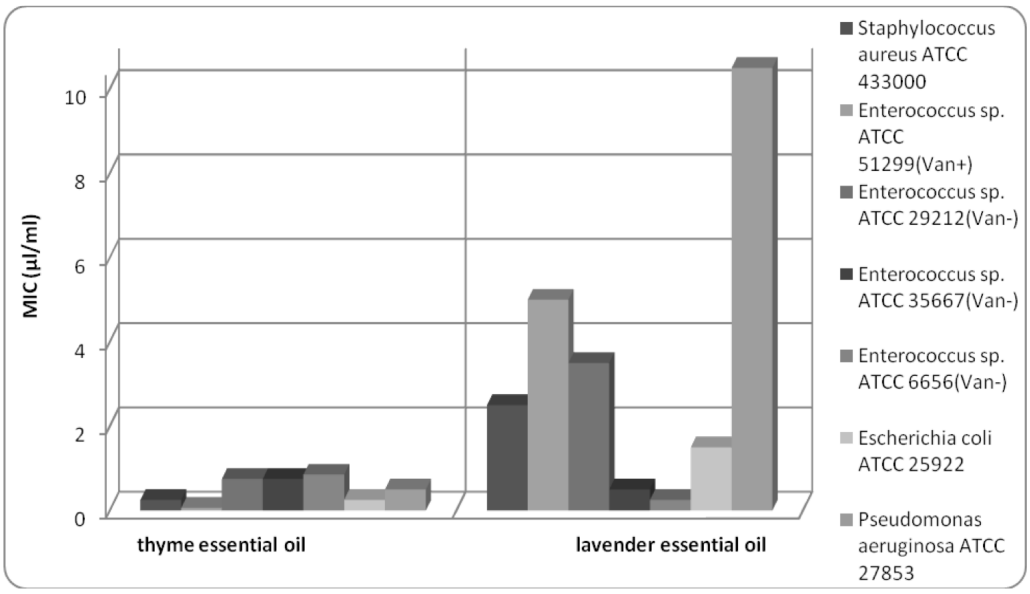

Fig. (1). Standard strains susceptibility to thyme and lavender essential oils.

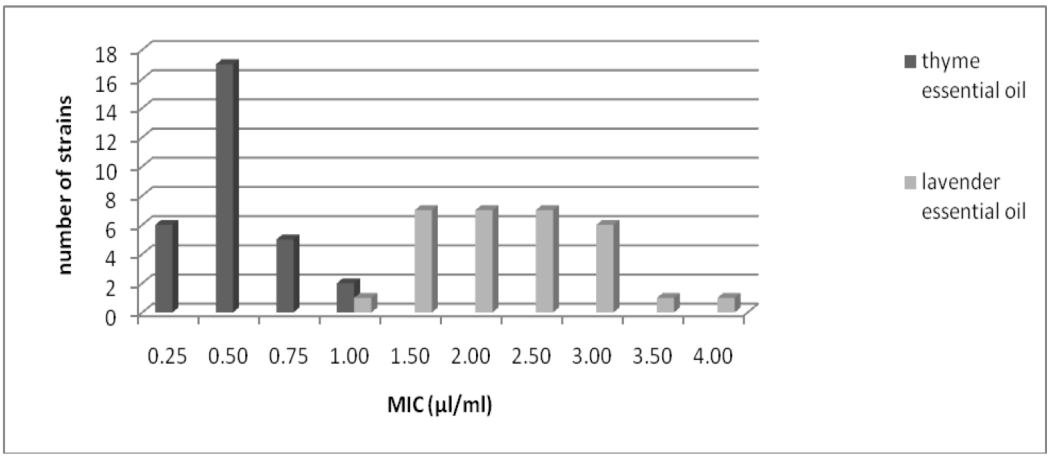

Fig. (2). Clinical strains of *Staphylococcus aureus* susceptibility to thyme and lavender essential oils.

Table **3** shows general characteristics of *Staphylococcus aureus* isolates.

6.3.2. Enterococcus sp. strains

Vancomycin resistant standard strain - *Enterococcus faecalis* Van B ATCC 51299 was the most sensitive strain to essential oil of thyme (MIC – 0.0625 µl / ml). The growth inhibition factor MIC was 0.75 µl / ml for vancomycin sensitive strains of *E. faecalis* ATCC 29212 and *E. faecium* ATCC 35667 and MIC was 0.85 µl / ml, slightly higher, for the standard strain of *E. durans* ATCC 6656. Lavender oil was active between 0.25 and 5.0 µl/ml concentration. Vancomycin resistant standard strain - *Enterococcus faecalis* Van B ATCC 51299 was the most resistant strain to essential oil of lavender (MIC – 5.0 µl/ml). Vancomycin sensitive strain of *E. faecalis* ATCC 29212 was sensitive to lavender oil at 3.5 µl/ml concentration. Standard strains were highly less resistant - *E. faecium* ATCC 35667 (MIC – 0.5 µl/ml) and *E. durans* (MIC – 0.25 µl/ml). The results are shown in Fig. (**1**).

The thyme oil was also very active against clinical strains, the most active against *E. faecium* strain isolated from the ulcers (MIC – 0.25 µl / ml). Oil at a concentration of 0.75 µl / ml inhibited significantly 16 clinical strains and at 0.5 µl / ml - 11 clinical strains. The value of the MIC – 0.5 µl / ml was characteristic for all strains of *E. faecalis* and *E. faecium* isolated from urine, and *E. faecalis* strains derived from the hospital environment. The concentration of 0.75 µl / ml inhibited the growth of *E. faecalis* clinical strains from wounds and throat and *E. faecium* strains from blood and from hospital environment.

Concentrations of 0.5 and 0.75 µl / ml differed in *Enterococcus* sp. growth inhibition, higher concentration proved to be more effective: ($\chi^2(1)=6.94$, $p<0.01$). The most sensitive to lavender oil was clinical strain of *E. faecalis* isolated from urine and *E. durans* isolated form hospital environment, the value of the MIC was 1.0 µl/ml. Clinical strains of the *E. faecalis* and *E. faecium* were inhibited by lavender oil at

Table 3. Characteristics of *Staphylococcus aureus* isolates

No.	*Staphylococcus aureus* strain / clinical material	MIC of thyme essential oil µl/ml	MIC of lavender essential oil µl/ml	Susceptibility to antibiotics and chemotherapeutics												Total		
				FOX	E	CC	F/M	VA	TEC	TE	C	CIP	SXT	FA	LZD	R	I	S
1.	swab/nose	0.25	2,0	R	S	R	-	S	S	R	S	I	S	S	S	3	1	7
2.	swab/nose	0.5	2,5	S	S	S	-	S	S	R	S	S	S	S	S	1	-	10
3.	swab/nose	0.75	2,5	R	S	R	-	S	S	R	S	I	R	R	S	5	1	5
4.	swab/nose	0.5	1,5	R	S	R	-	S	S	R	S	I	I	R	S	4	2	5
5.	swab/nose	0.5	2,0	R	S	R	-	S	S	R	S	I	S	R	S	4	1	6
6.	swab/nose	0.25	1,5	S	S	S	-	S	S	S	S	S	R	R	S	2	-	9
7.	swab/nose	0.5	2,0	S	S	S	-	S	S	R	S	S	R	S	S	2	-	9
8.	swab/nose	0.5	3,0	S	S	S	-	S	S	S	S	S	I	S	S	-	1	10
9.	swab/ear	0.5	1,5	R	R	R	-	S	S	R	S	R	R	R	S	7	-	4
10.	swab/hend	0.5	2,0	S	S	S	-	S	S	R	S	S	S	S	S	1	-	10
11.	swab/hend	0.5	3,0	R	R	R	-	S	S	R	S	R	R	R	S	7	-	4
12.	swab/wound	0.75	1,5	R	S	R	-	S	S	R	S	I	R	R	R	6	1	4
13.	swab/wound	0.75	2,5	R	S	R	-	S	S	R	S	I	R	S	S	4	1	6
14.	swab/wound	0.5	2,5	S	S	S	-	S	S	R	S	R	S	S	S	2	-	9
15.	swab/wound	0.5	3,0	I	R	R	-	S	S	R	R	S	S	S	S	4	1	6
16.	swab/wound	0.5	2,0	S	S	S	-	S	S	R	S	S	S	S	S	1	-	10
17.	swab/wound	1.0	3,0	R	R	R	-	S	S	R	S	R	S	R	S	6	-	5
18.	exudation /abdominal cavity	0.25	3,0	R	R	R	-	S	S	R	S	R	S	S	S	5	-	6
19.	exudation /abdominal cavity	0.5	2,5	I	R	R	-	S	S	R	S	R	S	S	S	4	1	6
20.	exudation /abdominal cavity	0.25	1,5	S	R	R	-	S	S	R	S	R	S	S	S	4	-	7
21.	swab/ulceration	0.5	1,5	R	R	R	-	S	S	R	S	R	I	R	S	6	1	4
22.	swab/ulceration	0.5	2,0	S	R	R	-	S	S	R	S	R	S	S	S	4	-	7
23.	swab/ulceration	0.75	3,5	R	R	R	-	S	S	S	R	R	R	R	S	7	-	4
24.	swab/ulceration	0.5	2,5	S	S	S	-	S	S	S	S	S	S	S	S	-	-	11
25.	swab/groin	0.5	3,0	S	S	S	-	S	S	S	S	S	S	S	S	-	-	11
26.	swab/groin	1.0	4,0	I	R	R	-	S	S	R	R	I	S	S	S	4	2	5
27.	swab/abscess	0.25	1,5	R	R	R	-	S	S	R	S	R	R	R	S	7	-	4
28.	urine	0.5	2,0	I	R	R	S	S	S	R	S	R	S	S	R	5	1	6
29.	urine	0.25	2,5	R	S	R	S	S	S	R	S	I	R	S	S	4	1	7
30.	swab/drain	0.75	1,0	S	S	S	-	S	S	R	S	S	S	S	S	1	-	10

R–resistant, I–intermediate susceptible strain, S- susceptible strain Control media containing alcohol (in concentration used to dilution thyme and lavender essential oils) were not inhibiting growth of *Staphylococcus aureus* strains.

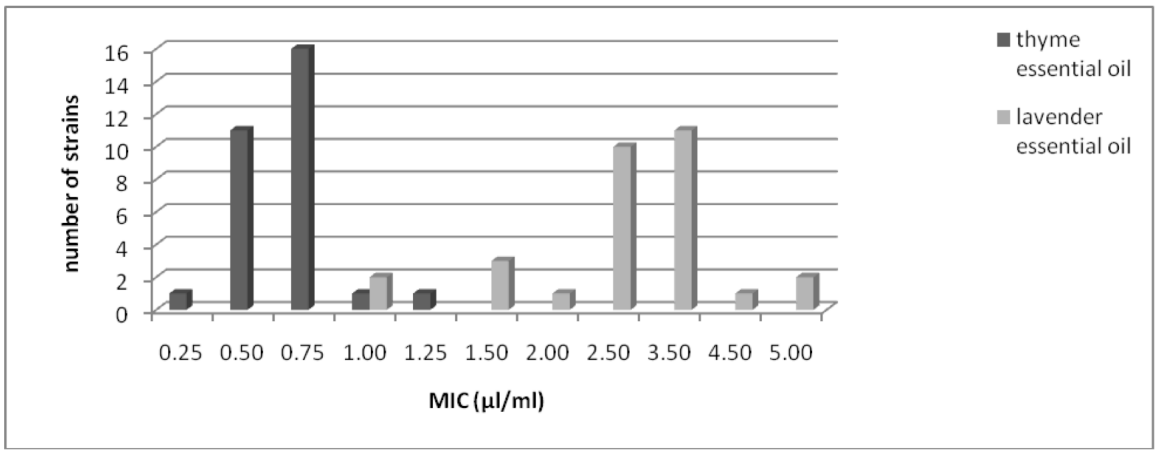

Fig. (3). Clinical strains of *Enterococcus* sp. susceptibility to thyme and lavender essential oils.

Table 4. Characteristics of *Enterococcus* sp. isolates

No.	*Enterococcus* spp. strain / clinical material	MIC of thyme essential oil µl/ml	MIC of lavender essential oil µl/ml	Susceptibility to antibiotics and chemotherapeutics														Total		
				AM	C	CIP	E	FOS	FM	GM	IPM	LNZ	P	S	SYN	TE	TEC	VA	R	I	S
1.	*E. faecalis*/urine	0.5	3,0	S	S	R	S	S	S	S	S	S	S	S	-	I	S	S	1	1	12
2.	*E. faecalis*/urine	0.5	1,0	R	S	R	R	S	S	R	R	S	R	R	-	R	S	S	8	-	6
3.	*E. faecalis*/urine	0.5	4,0	S	S	I	I	S	S	S	S	S	S	S	-	R	S	S	1	2	11
4.	*E. faecalis*/urine	0.5	3,0	S	R	R	R	S	S	R	S	S	R	R	-	R	S	S	7	-	7
5.	*E. faecalis*/urine	0.5	3,0	S	R	R	R	S	S	R	S	S	S	R	-	R	S	S	6	-	8
6.	*E. faecalis* /swab/wound	0.75	4,0	S	S	I	S	-	-	S	S	S	S	S	-	S	S	S	-	1	11
7.	*E. faecalis* /swab/wound	0.75	4,0	S	R	R	R	-	S	R	S	S	S	R	-	R	S	S	6	-	7
8.	*E. faecalis* /swab/wound	0.75	4,5	S	R	R	R	-	-	R	S	S	S	R	-	R	S	S	6	-	6
9.	*E. faecalis*/ swab/ulceration	0.75	4,0	S	R	S	R	-	-	S	S	S	S	I	-	R	S	S	3	1	8
10.	*E. faecalis*/bile	0.75	4,0	S	S	S	S	S	S	S	S	S	S	S	-	R	S	S	1	-	13
11.	*E. faecalis* /swab/bedsore	1.25	3,0	S	S	I	S	-	-	S	S	S	S	S	-	R	S	S	1	1	10
12.	*E. faecalis* /swab/pharynx	0.75	3,0	S	S	S	I	-	-	S	S	S	S	S	-	R	S	S	1	1	10
13.	*E. faecalis* /swab/pharynx	0.75	4,0	S	R	I	R	-	-	R	S	S	S	R	-	R	S	S	5	1	6
14.	*E. faecalis* /hospital staff	0.75	4,0	S	S	S	R	-	-	S	S	S	S	R	-	R	S	S	3	-	9
15.	*E. faecalis*/ drain	0.75	4,0	S	S	R	S	-	-	R	S	S	S	S	-	R	S	S	3	-	9
16.	*E. faecalis*/ cupboard	0.5	1,5	S	S	R	S	-	-	R	S	S	S	S	-	R	S	S	3	-	9

Table 4. contd…..

No.	*Enterococcus* spp. strain / clinical material	MIC of thyme essential oil µl/ml	MIC of lavender essential oil µl/ml	Susceptibility to antibiotics and chemotherapeutics															Total		
				AM	C	CIP	E	FOS	FM	GM	IPM	LNZ	P	S	SYN	TE	TEC	VA	R	I	S
17.	*E. faecalis*/ disinfecting dispencer	0.5	5,0	S	S	I	S	-	-	S	S	S	S	S	-	R	S	S	1	1	10
18.	*E. faecalis*/ disinfecting dispencer	0.5	3,0	S	S	R	S	-	-	R	S	S	S	S	-	R	S	S	3	-	9
19.	*E. faecium*/urine	0.5	2,0	R	S	R	R	-	S	R	R	S	R	R	S	R	S	S	8	-	6
20.	*E. faecium*/urine	0.5	4,0	R	S	R	R	-	R	R	R	S	R	R	S	S	S	S	8	-	6
21.	*E. faecium*/urine	0.5	4,5	R	S	R	R	-	I	R	R	S	R	R	S	R	S	S	8	1	5
22.	*E. faecium*/blood	0.75	3,0	R	S	R	R	-	-	S	R	S	S	R	S	R	S	S	6	-	7
23.	*E. faecium*/blood	0.75	4,0	R	S	R	R	-	-	S	R	S	R	R	S	R	S	S	7	-	6
24.	*E. faecium*/ swab/ulceration	0.25	3,0	R	I	R	R	-	R	R	R	S	R	R	S	R	S	S	9	1	4
25.	*E. faecium*/ hospital staff	0.75	3,0	R	S	R	R	-	-	S	R	S	R	R	S	R	S	S	7	-	6
26.	*E. faecium*/ hospital bed	0.75	4,0	R	S	R	R	-	-	R	R	S	R	R	S	S	S	S	7	-	6
27.	*E. faecium*/ hospital bed	0.75	3,0	S	S	S	S	-	-	S	S	S	S	S	S	S	S	S	-	-	13
28.	*E. faecium*/ cupboard	0.75	1,5	R	S	R	R	-	-	R	S	S	S	S	-	R	S	S	5	-	7
29.	*E. faecium*/ cupboard	0.75	1,5	R	S	R	R	-	S	R	R	S	R	R	S	R	S	S	8	-	6
30.	*E. durans*/ cupboard	1.0	1,0	R	S	R	R	-	S	R	R	S	R	R	S	R	S	S	8	-	6

R–resistant, I– intermediate susceptible strain, S- susceptible strain
Control media containing alcohol (in concentration used to dilution thyme and lavender essential oils) were not inhibiting growth of *Enterococcus* sp. strains.

concentration 2.5 µl/ml – 10 strains and at 3.5 µl/ml – 11 strains. The results are shown in Fig. (**3**).

Concentrations 2.5 µl / ml differed significantly in their ability to inhibit growth of *Staphylococcus aureus* strains from 1.0; 3.5 to 5.0 µl/ml concentrations: ($\chi^2(4)=11.25$, $p<0.05$).

It was found that most antibiotics resistant of enterococci clinical strains were most sensitive to the tested oils. *E. faecium* strain isolated from ulcers, resistant to 9 out of 14 antibiotics (sensitive to LNZ, SYN, TEC, VA), was sensitive to the essential oil of thyme in the lowest concentration of 0.25 µl / ml and to the oil of lavender at 2.5 µl / ml concentration. All *E. faecium* clinical strains isolated from urine, resistant to 8 antibiotics were sensitive to oil of thyme, MIC was 0.5 µl / ml. Multiresistant strains - *E. faecium* and *E. durans* isolated from hospital environment were sensitive to lavender essential oil at 1.5 and 1.0 µl/ml concentration. Over 45% of them were sensitive to oil of thyme - MIC (0.75 µl / ml) and over 40% of them were sensitive to oil of lavender – MIC (2.5 µl / ml). Table **4** shows general characteristics of *Enterococcus* sp. isolates.

6.3.3. Escherichia coli strains

Thyme oil showed bacteriocidal activity against *E. coli* ATCC 25922 standard strain at 0.25 µl / ml and lavender oil was active at 1.5 µl / ml concentration (Fig. **1**). The value of the MIC - 0.25 µl / ml for thyme oil was determined for 13 strains from the clinical material. It was effective against clinical strains isolated from the throat and most of the strains from wound swabs. The MIC – 0.5 µl / ml was obtained for 17 tested clinical strains of colon bacilli, isolated from anus, bedsore swabs and from urine.

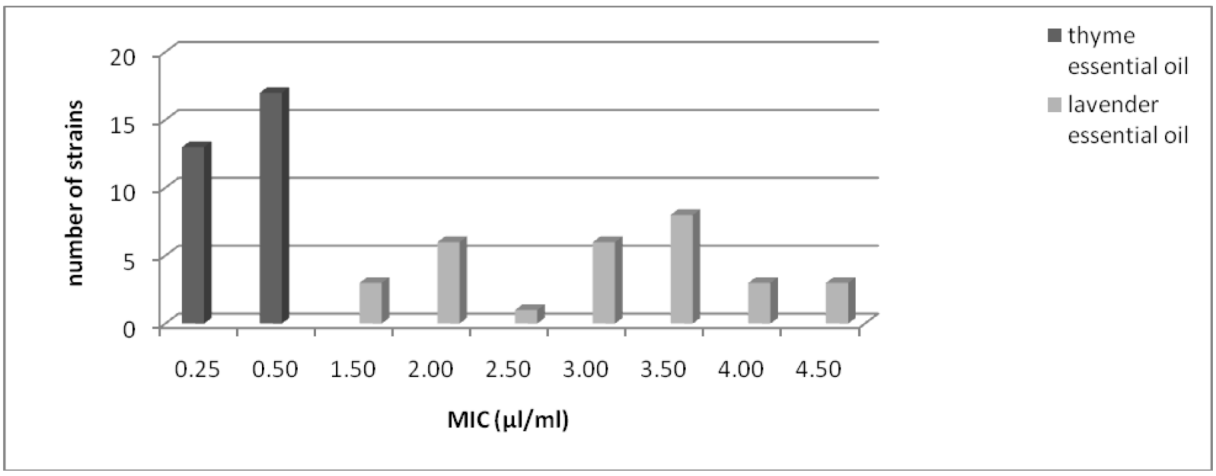

Fig. (4). Clinical strains of *Escherichia coli* susceptibility to thyme and lavender essential oils.

The statistical analysis showed a statistically significant difference between the concentrations of 0.25 µl / ml 0.5 µl / ml used. Higher concentration of the essential oil of thyme proved to be more effective in inhibiting the growth of *Escherichia coli* clinical strains ($\chi^2(1)=21.19$, $p<0.01$). Inhibiting concentrations of lavender oil against clinical strains of *Escherichia coli* were between 1.5 and 4.5 µl / ml. The most sensitive to lavender oil were 3 clinical strains isolated from bronchial aspirate, groins and ulcers swab - MIC (1.5 µl/ml). The largest number of *E. coli* strains were sensitive to 3.5 µl / ml lavender oil concentration, they were isolated from swabs and bedsores. The values of the MIC – 3.0 and 3.5 µl/ml were determined for clinical strains of *E. coli* isolated from wound swabs. Essential oil from lavender was least active against 3 strains isolated from abdominal exudates, anus swab and urine (MIC – 4.5 µl/ml). It is presented in Fig. (**4**).

There were no statistically significant differences in growth inhibition of *Escherichia coli* treated with lavender oil: ($\chi^2(5)=4.28$, $p=0.51$).

The tested clinical strains of *Escherichia coli* highly resistant to commonly used antibiotics showed the sensitivity to thyme and lavender oils. The multiresistant strain isolated from ulcers, resistant to 14 out of 16 antibiotics (sensitive to the ATM, IPM) was sensitive to thyme oil - MIC (0.25 µl / ml) and to lavender oil – MIC (1.5 µl / ml). The values of the MIC – 0.25 µl/ml for thyme oil and 3.0 µl/ml for lavender oil were obtained for two strains from wound and bedsore swabs, which were sensitive to only 5 out of the 16 tested antibiotics. MIC for thyme oil – 0.5 µl/ml and to lavender oil – 3.5 µl/ml obtained for multiresistant strains isolated from bedsores swabs. The growth of nearly 60% of the resistant strains was inhibited by thyme oil of 0.5 µl / ml and by lavender oil of 3.0 µl / ml. Table **5** shows general features of *Escherichia coli* isolates.

6.3.4. Pseudomonas aeruginosa strains

Strains of *Pseudomonas aeruginosa* were the most resistant to lavender oil. The standard strain - *Pseudomonas aeruginosa* ATCC 27853 was inhibited by thyme oil of 0.5 µl / ml and by lavender oil of 10.5 µl / ml. The results are shown in Fig. (**1**).

Number of clinical strains which were sensitive to the thyme oil of 1.5; 2.0; 2.5 µl / ml was similar. The concentration 1.5 and 2.0 µl / ml inhibited the growth of strains isolated mainly from wounds and bedsores. Values of the MIC – 2.0 and 2.5 µl / ml were obtained for blue pus bacilli isolated from ulcers. The highest concentration of thyme oil 2.5 µl / ml was effective against the bacteria from bronchial secretions and from the anus swabs.

There were no statistically significant differences in growth inhibition of *Pseudomonas aeruginosa* treated with thyme oil at 0.5; 1.5; 2.0 and 2.5 µl / ml concentrations ($\chi^2(3)=3.54$, $p=0.32$). Lavender oil was active at 10.0 µl/ml concentration against 2 clinical strains isolated from throat. The MIC – 18.0 µl / ml was obtained for 9 tested clinical strains of blue pus bacilli isolated from bronchial aspirate, anus and bedsore swabs. The highest values of the MIC – 18.5 and 19.0 µl / ml were for clinical strains of *Pseudomonas aeruginosa* isolated from ulcers and wounds. It is presented in Fig. (**5**).

Lavender oil concentrations ranging from 18.0 to 19.0 µl / ml differed significantly in their ability to inhibit growth of *Pseudomonas aeruginosa* strains from the 10.0 µl/ml and 14.5 µl/ml concentration: 10.0 and 14.5 µl/ml ($\chi^2(5)=19.54$, $p<0.01$).

It was found that the most resistant micro-organisms were isolated from bronchial challenge secretions. Two of them were found to be resistant to all 16 antibiotics and the third was sensitive to only 6 of them. MIC obtained for these strains for thyme oil ranged from 2.0 to 2.5 µl / ml and for lavender oil – 17.5-18.5 µl / ml. The higher activity of thyme oil (MIC-1.5 µl / ml) and lavender oil (MIC-14.5 µl / ml) were obtained against *Pseudomonas aeruginosa* strain isolated from the flank, which showed resistance to 12 antibiotics (sensitive to: AMC, TZP, IPM, NET). At the same time, these strains were sensitive in 40% to thyme oil used at 1.5 µl / ml concentration and were sensitive in 30% of lavender oil at 18.0 and 18.5 µl / ml concentrations. Table **6** shows general characteristics of *Pseudomonas aeruginosa* isolates.

Table 5. Characteristics of *Escherichia coli* isolates

No.	*Escherichia coli* strain /clinical material	MIC of thyme essential oil µl/ml	MIC of lavender essential oil µl/ml	Susceptibility to antibiotics and chemotherapeutics																				Total		
				AMC	CF	CZ	CXM	GM	AM	NOR	F/M	FOX	CTX	CAZ	ATM	IPM	CIP	NET	NN	C	TE	SXT	R	I	S
1.	swab /pharynx	0.25	2,0	R	R	R	R	S	-	-	-	S	S	S	S	S	S	S	S	S	I	R	5	1	10
2.	swab /pharynx	0.25	2,0	R	R	R	R	S	-	-	-	S	S	S	I	S	S	S	S	S	I	R	5	2	9
3.	swab/nose	0.5	2,5	S	S	S	S	S	-	-	-	S	S	S	R	S	S	S	R	S	R	S	3	-	13
4.	secretion/ bronchical	0.25	1,5	R	I	I	S	S	-	-	-	S	R	S	S	S	S	S	S	I	S	S	2	3	11
5.	secretion/ bronchical	0.5	4,0	R	R	I	S	S	-	-	-	S	R	S	S	S	S	S	S	I	S	S	3	2	11
6.	swab/groin	0.5	1,5	R	R	R	R	S	-	-	-	S	S	S	S	S	S	S	S	R	R	R	6	-	9
7.	swab/groin	0.25	3,0	R	I	S	S	S	-	-	-	S	S	S	S	S	S	S	S	S	I	S	1	2	13
8.	exudation/ abdominal cavity	0.5	4,5	R	R	S	S	S	-	-	-	R	R	R	S	S	S	S	R	R	R	R	9	-	7
9.	swab/anus	0.25	2,0	S	S	S	S	S	-	-	-	S	S	S	S	S	S	S	S	S	S	S	-	-	16
10.	swab/anus	0.5	2,0	R	I	I	I	S	-	-	-	S	S	S	S	S	S	S	S	S	I	S	1	4	11
11.	swab/anus	0.5	4,0	I	R	S	S	S	-	-	-	S	S	S	S	S	S	S	S	S	R	R	3	1	12
12.	swab/anus	0.5	4,5	S	S	S	I	S	-	-	-	S	S	S	S	S	S	S	S	I	R	S	1	2	13
13.	swab /bedsore	0.5	3,5	I	R	R	R	R	-	-	-	S	I	R	S	S	R	R	R	R	R	R	11	2	3
14.	swab /bedsore	0.5	3,5	S	I	S	I	S	-	-	-	S	S	I	S	S	S	S	S	S	S	S	-	3	13
15.	swab /bedsore	0.5	4,0	R	R	S	S	S	-	-	-	S	S	S	S	S	S	S	S	S	R	S	3	-	13
16.	swab /bedsore	0.5	3,5	R	R	R	R	S	-	-	-	R	R	R	S	S	R	R	S	S	R	R	11	-	5
17.	swab /bedsore	0.25	3,5	R	S	S	S	S	-	-	-	S	S	S	S	S	S	S	S	S	R	S	2	-	14
18.	swab /ulceration	0.5	3,0	R	R	R	R	R	-	-	-	R	R	R	S	S	R	R	R	R	R	R	14	-	2
19.	swab /ulceration	0.25	1,5	R	R	R	S	S	-	-	-	R	S	S	S	S	S	S	S	S	R	R	6	-	10
20.	swab /wound	0.25	2,0	S	S	S	S	S	-	-	-	S	S	S	S	S	S	S	S	S	R	S	1	-	15
21.	swab /wound	0.5	3,5	R	R	S	R	S	-	-	-	S	S	R	S	S	S	S	S	S	R	R	6	-	10
22.	swab /wound	0.5	3,5	R	R	S	R	S	-	-	-	S	S	S	S	S	S	S	S	R	R	R	6	-	10

Table 5. contd….

No.	*Escherichia coli* strain /clinical material	MIC of thyme essential oil µl/ml	MIC of lavenderessential oil µl/ml	Susceptibility to antibiotics and chemotherapeutics																		Total		
				AMC	CF	CZ	CXM	GM	AM	NOR	F/M	FOX	CTX	CAZ	ATM	IPM	CIP	NET	NN	C	TE	SXT	R	I	S
23.	swab /wound	0.25	3,5	I	I	S	S	S	-	-	-	S	S	S	R	S	S	S	R	S	R	R	4	2	10
24.	swab /wound	0.25	3,0	R	S	S	S	S	-	-	-	S	S	S	S	S	S	S	S	S	I	S	1	1	14
25.	swab /wound	0.25	3,0	R	R	R	R	R	-	-	-	S	R	S	S	S	R	R	R	S	R	R	11	-	5
26.	swab /wound	0.25	3,0	R	R	R	R	R	-	-	-	S	S	S	R	S	R	R	R	S	R	R	11	-	5
27.	urine	0.5	4,5	I	R	S	S	S	R	S	S	R	S	S	S	S	S	S	S	S	R	S	4	1	14
28.	urine	0.5	2,0	R	R	R	S	S	R	S	S	R	S	S	S	S	S	S	S	R	R	S	7	-	12
29.	urine	0.25	3,0	R	R	R	S	S	R	R	S	S	S	S	S	S	R	S	S	S	R	R	8	-	11
30.	urine	0.5	3,5	R	R	R	I	S	R	I	S	R	S	S	S	S	I	S	S	I	R	I	6	5	8

R–resistant, I– intermediate susceptible strain, S- susceptible strain
Control media containing alcohol (in concentration used to dilution thyme and lavender essential oils) were not inhibiting growth of *Escherichia coli* strains.

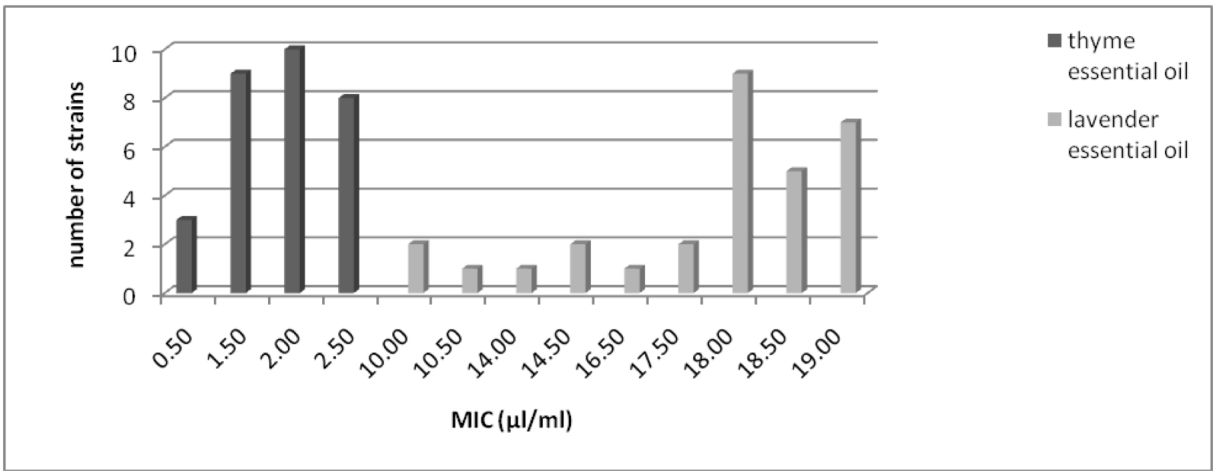

Fig. (5). Clinical strains of *Pseudomonas aeruginosa* susceptibility to thyme and lavender essential oils.

7. DISCUSSION

Knowledge about medicinal properties of substances obtained from plant materials goes back thousands of years. Because of the advances in science, many biologically active compounds of plant origin have been identified and their mechanisms of action have been understood. Essential oils derived from plants belonging to the *Lamiaceae* family are particularly valuable because of antibacterial and antioxidant properties. These oils are obtained from: *Mentha* sp., *Thymus* sp., *Origanum* sp., *Salvia* sp., *Lavandula* sp., *Rosmarinus* sp., *Ocimum* sp., *Majorana* sp., *Hyssopus* sp., *Melissa* sp. and *Satureja* sp. genus.

A lot of chemotypes were identified in red thyme *T. vulgaris*, of which the most important are thymol chemotype (65% thymol, 5-10% carvacrol) and carvacrol chemotype (85% carvacrol, 1-5% thymol) [15]. Polish Pharmacopeia and European Pharmacopoeia show flourishing, fresh herb of *Thymus vulgaris* L and *Thymus zygis* Loefl. ex L as the source of the essential oil of thyme. According to the requirements of FP and FE the oil should contain: thymol (36-55%) and carvacrol (1-4%) [16, 17]. Lavender oil is obtained from flourishing, fresh or dry herb of (*Lavandula angustifolia* Mill. syn. *L. officinalis* Chaix., *L. vera* DC) in accordance with the European Pharmacopoeia. It should contain: linalool from 20 to 45% and linallil acetate from 25 to 46%.

Table 6. Characteristics of *Pseudomonas aeruginosa* isolates

No.	*Pseudomonas aeruginosa* strain / clinical material	MIC of thyme essential oil µl/ml	MIC of lavender essential oil µl/ml	Susceptibility to antibiotics and chemotherapeutics																Total		
				MZ	PIP	CAZ	GM	NN	AMC	TZP	CTX	ATM	IPM	MEM	NET	CIP	SXT	C	CL	R	I	S
1.	swab/ear	1.0	14,5	S	S	S	R	S	R	S	S	S	S	S	S	S	S	S	S	2	-	14
2.	swab/ear	1.0	13,5	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	-	2	14
3.	swab /pharynx	2.5	10,0	R	S	S	S	S	R	S	R	S	S	S	S	I	R	R	S	5	1	10
4.	swab /pharynx	2.0	10,0	R	S	S	S	S	R	S	R	S	R	S	S	S	R	R	R	7	-	9
5.	swab/groin	1.5	14,5	R	R	R	R	R	I	S	R	R	S	R	S	R	R	R	R	12	1	3
6.	swab/toe	1.5	14,0	S	S	S	S	S	S	S	R	R	S	R	S	R	R	R	R	7	-	9
7.	swab/anus	2.5	18,0	S	S	S	S	S	S	S	R	S	S	S	S	S	R	R	R	4	-	12
8.	swab/anus	2.5	18,0	S	S	S	R	R	S	S	R	S	S	S	S	S	R	R	R	6	-	10
9.	sputum	1.0	17,0	S	S	S	S	S	R	S	S	S	S	S	S	S	S	R	I	2	1	13
10.	secretion /bronchical	2.0	18,0	R	R	S	I	S	R	S	R	R	R	R	S	R	R	R	S	10	1	5
11.	secretion /bronchical	2.5	18,0	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	16	-	-
12.	secretion /bronchical	2.5	18,5	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	16	-	-
13.	swab /bedsore	1.5	17,5	R	R	R	S	R	R	S	I	R	S	R	I	I	R	R	R	10	3	3
14.	swab /bedsore	1.5	17,5	R	R	S	S	S	R	S	R	S	S	S	R	R	R	R	S	8	-	8
15.	swab /bedsore	1.5	18,0	R	S	S	S	S	R	S	R	S	S	S	S	S	R	R	S	5	-	11
16.	swab /bedsore	2.0	18,0	R	S	S	S	S	R	R	R	S	S	S	S	S	R	R	R	7	-	9
17.	swab /bedsore	2.0	18,0	R	R	S	S	S	R	R	R	S	S	S	R	R	R	R	S	9	-	7
18.	swab /bedsore	2.0	18,0	R	S	S	S	S	R	S	R	S	S	S	S	S	R	R	R	6	-	10
19.	swab/wound	1.5	18,0	S	R	S	S	R	R	S	S	S	S	R	S	S	R	R	R	7	-	9
20.	swab/wound	1.5	18,5	R	R	S	S	I	R	S	S	S	S	R	S	S	R	R	S	6	1	9
21.	swab/wound	2.0	18,5	S	R	S	S	I	R	S	S	S	S	R	S	S	R	R	S	5	1	10
22.	swab/wound	2.0	19,0	R	S	S	S	S	R	S	R	S	S	S	S	S	R	R	R	6	-	10
23.	swab/wound	1.5	19,0	R	S	S	S	S	R	S	I	S	S	S	S	S	R	R	S	4	1	11
24.	swab/wound	1.5	19,0	R	S	S	S	S	R	R	R	S	S	R	S	S	R	R	R	8	-	8
25.	swab /ulceration	2.5	18,5	S	S	S	S	R	R	S	R	S	S	R	S	S	R	R	S	6	-	10
26.	swab /ulceration	2.5	18,5	R	S	S	S	S	R	R	R	S	S	R	S	S	R	R	R	8	-	8
27.	swab /ulceration	2.5	19.0	R	S	S	S	S	R	S	R	S	S	S	S	S	R	R	R	6	-	10
28.	swab /ulceration	2.0	19,0	R	S	S	S	S	R	S	I	S	S	S	S	S	R	R	S	4	1	11

Table 6. contd….

No.	*Pseudomonas aeruginosa* strain / clinical material	MIC of thyme essential oil µl/ml	MIC of lavender essential oil µl/ml	Susceptibility to antibiotics and chemotherapeutics																Total		
				MZ	PIP	CAZ	GM	NN	AMC	TZP	CTX	ATM	IPM	MEM	NET	CIP	SXT	C	CL	R	I	S
29.	swab /ulceration	2.0	19,0	R	R	S	S	I	R	S	S	S	S	R	S	S	R	R	S	6	1	9
30.	swab /ulceration	2.0	19,0	R	R	S	S	S	R	R	R	S	S	S	R	R	R	R	S	9	-	7

R–resistant, I– intermediate susceptible strain, S- susceptible strain
Control media containing alcohol (in concentration used to dilution thyme and lavender essential oils) were not inhibiting growth of *Pseudomonas aeruginosa* strains.

In the study we investigated antibacterial activity of thyme and lavender oils against standard and clinical strains of the bacteria: *Staphylococcus aureus*, *Enterococcus faecalis*, *Enterococcus faecium*, *Enterococcus durans*, *Escherichia coli* and *Pseudomonas aeruginosa*. The obtained results are in accordance with the literature and show that thyme oil has strong antimicrobial properties against all tested strains [6]. The activity is due to the high content of phenolic compounds with the antibacterial properties such as thymol and carvacrol which are over 40% of the ingredients of the oil. In accordance with the literature, *Thymus vulgaris L* oil showed growth inhibitory effect against *Staphylococcus aureus* strains isolated from respiratory infections. Using the discs-diffusion method, MIC - 0.0125 µl / ml was obtained for thyme oil and also for *Cinnamomum zeylanicum* Blume. and *Syzygium aromaticum* (L.) Merr. & Perry, rich in phenolic compounds. The tested strains of *Staphylococcus aureus*, sensitive to this essential oil were resistant to oxacylin, gentamycin and tobramycin and many of them to norfloxacin [18]. Oil obtained from *Thymus fontanesii* Boiss. Et Reut. containing carvacrol used at 0.3 µl / ml concentration inhibited the growth of the standard and clinical strains of *Staphylococcus aureus* isolated from clinical materials [19]. The oil was derived from carvacrol chemotype of *Thymus ciliatus* (Desf.) Benth. ssp. *eu-ciliatus* Maire. Thyme oil inhibited the growth of pristinamicin sensitive *Staphylococcus aureus* strains isolated from respiratory diseases with MIC 0.8 µl / ml, our results were similar [20]. Thyme oil of *T. vulgaris* L. because of the high content of active phenols and p-cymene showed a very strong activity against standard strains of *Enterococcus* genus range from 0.0625 to 0.85 µl / ml in our studies. MIC values obtained for the clinical strains cultured in the presence of the oil were also significantly lower (0.25 – 1.25 µl / ml).

Literature reports the inhibiting properties of thymol from *Thymus* sp species on adhesion of *Staphylococcus aureus* and *Escherichia coli* clinical strains to epithelial cells of genitourinary system, which may be an alternative to synthetic drugs in prevention of urinary tract infections [21]. Studies on antimicrobial properties of the essential oil obtained from *Thymus fontanesii* Boiss. Et Reut. containing carvacrol demonstrated its very strong activity against clinical strains of *Escherichia coli* with MIC – 0.35 µl / ml (19). Similar MIC values were obtained for carvacrol chemotype of *Thymus ciliatus* (Desf.) Benth. ssp. *eu-ciliatus* Maire *eu.* against clinical strains of colon bacilli isolated from the respiratory system [20]. In our tests clinical strains of *E. coli* were sensitive to thyme oil at 0.25 and 0.5 µl / ml concentrations. Similar results were obtained by Łysakowska et al., who investigated the antibacterial activity of thyme essential oil against multiresistant clinical strains of *Acinetobacter* genus. Strains of *Acinetobacter* sp. were sensitive to thyme oil at concentrations of 0.25, 0.5, 0.75 and 1 µl/ml, but MIC for most clinical strains was 0.5 µl/ml. *E. coli* strains are relatively sensitive to thyme oil, comparing to recent results concerning other Gram-negative rods, *Acinetobacter* isolates. However, *Acinetobacter* strains seem to be more resistant to that oil [22].

The most resistant to the oil of thyme proved clinical strains of *Pseudomonas aeruginosa*, inhibition of growth fell in the range 1.5 µl to 2.5 / ml. Similar MIC values were obtained using the disc-diffusion method for oil obtained from *Thymus persicus* L. (thymol - 10%, carvacrol - 25%) and *Thymus eriocalyx* (Ronniger) Jalas (thymol - 66%) [23]. The action of *Thymus spinulosus* Ten. essential oil, having much lower content of active phenolic compounds (thymol) compared to the oil derived from *Thymus vulgaris* L., was much weaker against blue pus bacilli. The obtained MIC values were within the limits of 4.5 – 9.0 ml / ml, which was according to the literature [24, 25].

Lavender oil shows less antibacterial activity against all tested bacterial strains. The tested oil contained linalool 34 %, linalil acetate 33 % and lavandulil acetate 3%. The values of the MIC for *Staphylococcus aureus* were between 1.5 and 3.0 µl / ml oil concentration. Clinical strains of *Enterococcus* sp. were inhibited by lavender oil at concentration of 2.5 µl/ml and 3.5 µl/ml. Strains of *Escherichia coli* were sensitive to this oil from 2.0 to 3.5 µl/ml. The most resistant were clinical strains of *Pseudomonas aeruginosa* – MIC (19 µl/ml), our results were similar with literature [26]. Lavender oil also shows strong antiseptic activity. It is used in the treatment of mouth, throat, upper respiratory tract and lung infections and in dermatology in the treatment of ulcers, burns and difficult to cicatrize wounds. Oil of lavender (*Lavandula angustifolia* Mill.) has strong antiseptic activity against methicillin resistant strains of *Staphylococcus aureus* (MRSA) and against vancomycin resistant strains of bacteria of the *Enterococcus* sp. genus (VRE) [27, 28]. Subject literature presents about inhibits selection of antibiotic resistant mutants activity under the influence of lavender oil against

clinical strains of *Salmonella typhimurium* [29]. Essential oil obtained from *Lavandula stoechas* ssp. *stoechas* Skill Level contains pulegon – 40 %, menton – 13 % and mentol – 18 % and shows antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* standard strains and has also cytotoxic activity [30]. Literature reports the antibacterial properties of essential oil obtained from *Lavandula luisieri* (Rozeira) Riv. Mart against Staphylococci and Streptococci [31]. Our results related to the activity of thyme oil against *Staphylococcus aureus* strains appears to be of particular interest. The largest number of multiresistant strains were inhibited at 0.5 µl/ml concentration. *Staphylococcus aureus* strain was resistant to 7 out of 11 tested antibiotics (cefoxitin, erythromycin, clindamycin, tetracyclin, ciprofloxacin, trimethoprim / sulfamethoxazole and fusidic acid), it was sensitive to thyme oil at 0.25 µl / ml. Thyme oil was active against multiresistant strains of *Enterococcus* sp. (MIC-0.5-0.75 µl/ml) and against *Escherichia coli* strains (MIC-0.25-0.5 µl/ml). Our studies may be of interest because the resistance to antibiotics and chemotherapeutics of the tested clinical strains of bacteria, especially, since many authors reported synergistic activity of antibiotics and essential oils. The use of essentials oil obtained from *Pelargonium graveolens* L′Her. ex Ait. (*Geraniaceae*) with norfloxacin against standard strains of *Staphylococcus aureus*, *Escherichia coli* and *Bacillus* sp. reduced antibiotic effective minimum inhibitory concentration [9, 10]. Thymol and also a mixture of biologically active compounds contained in oil from *Thymus magnus* (Nakai) Nakai increases norfloxacin activity against resistant strains of *Staphylococcus aureus* [11]. The use of cinnamon aldehyde obtained from *Cinnamomum zeylanicum* Blume demonstrated 8 fold lower clindamycin baktericidal dose against clinical strains of *Clostridium difficile* [32]. Antimicrobial activity of essential oils and also another plant metabolites may be used against resistant to commonly used antibiotics and chemotherapeutics bacterial strains isolated from patients with persistent bacterial infection. In recent years, the number of resistant strains isolated from clinical materials is growing rapidly. It is particularly vital in case of clinical strains of *Staphylococcus aureus* (MRSA, VRSA) and bacteria of the *Enterococcus* sp. (VRE, HLAR) as well as of *Enerobacteriaceae* which become more and more resistant to carbapenems, cephalosporins III generation and quinolones [1]. Synergy of action of essential oils and antibiotics is a chance for significant reduction of therapeutic doses and reduction of adverse effects of antibiotic therapy, as well as the deceleration selection of resistant strains. The values of the LD_{50} for most essential oils are greater than 5 g / kg body weight, therapeutic doses are usually only a few drops per day. Although plant medicines are considered to be safe, possibility of overdose and their interactions with synthetic drugs administered orally must be considered. However, the positive findings in these studies support carrying on research on plant medicines and essential oils and their safe use in medical treatment.

8. CONCLUSIONS

8. 1. Tested oils inhibit the growth of standard and clinical strains belonging to: *Staphylococcus* sp., *Enterococcus* sp., *Escherichia* sp. and *Pseudomonas* sp.,

8. 2. Thyme oil shows stronger activity against tested bacterial strains than lavender essential oil,

8. 3. The oils shows lower efficacy against clinical strains of *Pseudomonas aeruginosa*.

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