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Original paper

Assessment of the antimicrobial activity of some essential oils against multi-resistant bacterial strains

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Abstract

Volatile oils, also called essential oils, are secreted by plant-specific secretory cells and tissues. These components are a mixture of different substances, primarily mono and sesquiterpenes. Our aim was to assess the antimicrobial activity of different essential oils, i.e. lavender, cloves, eucalyptus, rosemary, thyme and oregano against multidrug resistant bacterial strains belonging to the ESKAPE group.
The most effective oils proved to be the thyme and oregano which induced the occurrence of the largest diameters of the growth inhibition zones against all tested bacterial species. Oregano oil showed the best inhibitory activity against *Pseudomonas aeruginosa*, followed by clove, while thyme oil was the most effevtive against *Staphylococcus aureus* strains.

Keywords essential oils, nosocomial infections, antimicrobial activity, ESKAPE

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Introduction

Volatile oils, also called essential oils, are secreted by plant-specific secretory cells and tissues.These components are a mixture of different substances, primarily mono and sesquiterpenes [1, 2]. More than 5,000 compounds have been identified in the composition of volatile oils, such as mono and sesquiterpenes, aromatic compounds, phenylpropanes and diterpenes. Terpenic compounds are hydrocarbons and oxygenated derivatives such as oxides, ketones, aldehydes, alcohols or their acids, and terpenic alcohols can be esterified [2].

From vegetable products, volatile oils can be obtained by entrainment (distillation) with water vapor, with volatile apolar solvents, by means of fats or by pressing [3].

Volatile oils provide important pharmacological properties, incuding the inhib ition of antibiotic-resistant bacteria growth [4, 5]. Our aim was to assess the antimicrobial activity of different essential oils, i.e. lavender, cloves, eucalyptus, rosemary, thyme and oregano against multidrug resistant bacterial strains belonging to the ESKAPE (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter spp*) group. The oils tested in the present study, including lavender, cloves, eucalyptus, rosemary, thyme and oregano were selected for their previously reported antibacterial, bacteriostatic and antiseptic properties [2, 6].

Materials and methods

The study included 30 bacterial strains, both Gram positive and Gram negative, isolated from patients admitted to the Cardiovascular Surgery section of a hospital in Bucharest.

The strains belonged to the *Klebsiella* (12 strains), *Acinetobacter* (8 strains), *Staphylococcus* (5 strains) and *Pseudomonas* (5 strains) genera. The sources of isolation are specified in the tables below (tables 1, 2, 3, 4).

For the study, fresh cultures of 18-20 hours were obtained by seeding the strains studied on solid TSA media (*Tripticase Soy Agar*) and incubation at 37 °C.

The tested essential oils were acheved from commercial producers, i.e. Fares and TrioVerde (Table 5).

Table 1. Bacterial strains belonging to the *Staphylococcus aureus*

Number	Bacterial strains	Source of isolation
1	*Staphylococcus aureus* 882	Blood culture
2	*Staphylococcus aureus* 3118	Nasal exudate
3	*Staphylococcus aureus* 1003/1663	Wound secretion
4	*Staphylococcus aureus* 2971	Nasal exudate
5	*Staphylococcus aureus* 3051	Nasal exudate

Table 2. Bacterial strains belonging to the *Pseudomonas aeruginosa*

Number	Bacterial strains	Source of isolation
1	*Pseudomonas aeruginosa* 5034	Tracheal secretions
2	*Pseudomonas aeruginosa* 914	Central venous catheter
3	*Pseudomonas aeruginosa* 1044	Wound secretion
4	*Pseudomonas aeruginosa* 1441	Urinalysis
5	*Pseudomonas aeruginosa* 845	Blood culture

Table 3. Bacterial strains belonging to the *Acinetobacter baumannii*

Number	Bacterial strains	Source of isolation
1	*Acinetobacter baumannii* 1989	Wound secretions
2	*Acinetobacter baumannii* 1177	Wound secretions
3	*Acinetobacter baumannii* 475	Tracheal secretions
4	*Acinetobacter baumannii* 437	Tracheal secretions
5	*Acinetobacter baumannii* 863	Wound secretions
6	*Acinetobacter baumannii* 693	Wound secretions
7	*Acinetobacter baumannii* 621	Tracheal secretions
8	*Acinetobacter baumannii* 676	Tracheal secretions

Table 4. Bacterial strains belonging to the *Klebsiella pneumoniae*

Number	Bacterial strains	Source of isolation
1	*Klebsiella pneumoniae* 2583	Anal portage
2	*Klebsiella pneumoniae* 1526	Central venous catheter
3	*Klebsiella pneumoniae* 2576	Anal portage
4	*Klebsiella pneumoniae* 2268	Urinalysis
5	*Klebsiella pneumoniae* 5662	Urinalysis
6	*Klebsiella pneumoniae* 5972/8850	Urinalysis
7	*Klebsiella pneumoniae* 6984	Wound secretions
8	*Klebsiella pneumoniae* 5670/8822	Urinalysis
9	*Klebsiella pneumoniae* 6003	Wound secretions
10	*Klebsiella pneumoniae* 6980	Urinalysis
11	*Klebsiella pneumoniae* 2460	Anal portage
12	*Klebsiella pneumoniae* 6982/4091	Urinalysis

Table 5. Bacterial strains belonging to the *Klebsiella pneumoniae*

Code	Name	Provider
1	Lavender	Fares
2	Cloves	Fares
3	Eucalyptus (contains *Eucalyptus globulus* and limonene leaf oil)	TrioVerde
4	Rosemary	Fares
5	Thyme	Fares
6	Oregano (contains essential oil of *Origanum vulgare*, carvacrol, linalol, thymol and limonene)	TrioVerde

Method of qualitative testing of the antimicrobial activity of the essential oils

The testing was performed using the adapted diffusimetric method (according to CLSI recommendations, 2019).

On the surface of the medium Mueller Hinton (without glucose) agarized 2% (pH = 7.2 - 7.4), with the thickness of 4 mm distributed in Petri dishes (Ø = 10 cm) a standardized inoculum (a suspension in physiological water) was seeded "in the canvas" sterile - AFS) from cultures of 18-20 h belonging to the studied bacterial strains, density 1.5 x 108 CFU / ml nephelometrically adjusted with McFarland 0.5 standard.

Subsequently, 10 µl of each essential oil sample was placed in the spot on the surface of the seeded medium. After their free diffusion, the plates were incubated for 16-18 h at 37°C.

The sensitivity was evaluated by measuring the diameters of the growth inhibition zones that appeared around the spot. For some oil samples dilutions were performed in dimethylsulfoxide (DMSO).

Method of the quantitative testing of the antimicrobial activity of the essential oils

The serial microdilution method was performed in liquid medium using the 96-well plates and liquid broth. The intensity of bacterial growth has been appreciated by the absorbance value read spectrophotometrically at 620 nm allowing to establish the minimal inhibitory concentration (MIC) expressed in % of the undiluted essential oil. The MIC was considered as the last concentration at which no microbial growth was observed [7].

Results and Discussions

The results of the qualitative tests to evaluate the antimicrobial activity of the essential oils

The qualitative tests performed by the adapted disc difusion method allowed the evaluation of the degree of sensitivity of all 30 strains to the testes essential oils, by measuring the values of all diameters of inhibition zones and calculating an average value/genus studied (tables 6 - 8). Thus, two suggestive graphs could be drawn to highlight the comparative results of this test (Figures 1, 2).

Analyzing the Figures 1 and 2, it can be seen that the most effective oils proved to be the essential oils of thyme and oregano which exhibited the largest diameters of the growth zones of inhibition against all tested bacterial species (fig. 2).

Oregano oil showed the best inhibitory activity against *P. aeruginosa* strains, while thyme oil induced the largest

Table 6. Diameter values of growth inhibition zones for *S. aureus* strains

***S. aureus*-Diameter of the inhibition zone (mm)**						
Bacterial strain codes	Lavender	Clove	Eucalyptus	Rosemary	Thyme	Oregano
S. aureus 882	11	12	8	10	22*	27*
S. aureus 3118	12	19	15	10	23*	22*
S. aureus 1003/1663	20	15	10	8	22*	31*
S. aureus 2971	9	13	13	14	40*	33*
S. aureus 3051	10	12	16	12	45*	33*
Average diameters	12,42857143	15,1428571	13,285714	10,71428571	28,285714	30,8571429
** oil diluted in DMSO (1/4)*						

Table 7. Diameter values of growth inhibition zones for *P. aeruginosa* strains

***P. aeruginosa* – Diameter of the inhibition zone (mm)**						
Bacterial strain codes	Lavander	Clove	Eucalyptus	Rosemary	Thyme	Oregano
P. aeruginosa 5034	11	14	15	11	23**	32**
P. aeruginosa 914	9	27	12	12	28*	40*
P. aeruginosa 1044	9	11	10	9	19*	36*
P. aeruginosa 1441	10	28	12	11	26**	40**
P. aeruginosa 845	11	20	11	11	22**	34**
Average diameters	10,5	20	11,833333	10,83333333	24,666667	37,6666667
** oil diluted in DMSO (1/4) ; ** oil diluted in DMSO (1/6)*						

Table 8. Diameter values of growth inhibition zones for *A. baumannii* strains

baumannii- Diameter of the inhibition zone (mm)						
Bacterial strain codes	Lavander	Clove	Eucalyptus	Rosemary	Thyme	Oregano
A. baumannii 1989	13	16	20	12	32*	38*
A. baumannii 1177	10	12	9	10	21*	22*
A. baumannii 475	13	16	9	9	25**	15**
A. baumannii 437	11	14	10	10	26*	39*
A. baumannii 863	17	11	19	10	17*	30*
A. baumannii 693	16	20	20	23	40**	39**
A. baumannii 621	15	18	17	10	23**	28**
A. baumannii 676	11	9	10	10	35*	36*
Average diameters	13	13,8947368	12,736842	10,89473684	24,473684	29,8421053
* oil diluted in DMSO (1/4) ; ** oil diluted in DMSO (1/6)						

Table 9. Diameter values of growth inhibition zones for *K. pneumoniae* strains

K. pneumoniae- Diameter of the inhibition zone (mm)						
Bacterial strain codes	Lavander	Clove	Eucalyptus	Rosemary	Thyme	Oregano
K. pneumoniae 2583	12	12	9	11	30*	20*
K. pneumoniae 1526	11	10	9	9	19*	18*
K. pneumoniae 2576	10	12	10	9	15*	13*
K. pneumoniae 2268	11	10	9	10	42*	32*
K. pneumoniae 5662	14	11	10	9	30*	26*
K. pneumoniae 5972/8850	17	11	10	9	15*	16*
K. pneumoniae 6984	8	10	8	10	16*	11*
K. pneumoniae 5670/8822	14	10	9	9	24*	29*
K. pneumoniae 6003	10	12	8	10	23*	30*
K. pneumoniae 6980	8	9	9	9	19*	14*
K. pneumoniae 2460	12	11	10	11	17*	18*
K. pneumoniae 6982/4091	11	16	10	10	25*	38*
Average diameters	11,81481481	11,7037037	9,4814815	9,814814815	22,777778	24,2592593
* oil diluted in DMSO DMSO (1/4)						

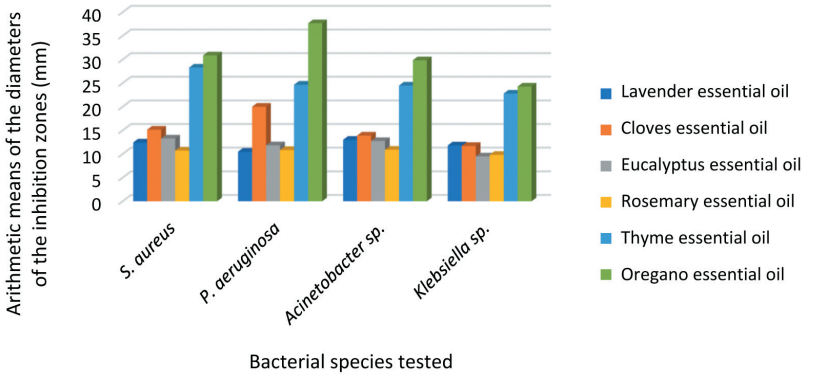

Figure 1. Graphic representation of the values of the arithmetic means of the diameters of the growth inhibition zones.

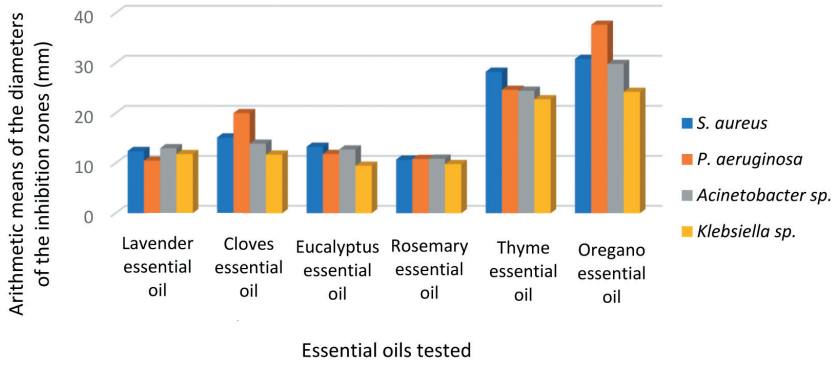

Figure 2. Graphic representation of the values of the arithmetic means of the growth diameters of the inhibition zones.

inhibitory diameter against *S. aureus* strains. Clove essential oil also showed a more pronounced inhibitory effect on the clinical strains of *P. aeruginosa.*

The binary serial dilution method in liquid growth medium allowed the determination of the MIC values for each sample of essential oil separately, at the end also establishing an average CMI value / species tested. Analyzing fig. 3, it can be seen that the lowest CMI values were also recorded for the essential oils of oregano, cloves and thyme, which confirms the qualitative tests on the most effective oils.

Regarding the degree of sensitivity of bacterial strains, it can be seen that the susceptibility profiles of the bacterial strains to the tested essential oils is similar, with a very good activity of oregano, followed by thyme and cloves demonstrated by the low MIC vales, while rosemary, eucaliptus and lavender expressed much higher MIC values (fig. 3).

Among the tested bacterial strains, the most susceptible prove to be the *S. aureus* strains.

Conclusions

Following the interpretation of the results obtained in this study, we can conclude that the most effective oils proved to be the essential oils of thyme, oregano and cloves, which exhibited the largest diameters of the growth zones of inhibition against all tested bacterial strains belonging to the ESKAPE group.

Oregano and clove oil showed the best inhibitory activity against *P. aeruginosa* strains, while thyme oil showed the largest inhibitory diameter against *S. aureus* strains.

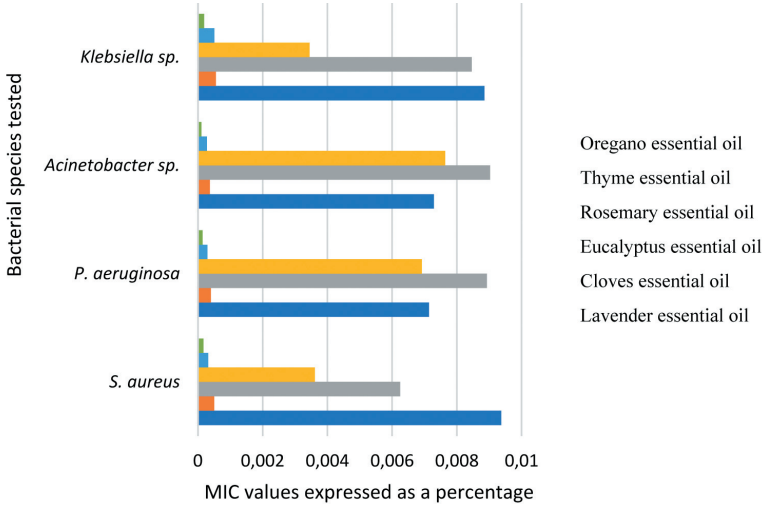

Figure 3. Graphical representation of the degree of sensitivity to the action of different essential oils of clinical bacterial strains.

Regarding the results of the quantitative assay, the susceptibility profiles of the bacterial strains to the tested essential oils was relatively similar, the most active bein, similarly to the qualitative assay results, the oregano, thyme and cloves. This experimental approach may open new perspectives for developing novel strategies to combat the emerging global threat of multiple drug resistance.

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