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The activity of thyme essential oil against *Acinetobacter* spp.

Research Article

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Abstract: The aim of this work was to investigate the antimicrobial properties of thyme essential oil against clinical multiresistant strains of *Acinetobacter* spp. The antibacterial activity of oil was tested against standard and clinical bacterial strains of *Acinetobacter* genus. The agar diffusion method was used to check the inhibition of microbial growth at various concentrations of the oil from *Thymus vulgaris*. Susceptibility testing to antibiotics and chemotherapeutics was prepared using the disc-diffusion method. Identification of bacterial strains was carried out with the Vitek system and confirmed by PCR for *Acinetobacter baumanii gyrB* gene. The results of experiments showed that the oil from *T. vulgaris* exhibited an extremely strong activity against all of the clinical strains of *Acinetobacter*. Thyme oil demonstrated a very good efficacy against multiresistant strains of tested bacteria. Essential oils seems to be an excellent alternative for synthetic preparations and that is reason for an extensive assessment of their antimicrobial activity.

Keywords: *Antibacterial activity • Thyme oil • Minimal inhibitory concentration • Multiresistant strains*

1. Introduction

Effective control of many severe bacterial infections has been made possible due to introduction of antibiotics in medicine. However, the fight continues because of growing resistance to antibiotics commonly used in clinical practice [1].

Essential oils, a diverse group of plant metabolites, seem to be interesting: they have long been used in aromatherapy, dermatology and cosmetics [2]. Nowadays, experimental research confirms the additional pharmaceutical activity of oils. Various essential oils produce pharmacological effects, demonstrating anti-inflammatory, antioxidant and anticancerogenic properties. Their broad and complex activity, along with their synergy of action in combination with antibiotic therapy make them a valued complement to infection therapy in human diseases, not to mention the lack of reports about emergence of resistance mechanisms of bacteria to these compounds [3-6]. What is of particular interest is that many of them appear to have a wide spectrum of antimicrobial activity against microflora which usually cause intrahospital infections, such as *Acinetobacter* species.

Opportunistic infections caused by *Acinetobacter* spp. are of great concern because of multidrug resistance typical to these bacteria. The majority of infections affect the respiratory tract of hospitalised patients [7]. Besides, bacteria may cause meningitis, bacteremia, wound infections and urinary tract infections [8]. The most common *Acinetobacter* pneumonia happens among at-risk populations such as patients of ICUs (Intensive Care Units) or immunocompromised people. Infections are characterized with a high mortality rate [9]. *Acinetobacter baumannii* is a particularly serious threat within the hospital environment, both due to its innate and acquired antimicrobial resistance, its tendency for epidemic spread and ability to persist for long time on unanimated surfaces [10,11]. The *Acinetobacter* genomic sp. 13TU and sp. 3 and are also responsible for causing nosocomial infections, but to a lesser extent. Phenotypic commercial identification

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systems do not differentiate among these species efficiently (misidentification of c. 25% of *Acinetobacter* isolates belonging to the *A. calcoaceticus –A. baumannii* complex as *A. baumannii* [12] but using primers for *Acinetobacter gyr* B gene enable differentiation of the most important clinically strains of *Acinetobacter baumannii* and *Acinetobacter* genomic sp. 13TU [13].

The aim of this work was to investigate the antimicrobial properties of thyme essential oil obtained from thyme (*Thymus vulgaris* L.) against standard and clinical strains isolated from patients and clinical staff, as well as from the hospital environment.

2. Experimental Procedures

2.1 Bacterial strains

The standard bacterial strain, *Acinetobacter baumanii* ATCC 19606, used both in the agar dilution method and PCR reactions came from collection of Medical and Sanitary Microbiology Department, Medical University of Lodz. Clinical *Acinetobacter* isolates were collected from different materials from patients (29 isolates) and their environment (n=1). They came from the wards of intensive care unit (n=17), orthopaedics (n=3), nephrology (n=3), neurology (n=1), cardiology (n=1), surgery (n=1), urology (n=1), laryngology (n=1) and outpatient clinic (n=1) from one of Lodz hospitals. Clinical bacterial strains were isolated from bronchial washings (n=8), urine (n=6), wounds (n=4), intubation tubes (n=4), drain (n=2), bedsore (n=2), respiratory exudates (n=1), abdominal exudates (n=1) and ear (n=1). One strain came from environmental swab. Both standard and clinical strains were stored at -70°C for further investigation.

2.2 Bacteriological media

Several microbiological media were used including Columbia Agar (bioMerieux, France), McConkey Agar (Graso, Poland), Muller Hinton Agar II (bioMerieux, France).

2.3 Essential oil and its analysis

Commercial essential oil was 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) with FID and 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 [14,15] 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.

The standard and clinical strains used for oil activity testing were cultivated on Columbia agar medium and incubated at 37°C for 48 h in aerobic conditions. Bacterial suspensions with an optical density of 0.5 McFarland scale were prepared. bioMerieux densitometer was used.

Antibacterial analysis of oil activity was carried out by using agar dilution. The essential oil was diluted in ethanol. This solution was mixed with a nutrient broth to obtain concentrations from 0.125 to 1 µl/ml and poured into petri dishes. Inoculum containing 1.5×10^8 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 5 days of incubation at 37°C in aerobic conditions. Antibacterial analysis of oil activity was performed three times independently.

2.4 Phenotypic and genetic identification of bacterial strains

Acinetobacter strains were identified to the genus by using standard microbiological methods. Speciation was performed with the Vitek system and then confirmed by PCR for *Acinetobacter gyrB* gene [13]. Bacterial DNA was isolated with a Genomic Mini Kit (A&A Biotechnology, Poland). PCR reactions were carried out in a total volume of 25 µl in a Biometra cycler. The mixture consisted of 0.5 U Hypernova DNA polymerase (DNA Gdańsk), 600 nM of each primer [13] (IBB, Warsaw), 2 mM $MgCl_2$ (DNA Gdańsk), 2.5 µl PCR buffer and 400 µM dNTP (Fermentas). The products were separated by 1.4% agarose gel (Prona) in 1xTAE buffer (Fermentas) and stained with ethidium bromide (Sigma).

2.5 Susceptibility testing

The following antibiotics and chemioterapeutics (*Becton Dickinson*) were used for susceptibility testing of

Acinetobacter spp. strains: AN - amikacin (30 µg), SAM - ampicillin/sulbactam (10/10 µg), ATM – aztreonam (30 µg), CAZ – ceftazidim (30 µg), FEP - cefepim (30 µg), CTX - cefotaxim (30 µg), C – chloramphenicol (30 µg), CIP – ciprofloxacin (5 µg), GM – gentamicin (10 µg), IPM – imipenem (10 µg), MEM – meropenem (10 µg), TIM - tikarcilin/clavulanic acid (75/10 µg), NN – tobramycin (10 µg), TZP - piperacillin/tazobactam (100/10 µg), SXT - trimethoprim/sulfamethoxazole (1.25/23.75 µg), TE - tetracycline (30 µg). Analysis was carried out by using disc-diffusion method on Mueller-Hinton II Agar (bioMerieux). Cultures were incubated at 35°C for 16-18 h. The results were interpreted according to Clinical and Laboratory Standard Institute (CLSI) [16].

2.6 Statistical analysis

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

3. Results

3.1 Phenotypic and genetic identification

It was shown that not all *Acinetobacter* strains determined as *A. baumanii* by using Vitek system were assigned to that species by using PCR with primers for *gyrB* gene. 16 isolates assigned to the species *A. baumannii* turned out to be *Acinetobacter* genomic species 13TU. What is more, there were 7 isolates which proved to be other than *A. baumannii* and *Acinetobacter* genomic species 13TU strains, which had been determined previously as *A. baumannii* strains. Incubation at 41°C and 44°C revealed that among these strains there were no genomic sp. 3 isolates. Two isolates described biochemically as *A. lwoffii* yielded no products in PCR for *gyrB* gene.

3.2 Susceptibility testing

It was revealed that most of the *Acinetobacter baumannii* clinical strains were resistant to many antibiotics and chemioterapeutics. An especially high number of isolates were resistant to amikacin (43.3%), aztreonam (63.3%), cefotaxim (70%) as well as ciprofloxacin (86.6%), piperacillin/tazobactam (46.6%) and tetracycline (76.7%). It was shown that the most resistant microorganisms were isolated from bronchial secretions and intubation tubes. Two strains of *A. baumannii* (from bronchial washings and wound) and two genomic species 13TU (from bronchial washings) were susceptible only to one antibiotic – meropenem. Three other (from bronchial washings, intubation tube and vascular catheter) showed susceptibility to two antibiotics – ampicillin/sulbactam and imipenem. Most of the *Acinetobacter* strains were multiresistant, only *A. lwoffii* isolates showed a high susceptibility to tested drugs. What is more, *A. baumannii* strains and genomic species 13TU were more resistant than other isolates of *Acinetobacter*. Table 1 shows general characteristics of *Acinetobacter* isolates and Table 2 the number of isolates resistant to antibiotics.

3.3 Chemical composition of the tested oil

The analysis of the tested essential oil derived from *T. vulgaris* revealed that its composition meets the requirements of the Polish Farmacopoeia VIII and the European Farmacopoeia [17,18]. The content of thymol amounts to 38.1%, and carvacrol to 2.3%. Besides, there were other prevailing compounds as *p*-Cymene (29.1%), γ-terpinene (5.2%) and linalool (3.7%). The chemical composition of the tested oil is shown in Table 3.

3.4 The activity of thyme oil against Acinetobacter sp. strains

The values of the MIC for *Acinetobacter* spp. were between 0.25 and 1.0 µl/ml. MIC was 0.25 µl/ml for the standard strain of *Acinetobacter baumanii* ATCC 19606 and 3 clinical strains. Most *Acinetobacter* spp. strains (n=24) were sensitive to 0.5 µl/ml oil concentration. Figure 1 presents susceptibility of *Acinetobacter* strains to thyme essential oil. The tested clinical strains of multiple antibiotic-resistant *Acinetobacter* were sensitive to thyme oil at low concentrations. Control media containing alcohol (in concentration used to dilution) did not inhibit the growth of bacterial strains. Concentrations ranging from 0.5 to 1.0 µl/ml differed significantly in their ability to inhibit growth of *Acinetobacter* spp. strains from the 0.25 µl/ml concentration ($c^2(3)=12.62$, $P<0.01$).

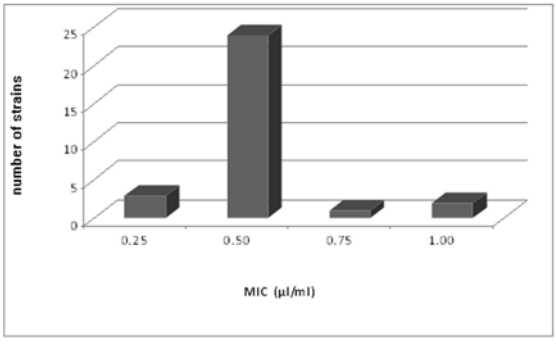

Figure 1. Susceptibility of clinical strains of *Acinetobacter* spp. to thyme essential oil.

No	Species	No of isolate	Source of isolation	MIC of thyme oil	Susceptibility to antibiotics															
					AN	SAM	ATM	CAZ	FEP	FOX	CIP	GM	IMP	MEM	TIM	TZP	NN	SXT	C	TE
1.	Genomic sp. 13TU	539	wound	0.75	R	S	S	S	R	R	R	S	R	S	R	R	S	R	R	R
2.	*A. lwoffii*	486	environment	0.25	S	S	S	S	S	I	S	S	S	-	S	S	S	S	S	S
3.	*A. baumannii*	448	wound	0.5	I	S	R	R	I	R	R	R	S	-	I	S	R	R	I	R
4.	*A. baumannii*	413	drain	0.5	I	S	R	R	I	R	R	R	S	-	I	I	R	R	R	R
5.	-	575	intubation tube	0.5	R	S	R	I	S	R	R	S	R	S	R	I	R	I	S	R
6.	*A. baumannii*	564	wound	0.5	R	S	I	R	S	R	R	R	S	-	R	S	R	R	R	R
7.	Genomic sp. 13TU	690	intubation tube	0.5	I	S	R	S	R	R	R	S	R	S	R	I	S	R	R	R
8.	Genomic sp. 13TU	694	urine	1.0	R	S	R	R	R	R	R	S	S	-	S	R	S	S	R	R
9.	Genomic sp. 13TU	783	bronchial washings	0.5	S	S	I	I	S	R	R	R	R	S	R	I	S	R	R	R
10.	Genomic sp. 13TU	784	bronchial washings	0.5	I	R	R	I	S	R	R	S	S	-	R	R	S	R	R	R
11.	-	769	ear	0.5	S	S	S	S	S	I	R	S	S	-	R	R	S	R	S	S
12.	Genomic sp. 13TU	815	urine	0.5	R	R	I	S	I	R	R	R	S	-	S	R	R	I	I	I
13.	Genomic sp. 13TU	942	urine	1.0	R	S	I	S	I	R	S	S	S	-	S	S	S	S	I	I
14.	Genomic sp. 13TU	1206	bronchial washings	0.5	S	S	R	R	I	I	R	S	R	S	R	R	S	R	R	R
15.	-	1251	drain	0.5	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	I
16.	-	1280	bronchial washings	0.25	I	S	R	S	R	I	R	S	R	S	S	I	S	I	R	R
17.	Genomic sp. 13TU	1371	abdominal exudates	0.5	S	S	R	I	S	R	R	R	S	-	R	R	S	R	I	R
18.	Genomic sp. 13TU	1392	bronchial washings	0.5	R	R	R	R	R	I	R	R	R	S	R	R	R	R	R	R
19.	*A. baumannii*	1395	intubation tube	0.5	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R
20.	Genomic sp. 13TU	1657	bronchial washings	0.5	R	R	R	R	R	R	R	R	R	S	R	I	R	R	R	R
21.	Genomic sp. 13TU	1724	urine	0.5	I	R	R	S	R	R	R	R	S	-	I	S	R	R	R	R
22.	-	1815	bedsore	0.5	R	R	R	S	R	I	R	R	R	S	R	I	R	R	S	R
23.	*A. baumannii*	1855	wound	0.5	I	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R
24.	*A. lwoffii*	2062	urine	0.25	S	S	S	S	S	S	S	S	S	-	S	S	S	S	S	S
25.	-	2134	bedsore	0.5	R	R	R	S	R	I	R	I	S	-	I	R	R	R	S	S
26.	-	2158	bronchial washings	0.5	S	S	R	S	R	R	R	S	R	S	R	R	S	S	R	R
27.	Genomic sp. 13TU	1741	urine	0.5	S	S	I	I	S	R	R	R	S	-	I	S	S	R	S	R
28.	Genomic sp. 13TU	1786	bronchial washings	0.5	R	S	I	R	S	R	R	R	S	-	R	R	S	R	R	R
29.	Genomic sp. 13TU	19	intubation tube	0.5	I	S	R	R	I	R	R	I	S	-	I	R	R	R	R	R
30.	Genomic sp. 13TU	334	respiratory exudates	0.5	R	S	R	R	R	R	R	R	S	-	R	I	R	R	R	R

Table 1. General characteristics of *Acinetobacter* isolates.

R – resistant strain I – intermediate susceptible strain S – susceptible strain,

4. Discussion

The species that form the *A. calcoaceticus–A. baumannii* complex have emerged as clinically very important pathogens because of their innate resistance and ability to acquire resistance genes. Because they possess a wide array of β-lactamases and other enzymes for resistance encoded by transposable elements, plasmids or chromosomes [19], they may cause life-threatening infections. Therefore it would be interesting to know whether there are any other than antibiotic compounds which may show strong antimicrobial activity against those species.

Knowledge about the 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. Immunostimulatory, antioxidant and antimicrobial activity of many plant metabolites have been described. Essential oils derived from plants belonging to the *Lamiaceae* family are particularly valuable because of their antibacterial and antioxidant properties. Such 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 [20].

Thyme as a wild plant is common in the Iberian Peninsula, France, Italy, Greece and North Africa. It is cultivated throughout Europe and America. A lot of chemotypes have been identified in red thyme *T. vulgaris*, the most important are thymol chemotype (65% thymol, 5-10% carvacrol) and carvacrol chemotype (85% carvacrol, 1-5% thymol) [21]. According to the requirements of the FP and FE the oil should contain thymol (36-55%) and carvacrol (1-4%). The Polish Pharmacopeia and European Pharmacopoeia [17,18] show the flourishing, fresh herb of *Thymus vulgaris* L and *Thymus zygis* Loefl. ex L. as a source of the essential oil of thyme. The thyme oil used by us was obtained from *Thymus vulgaris* L. by the Technical University of Lodz and meets the requirements of the Polish Pharmacopoeia VIII and European Pharmacopoeia. In our study, the oil showed antimicrobial activity against standard and clinical strains of *Acinetobacter* sp. Our results show that thyme oil has strong antimicrobial properties against all tested strains. 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 [2].

Antibiotics (μg)	*Acinetobacter baumanii* (n=5 strains)		*Acinetobacter genomic sp. 13 TU* (n=16 strains)		*Other Acinetobacter spp.* (n=9 strains)		Total number of resistant strains	
	R	I	R	I	R	I	R	I
Amikacin (30)	2	3	8	4	3	1	13	**8**
Ampicillin/sulbactam (10/10)	2	0	5	0	2	0	9	**0**
Aztreonam (30)	4	1	8	5	5	0	19	**6**
Ceftazidim (30)	5	0	7	4	0	1	12	**5**
Cefepim (30)	3	2	7	4	4	0	13	**6**
Cefotaxim (30)	5	0	14	2	2	5	21	**7**
Chloramfenikol (30)	4	1	12	3	2	0	18	**4**
Ciprofloxacin (5)	5	0	15	0	6	0	26	**0**
Gentamicin (10)	5	0	9	1	1	1	15	**2**
Imipenem (10)	2	0	6	0	5	0	13	**0**
Meropenem (10)	0	0	0	0	0	0	0	**0**
Tikarcillin/clavulanic acid (75/10)	3	2	10	3	4	1	17	**6**
Tobramycin (10)	5	0	6	0	3	0	14	**0**
Piperacillin/tazobactam (100/10)	2	1	8	4	3	3	14	**8**
Tetracycline (30)	5	0	13	2	4	1	23	**3**
Trimetoprim/sulfametoxazol (1.25/23.75)	5	0	13	1	3	2	21	**3**

Table 2. Resistance of *Acinetobacter* spp. (n=30) to antibiotics and chemoterapeutics.

R – resistant strain I – intermediate susceptible strain

In our tests, clinical strains of *Acinetobacter* sp. were sensitive to thyme oil at concentrations of 0.25, 0.5, 0.75 and 1 µl/ml, therefore relatively low compared to the high concentrations of antibiotics usually required. MIC for most strains (n=24) was 0.5 µl/ml. These strains came from diverse materials and hospital wards. They belonged both to genomic sp. 13TU and *Acinetobacter baumannii* as well as to other species. As far as susceptibility to antibiotics was concerned, most isolates were resistant to ATM (n=23), CTX (n=24), CIP (n=29), TIM (n=21), STX (n=25) and TE (n=25). However, most strains were susceptible to ampicillin/sulbactam, tobramicin and cefoxitin. Both strains with MIC at 1 µl/ml were isolated from urine but in different wards (nephrology and ICU). They were also susceptible to several antibiotics: SAM, GM, IMP, TIM, NN, SXT. The only isolate for which the MIC was 0.75 µl/ml, isolated from a wound in the surgery ward, was susceptible to SAM, ATM, GM, CAZ, MEM, NN.

Resistance to meropenem is more and more often found among *Acinetobacter* strains [22], but in our study there were no isolates demonstrating that kind of resistance. Because carbapenems are thought to be the only independent risk factor for the appearance of imipenem resistant MDRAB (Multidrug Resistant *A. baumannii*), other antimicrobial agents should be evaluated for the efficacy of eradicating those strains sensitive to imipenem, but multiresistant [19]. As other authors suggested, beside tigecycline, old antibiotics like aminoglycosides and colistin will have to be re-employed. What is more, there is a need to search for drugs such as essential plant oils rather than antibiotics and chemotherapeutics. Additionally other procedures such as reducing selection pressure by shortening duration of treatment should be used [23,24].

According to many manuscripts, multidrug resistant *Acinetobacter baumannii* is defined as resistant to more than three classes of antibiotics [25,26]. Dent *et al.* reported that 58% of the *A. baumannii* strains were resistant to imipenem, amikacin, and ampicillin-sulbactam, which were previously very effective against *A. baumannii*. It was later reported that only 17% of the isolates were sensitive to all three of the above antimicrobial agents and 46% of the isolates were resistant to all commonly-used antibiotics [25]. Resistant strains were a significant problem also in our study. However, 13% of the strains were resistant at the same time to IMP, AN, SAM, but ampicillin/sulbactam remained generally effective against tested isolates and 16.6% of the strains were susceptible to all three drugs. Our tests revealed that 13 isolates (43%) were resistant to imipenem and 26 of 30 were resistant to most tested drugs. This study is limited because it did not include the

No	Compound	Total oil %	Retention Index
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	Terpinen-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
27	β-**Caryophyllene**	**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 3. Components of the essential oil obtained from thyme - *Thymus vulgaris* L. (*Lamiaceae*).

high number of strains tested for antibiotic susceptibility but the goal was to determine the impact of thyme oil on *Acinetobacter* strains. Most of the resistant isolates occurred in the ICU and the major site of isolation was the respiratory tract, which is in accordance with the literature [25]. The sites of *A. baumannii* isolation in ICUs (n=17) were mainly respiratory tract (n=13, 43.3%), wounds (n=2, 6.6%), urinary tract (n=1, 3.3%), and vascular catheters (n=1, 3.3%).

In our investigation 30 Acinetobacter clinical strains were tested for the presence of gyrB gene. Five of 30 clinical strains and standard *Acinetobacter baumannii* ATCC 19606 strain produced two clear bands, then proved to be *A. baumannii* strains. All those isolates were biochemically determined to be *A. baumannii* strains. However, 16 strains identified previously as *A. baumannii* were proved to be genomic sp. 13TU. These species are very difficult to differentiate with biochemical tests. What is more, seven strains yielded no PCR products for the *gyrB* gene even though being earlier classified as *A. baumannii* isolates. Two strains determined as *A. lwoffii* also failed to produce PCR products. Regarding susceptibility to thyme oil there were no significant differences in MICs between species of *Acinetobacter*.

In accordance with the literature, *Thymus vulgaris L* oil showed an inhibitory effect against the growth of *Staphylococcus aureus* strains isolated from respiratory infections. Using the disc-diffusion method an MIC of 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 *S. aureus* sensitive to this essential oil were resistant to oxacillin, gentamicin and tobramycin and many of them to norfloxacin [27]. Studies on the antimicrobial properties of the essential oil obtained from *Thymus fontanesii* Boiss. Et Reut. containing carvacrol demonstrated its very strong activity against Gram-negative bacteria, clinical strains of *Escherichia coli,* with an MIC of 0.35 µl/ml [28]. The action of *Thymus spinulosus* Ten. essential oil, having much a 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 in accordance with the literature [29]. The essential oil of *Origanum vulgare*, containing phenols as predominant compounds, in low concentration was capable of preventing the growth of the nosocomial bacteria *A. baumannii*, *P. aeruginosa,* (resistant to ceftazidime and carbapenems)*, E. coli, K. pneumonia, E. faecalis,* extended spectrum beta-lactamases producers (ESBL) and methicillin resistant *S. aureus* (MRSA) [30]. To our knowledge, this study is the first one presenting the considerable activity of essential thyme oil against diverse clinical species of *Acinetobacter*, which is becoming more crucial because of the increasing resistance of those species to commonly-used antibiotics. More importantly, thyme essential oil may be used as a drug in respiratory tract infections often caused by *Acinetobacter* spp. Essential oils are to be an excellent alternative for synthetic preparations and used in combination with antibiotics may prevent antibiotic-resistant strain development.

5. Conclusions

Thyme oil obtained from *Thymus vulgaris* L.:

- shows very strong activity against standard and clinical strains belonging to *Acinetobacter* genus.
- is active at low concentrations against clinical strains resistant to most tested antibiotics.

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References

[1] Chastre J., Evolving problems with resistant pathogens, Clin. Microbiol. Infect., 2008, 14, 3-14

[2] Kalemba D., Kunicka A., Antibacterial and antifungal properties of essential oils, Curr. Med. Chem., 2003, 10, 813-829

[3] Giordani R., Regli P., Kaloustian J., Portugal H., Potentation of antifungal activity of amphoteracin B by essential oil from Cinnamonium cassia, Phytother. Res., 2006, 20, 58-61

[4] Lis-Balchin M., Deans S., Hart S., Bioactive Geranium oils from different commercial sources, J. Essent. Oil Res., 2007, 8, 281-290

[5] Rosato A., Vitali C., De Laurentis N., Armenise D., Antonietta Milillo M., Antibacterial effect of some essential oils administered alone or in combination with Norfloxacin, Phytomedicine, 2007, 14, 727-732

[6] Shin S., Kim J., In vitro inhibitory activities of essential oils from two Korean Thymus species

against antibiotic-resistant pathogens, Arch. Pharm. Res., 2005, 28, 897-901
[7] King L., Swiatlo E., Swiatlo A., Swiatlo A., McDaniel L.S., Serum resistance and biofilm formation in clinical isolates of Acinetobacter baumannii, FEMS Immunol. Med. Microbiol., 2009, 55, 414-421
[8] Gaynes R., Edwards J., Overview of nosocomial infections caused by gram-negative bacilli, Clin. Infect. Dis., 2005, 41, 848-854
[9] Falagas M., Karveli E., The changing global epidemiology of Acinetobacter baumannii infections: a development with major public health implications, Clin. Microbiol. Infect., 2007, 13, 117-119
[10] Bernabeu-Wittel M., Pichardo C., Garcia-Curiel A., Pachón-Ibáñez M., Ibáñez-Martínez J., Jimenez-Mejias M., et al., Pharmacokinetic/pharmacodynamic assessment of the in-vivo efficacy of imipenem alone or in combination with amikacin for the treatment of experimental multiresistant Acinetobacter baumannii pneumonia, Clin. Microbiol. Infect., 2005, 11, 319-325
[11] Ecker J., Massire C., Hall T., Ranken R., Penella T., Agasino Ivy C., et al., Identification of Acinetobacter species and genotyping of Acinetobacter baumannii by multilocus PCR and mass spectrometry, J. Clin. Microbiol., 2006, 44, 2921-2932
[12] Wisplinghoff H., Edmond M., Pfaller M., Jones R., Wenzel R., Seifert H., Nosocomial bloodstream infections caused by Acinetobacter species in United States hospitals: clinical features, molecular epidemiology, and antimicrobial susceptibility, Clin. Infect. Dis., 2000, 31, 690-697
[13] Higgins P., Wisplinghoff H., Krut O., Seifert A., PCR-based method to differentiate between Acinetobacter baumannii and Acinetobacter genomic species 13TU, Clin. Microbiol. Infect., 2007, 13, 1199-1201
[14] Adams R.P., Identification of essential oil components by gas chromatography/mass spectroscopy, 4th Ed., Allured Publishing Corporation, Carol Stream, IL, USA, 2007
[15] Joulain D., König W.A., The atlas of spectral data of sesquiterpene hydrocarbons, E.B.-Verlag, Hamburg, 1998
[16] Clinical and Laboratory Standard Institute (CLSI), Performance standards for antimicrobial susceptibility testing: sixteenth informational supplement. CLSI document M100-S16, Clinical and Laboratory Standard Institute, Wayne, Pennsylvania, USA, 2006
[17] Polish Pharmacopeia, 8th Ed., Polish Pharmaceutical Society, Warsaw, 2008
[18] European Pharmacopoeia, 6th Ed., Council of Europe, Strasbourg, 2008
[19] Ye J.-J., Huang C.-T., Shie S.-S., Huang P.-Y., Su L.-H., Chiu C.H., Multidrug resistant Acinetobacter baumannii: Risk factors for appearance of imipenem resistant strains on patients formerly with susceptible strains, PLoS ONE, 2010, 5, e9947
[20] Lis-Balchin M., Aromatherapy Science. A guide for healthcare professionals, 2nd Ed., Pharmaceutical Press, London, 2006
[21] Price A., Price L., Aromatherapy for health professionals, 3rd Ed., Churchill Livingstone, London 1999
[22] Routsi C., Pratikaki M., Platsouka E., Sotiropoulou C., Nanas S., Markaki V., et al., Carbapenem-resistant versus carbapenem-susceptible Acinetobacter baumannii bacteremia in a Greek intensive care unit: risk factors, clinical features and outcomes, Infection, 2010, 38, 173-180
[23] Chastre J., Wolff M., Fagon J., Chevret S., Thomas F., Wermert D., et al., Comparison of 8 vs 15 days of antibiotic therapy for ventilator-associated pneumonia in adults: a randomized trial, J. Am. Med. Assoc., 2003, 290, 2588-2598
[24] Meyer E., Schwab F., Schroeren-Boersch B., Gastmeier P., Dramatic increase of third-generation cephalosporin-resistant E. coli in German intensive care units: secular trends in antibiotic drug use and bacterial resistance, 2001 to 2008, Crit. Care, 2010, 14, R113-R121
[25] Dent L., Marshall D., Pratap S., Hulette R., Multidrug resistant Acinetobacter baumannii: a descriptive study in a city hospital, BMC Infect. Dis., 2010, 10, e196
[26] Falagas M., Koletsi P., Bliziotis I., The diversity of definitions of multidrug-resistant (MDR) and pandrug-resistant (PDR) Acinetobacter baumannii and Pseudomonas aeruginosa, J. Med. Microbiol., 2006, 55, 1615-1617
[27] Fabio A., Cermelli C., Fabio G., Nicoletti P., Quaglio P., Screening of the antibacterial effects of a variety of essential oils on microorganisms responsible for respiratory infections, Phytother. Res., 2007, 21, 374-377
[28] Bekhechi C., Bekkara F.A., Abdelouahid D.E., Tomi F., Casanova J., Composition and Antibacterial Activity of the Essential Oil of Thymus fotanesii Boiss. et Reut. from Algeria, J. Essent. Oil Res., 2007, 19, 594-596
[29] Rosooli I., Mirmostafa S., Bacterial susceptibility to and chemical composition of essential oils from Thymus kotschyanus and Thymus persicus, J. Agr. Food Chem., 2003, 51, 2200-2205

[30] Coelho da Costa A., Cavalcanti dos Santos B., Filho L., de Oliveira Lima E., Antibacterial activity of the essential oil of Origanum vulgare L. (Lamiaceae) against bacterial multiresistant strains isolated from nosocomial patients, Rev. Brasil. Farmacogn., 2009, 19, 236-241