Efficacy of Essential Oils in *In Vitro* Control of *Acinetobacter baumannii*

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Abstract: Although *Acinetobacter baumannii* is common in nature and considered human skin and respiratory tract commensal, it has also been associated to serious infectious diseases, such as pneumonia, urinary tract infection, endocarditis, wound infection, meningitis and septicemia. The present study aimed to evaluate the intrinsic antimicrobial activity of essential oils on the *Acinetobacter baumannii* ATCC 17978 strain. We used essential oil of rosemary, artemisia, cinnamon, camphor, citronella, Indian clove, *Eucalyptus globulus*, *Eucalyptus staigeriana*, *Eucalyptus citriodora*, tea tree (also known as melaleuca), mint, oregano and sage, in concentrations that ranged from 0 to 100%. The antibacterial activity was determined through the broth microdilution method and through bactericidal kinetics of essential oils. All the oils performed antibacterial activity against *Acinetobacter baumannii*. citronella, *Eucalyptus staigeriana* and mint oils presented lower minimum inhibitory concentration and minimum bactericidal concentration. Oregano, tea tree and Indian clove oils presented higher bacterial death rate, and they canceled the microbial load of *A. baumannii* in up to 60 minutes.

Keywords: Medicinal plants, Essential compounds for treatment, Antimicrobials, Mechanisms of action, *Acinetobacter baumannii*, Infectious diseases.

1. INTRODUCTION

The genus *Acinetobacter* is a gram-negative, ubiquitous, aerobic, non-fermenting, non-demanding, immobile, catalase positive and oxidase negative bacillus, with the ability to grow under various pH and temperature conditions and uses many different types of substrates for its development [1,2]. The virulence factors that allow the survival an adaptation of such agent to the hospital environment are: the ability to capture iron from the environment, which allows it to survive in iron deficiency conditions, the resistance to drying, the production of a polysaccharide capsule in some strains, the ability to adhere to different surfaces by the formation of biofilms [3,4], and the adherence to respiratory epithelial cells by pili [5].

The ability of *A. baumannii* to adhere and form biofilms on inanimate objects and surfaces can explain its permanence in hospital environment. The structure of the biofilm formed by this pathogenic bacterium enhances its ability to resist antimicrobial treatments, as well as other stress conditions such as dehydration and limited availability of nutrients. The formation of biofilm by *A. baumannii* is associated to its ability to produce exopolysaccharides and to the presence of pilis [6,7].

Acinetobacter baumannii came as a clinically important pathogen due to an increasing number of infections produced by it and to the global dissemination of strains resistant to many types of antibiotics [8]. *Acinetobacter* is a cutaneous microbiota commensal microorganism that presents a higher level of skin and respiratory epithelium colonization in hospitalized individuals. The patients with this agent have a leading role on the contamination of the health professionals' hands and the hospital equipment, which contributes to the perpetuation of outbreaks by this bacterial species. Besides dermal transmission, it has also been described the aerial and digestive contamination through aerosols, sputum and feces [9-11].

Due to the acquisition of mechanisms of resistance to antimicrobials and disinfectants, as well as the ability to survive in the hospital environment, *A. baumannii* has become persistent. The continuous and exaggerated exposition to broad-spectrum antibiotics performs a selective pressure over *Acinetobacter*, causing the survival of more resistant strains and the dissemination of mechanisms of resistance among

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different bacteria. Therefore, it is fundamental the judicious usage of antibiotics with combined therapeutic regimens, to prevent emergence of resistance [1,8].

Currently, the infectious bacteria resistant to antibiotics imply a high risk and, therefore, they are a challenge when treating patients in hospitals. The characterization of these species and particular strains is a priority in order to establish diagnostic tests and preventive procedures. The relevance of *Acinetobacter baumannii* as a problematic microorganism in hospitals, especially in ICUs, has increased throughout time [12].

The development of pathogenic bacteria resistance to antibiotics demanded the search for new antimicrobial substances from new sources, including plants. The extracts and essential oils from many aromatic and medicinal plants present biological activities important to the pathogen control [13-15]. Essential oils have broad antimicrobial effects, acting as an antibacterial, antifungal, antiparasitic, antioxidant, antiviral, anticancer, among others. However, there is not enough information about the bioactivity and toxicity of essential oils. In spite of that, the commercial use and applications of essential oils are have been growing, and they are used in household cleaning products, cosmetics, perfumery, insecticides, disinfectants, in the management of animal and human infections [16,17].

The mechanisms of action of the natural compounds are associated to the disintegration of the cytoplasmic membrane, destabilization of the proton-motive force (PMF), electrons flow, active transport and coagulation of cellular content. Not all the mechanisms of action focus on specific targets, and some sites may be affected by other mechanisms not yet determined. The essential oils are acknowledged by their strong antibacterial potential, biological activity, which is due to its composition, including aromatic and terpene compounds [18,19]. According to Saviuc *et al*. [20] some natural compounds, including 1,8-cineole (eucalyptol), induce the inhibition of permeabilization of cell walls and efflux pumps, suggesting its potential use for the restoration of antibiotic efficiency in resistant strains carrying efflux mechanism.

Although the biological properties of the essential oils are closely associated with their main components, the extent of its effects can be due to its high concentration on the original oil, which can hide the effect of smaller components when the concentration is high. Therefore, the interactive functions of the various components of an essential oil when compared to the action of one or two major components of the oil appear to have not been resolved. Therefore, the pure essential oils exert higher antibacterial activity when compared to the main components used individually [21].

Due to the importance of *Acinetobacter baumannii* as etiological agent of significant nosocomial infections and multiresistance to different antibiotics, we carried out research to evaluate the efficacy of essential oils in the control of this bacterium [13,15,17,22-28]. In this respect, the present study aimed to evaluate the intrinsic antimicrobial activity of essential oils on the *Acinetobacter baumannii* ATCC 17978 line.

2. MATERIAL AND METHODS

2.1. Bacterial Line and Culture Media

We used the *Acinetobacter baumannii* ATCC 17978 strain (American Type Culture Collection). For the culture, it was used agar blood agar (Oxoid®), and incubation at 35°C for 48 hours. The bacterial suspensions were performed on three to four *A. baumannii* colonies cultured on blood agar and inoculated into Brain Heart Infusion Broth (BHI, Oxoid®) medium and incubated at aerobic conditions for 24 hours at 37°C, when it was performed the centrifugation (4000 rpm) for five minutes, afterwards the supernatant was excluded and the precipitate material resuspended in sterile NaCl solution (0.85%) and subjected again to centrifugation. This procedure was repeated five times with the aim to remove the components from the culture medium.

After this procedure, the bacterial suspension was diluted in sterile saline solution (NaCl 0.85%) until reaching the turbidity corresponding to the 0.5 tube of the Mac-Farland scale, equivalent to the concentration of $1{,}5x10^8$ UFC/mL. This bacterial suspension constituted the inoculum for the antibacterial evaluation of the oils by the microdilution technique [29], to obtain minimum inhibitory concentration (MIC), minimal bactericidal concentration (MBC) and bactericidal kinetics of essential oils.

2.2. Essential Oils

We used thirteen essential oils, which were acquired from FERQUIMA Industria and Comercio Limitada (Varginia Grande Paulista - SP, Brazil). Table

1 describes the characteristics of the essential oils. The experiments were carried out using concentration ranges from 0.00 to 100.00%: 0.39%; 0.78%; 1.56%; 3.12%; 6.25%; 12.50%; 25.00%; 50.00%; 100.00% and the negative control [30].

2.3. Essential Oil Sterility Test

In order to ensure the sterility of the essential oils, it was prepared dilutions of the essential oils in a 96-well microtiter plate, including a growth control (BHI + Tween 80) and a sterility control (test oil BHI + Tween 80+). The plates were incubated at 37°C for 24 hours. The contaminant bacterial growth was indicated by the presence of a white pellet at the bottom of the well, the oil was considered sterile when there was no bacterial growth or absence of pellet [31].

2.4. Determination of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

All the evaluations were performed in BHI broth supplemented with Tween 20 detergent (final concentration 0.5% (v / v). The *A. baumannii* line was suspended in BHI broth to provide a final density of 106 CFU mL^{-1}, and they were confirmed by viable cell counting. The Minimum inhibitory concentration (MIC) and minimal bacterial concentration (MBC) were evaluated according to the procedure recommended by CLSI [29]. The MIC was determined by a ninety-six-well microdilution method. After incubation at 37°C for 24h, the MIC was evaluated, and the presence of viable bacterial cells at the noninhibitory concentrations was determined by the addition, in each sample, of the dye 2,3,5 - Triphenyltetrazolium Chloride, in the volume

Table 1: Common and Scientific Names of Medicinal Plants and Characteristics of Essential Oils

Characteristics of essential oils*.							
Common name	**Scientific name**	**CAS** No**	**Batch**	**Color**	**Density (20ºC)**	**RI***(20ºC)**	**Main components**
Rosemary	*Rosmarinus officinais* L. (*Lamiaceae*)	8008-45-5	158	Straw yellow	0.915	1.467	Beta-pinene (8%); 1.0 cineol (40%) Camphor (12%)
Artemisia	*Artemisia vulgaris* L. (*Asteraceae*)	84775-45-1	218	Yellow	0.942	1.466	Alpha-tujone (47%); Beta-Tujone (10%)
Chinese cassia	*Cinnamomum cassia* (L.) Presl (*Lauraceae*)	84961-46-6	216	Yellow-brown	1.053	1.609	Cinnamic aldehyde (81%); coumarin (3%); Benzaldehyde (3%); Cinnamic alcohol (3%); Styrene (3%)
White Camphor	*Cinnamomum camphora* (L.) J. Presl camphortree (*Lauraceae*)	8008-51-3	113	Colorless	0.875	1.468	Limonene (26%); 1,8-cineole 30%); Alpha-pinene (13%)
Citronella	*Cymbopogon winterianus* Jowitt (*Poaceae*)	8000-29-1	142	Light yellow	0.885	1.4670	Not determined
Clove (Leaf)	*Syzygium aromaticum* (L.) Merr. & L. M. Perry (*Myrtaceae*)	8015-87-2	218	Yellow	1.047	1.536	Eugenol (86%)
Eucalyptus staigeriana	*Eucalyptus staigeriana* F. Muell. ex F. M. Bailey (*Myrtaceae*)	92502-70-0	116	Yellow	0.878	1.475	Limonene (9%)
Eucalyptus globulus	*Eucalyptus globulus* Labill (*Myrtaceae*)	8000-46-4	158	Colorless	0.913	1.461	1.8 Cineol (80%); Limonene (9%); Alpha pinene (9%)
Eucalyptus citriodora	*Eucalyptus citriodora* Hook (*Myrtaceae*)	92502-70-0	107	Yellow	0.864	1.454	Citronellal (76%)
Tea Tree	*Melaleuca alternifolia* Cheel (*Myrtaceae*)	68647-73-4	194	Straw yellow	0.896	1.477	Terpinen-4-ol (42%); Gamma terpinene (22%); Alpha terpinene (10%); Cineol (1.5%)
Peppermint	*Mentha piperita* L. (*Lamiaceae*)	84082-70-2	185	Straw yellow	0.902	1.460	l-menthol (33%); Mentone (30%); Methyl acetate (4%); Eucalyptol (6%)
Oregano	*Origanum vulgaris* L. (*Lamiaceae*)	84012-24-8	224	Brown	0.954	1.511	Carvacrol (71%); Thymol (3%); Gamma-terpinene (4.5%); Para-cymene (3.5%); Beta-caryophyllene (4%)
Sage	*Salvia sclarea* L. (*Lamiaceae*)	8016-63-5	215	Straw yellow	0.892	1.461	Linalyl acetate (64%); Linalool (20%)

*Data provided by FERQUIMA.
**CAS No: Chemical Abstracts Service.
***RI: Refractive index.

of 50 µL. Because of this, it was possible to distinguish the live samples, with red color, from the dead ones that kept their color. The minimal inhibitory concentration was considered as the lowest concentration of essential oil capable of inhibiting bacterial growth [32].

The minimum bactericidal concentration (MBC) was determined after the MIC results were obtained. Microdilution plates containing wells with visible or non-visible growth were shaken vigorously with the aid of the micropipettor, and then 100µl of the solution from each well was transferred to Petri dishes with blood agar medium and incubated at 37°C for 24 h. The MBC was defined as the minimum concentration at which bacterial growth did not occur [33].

2.5. Bactericidal Kinetics of Essential Oils

We used the methodology described by Allahghadri *et al*. [31]. It was added in tubes 40 mL of each essential oil at the dilution determined by MBC to each 5 mL of BHI broth containing bacterial suspension of 106 UFC mL^{-1} and then were incubated at 37°C. Samples (0.1 mL) were removed every 10 minutes for a period of 180 min. Samples were immediately washed with sterile phosphate buffer, pH 7.0, centrifuged at 10000 rpm, resuspended in the buffer and then cultured on BHI agar for 24 h at 37°C. All valuations were performed in triplicate. The microbial colonies were counted after the incubation period. An evaluation was made on the variation of the microbial load in order to observe which essential oil presented greater negative variation (drop) in the microbial count. This analysis showed that the greater the negative variation, the greater the efficacy of the essential oil. In this context, the percentage variation of microbial counts consisted of the following relation:

$$Microbial\ count_{essential\ oil}(\%) = \frac{(Count_{0.039\%} - Count_{0.0\%})}{Count_{0.0\%}} \times 100$$

This association was used for all evaluated essential oils and for all the concentrations used. According to the expression above, negative variations show a decrease in microbial counts and positive variations show an increase in microbial counts as the concentration of the respective essential oil increases.

2.6. Statistical Analysis

In order to analyze the data, a descriptive analysis of the microbial count was performed according to the varied concentrations of several essential oils evaluated, microbial counting data were analyzed using line graphs to observe the evolution of the microbial count with the increase of the concentration of the essential oil. We used the Kruskal-Wallis test to compare the variation of the microbial count in relation to the concentration and the time of analysis, the latter referring to the minimum bactericidal concentration (MBC). All statistical tests were applied with a significance level of 5% ($p < 0.05$).

Table 2: Means of Microbial Count for each of the Concentrations of Essential Oils Evaluated in the Control of *Acinetobacter baumannii*

Essential oil	0%	0.39%	0.78%	1.56%	3.12%	6.25%	12.5%	25%	50%	100%	MIC[1]	MBC[1]
Rosemary	$1.0.10^6$	$1.6.10^4$	$1.5.10^3$	$1.9.10^2$	$4.6.10^1$	2.0	0.0	0.0	0.0	0.0	6.25 %	12.5 %
Artemisia	$1.0.10^6$	$7.6.10^3$	$1.8.10^3$	$9.0.10^2$	$1.4.10^2$	5.0	0.0	0.0	0.0	0.0	3.12 %	6.25 %
Cinnamon	$1.0.10^6$	$1.1.10^5$	$8.8.10^4$	$1.8.10^3$	$6.1.10^2$	8.6	1.0	0.0	0.0	0.0	12.5 %	12.5 %
Camphor	$1.0.10^6$	$3.1.10^5$	$3.6.10^4$	$3.2.10^3$	$8.8.10^2$	$1.2.10^2$	$4.2.10^1$	4.0	0.0	0.0	25 %	25 %
Citronella	$1.0.10^6$	$2.3.10^1$	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.39 %	0.78 %
Clove	$1.0.10^6$	$1.8.10^4$	$1.6.10^4$	$1.2.10^3$	$8.1.10^2$	$5.0.10^1$	3.3	0.0	0.0	0.0	12.5 %	12.5 %
Eucalyptus citriodora	$1.0.10^6$	$3.2.10^4$	$4.3.10^3$	$5.6.10^2$	$6.5.10^1$	6.6	0.0	0.0	0.0	0.0	3.12 %	6.25 %
Eucalyptus globulus	$1.0.10^6$	$1.2.10^5$	$1.1.10^4$	$2.4.10^3$	$1.3.10^2$	$2.6.10^1$	2.3	0.0	0.0	0.0	12.5 %	12.5 %
Eucalyptus staigeriana	$1.0.10^6$	$5.4.10^3$	$3.2.10^2$	$9.2.10^1$	0.0	0.0	0.0	0.0	0.0	0.0	1.56 %	3.12 %
Tea tree	$1.0.10^6$	$1.1.10^4$	$2.1.10^3$	$8.3.10^2$	2.0	0.0	0.0	0.0	0.0	0.0	3.12 %	6.25 %
Mint	$1.0.10^6$	$1.7.10^4$	$2.0.10^2$	8.6	0.0	0.0	0.0	0.0	0.0	0.0	1.56 %	3.12 %
Oregano	$1.0.10^6$	$5.6.10^4$	$9.4.10^3$	$1.2.10^3$	$9.5.10^2$	$1.2.10^2$	4.3	0.0	0.0	0.0	12.5 %	12.5 %
Sage	$1.0.10^6$	$4.3.10^4$	$1.4.10^4$	$1.1.10^3$	$2.9.10^2$	$5.5.10^1$	1.3	0.0	0.0	0.0	6.25 %	12.5 %

[1]MIC: minimum inhibitory concentration and MBC: minimum bactericidal concentration.

3. RESULTS

Table **2** shows the mean of the microbial counts for each of the essential oils at the concentrations evaluated in the study. It was possible to determine the minimum bactericidal concentrations for each of the essential oils studied, highlighting the efficacy of the essential oils of citronella, mint and *Eucalyptus staigeriana*, since they abolished the microbial load in concentrations below 3.12%; and the scarce antimicrobial effect of the essential oils of camphor, cinnamon, clove, *Eucalyptus globulus* and oregano. The other essential oils presented an intermediate antimicrobial effect.

It was verified that the microbial count with the use of citronella oil in the concentration of 0.78% had positive effects regarding the antimicrobial effect. The essential oil of peppermint also presented high efficacy, and its antimicrobial activity was effective at 1.56%. The least effective essential oils were cinnamon, cloves, eucalyptus globules and oregano with MICs and MBCs of 12.5%, while for essential camphor oil, it was 25%. The essential oils with intermediate antimicrobial effect were those of tea tree, *Eucalyptus citriodora*, artemisia, rosemary and sage.

The percent variation of microbial counts was determined by means of descriptive statistics, in order to observe which essential oils obtained greater efficacy in the reduction of microbial counts (Table **3**).

The results of the means of the microbial counts differed significantly from the results of the medians, which requires the application of a nonparametric comparison test based on the position of the medians of the variations of the microbial count. In this context, the results show significant differences in the percentage variations of the microbial count ($p=0.033$), since the p value was lower than the significance level adopted for the statistical test ($p<0.050$).

Figure **1** shows the behavior of the percentage variations of the microbial count of each of the essential oils evaluated. It is possible to observe that the confidence intervals of the citronella and mint oils overlap evidencing the absence of significant differences in the variation of the microbial count of these essential oils. This result showed that the variation of the microbial count median was higher for the citronella and mint oils, and the variation of the oils mentioned did not differ significantly.

The *Eucalyptus staigeriana* and tea tree oils presented a prominence in the analysis of the variation of the microbial count, evidencing its high efficiency in the reduction of the microbial load as the concentration of the essential oil increases. The essential oils of *Eucalyptus citriodora*, oregano, cinnamon and camphor presented the smallest variations of the microbial load, assuming their lower efficacy in the reduction of the microorganisms evaluated, since the variation of the

Table 3: Percentage Variation (%) of the Microbial Count in Relation to the Essential Oils Used in the Control of *Aceinetobacter baumannii*

Essential oil	N	Mean±SD	Median (Md)[2]	Value p[1]
Rosemary	18	-91.32±8.53	-92.29 c	
Artemisia	18	-84.18±18.37	-90.52 cd	
Cinnamon	21	-80.67±25.98	-88.89 cd	
Camphor	24	-82.73±12.15	-87.32 d	
Citronella	6	-100.0±0.001	-100.0 a	
Clove	21	-74.19±35.50	-93.83 cd	
Eucalyptus citriodora	18	-91.43±5.31	-88.32 cd	0.003
Eucalyptus globulus	21	-88.88±7.49	-90.00 cd	
Eucalyptus staigeriana	15	-91.82±11.13	-94.68 bc	
Tea tree	15	-88.04±16.22	-98.90 bc	
Mint	12	-97.93±2.53	-98.30 ab	
Oregano	21	-81.44±25.80	-88.54 cd	
Sage	21	-86.75±12.34	-92.14 cd	

[1]Value p regarding the Kruskal-Wallis test P<0,05. [2]Different letters in the same column indicate significant difference in variation by Dunn's multiple comparison test at P <0.05.

microorganism count of these essential oils was superior to -90 % (dashed line in Figure **1**).

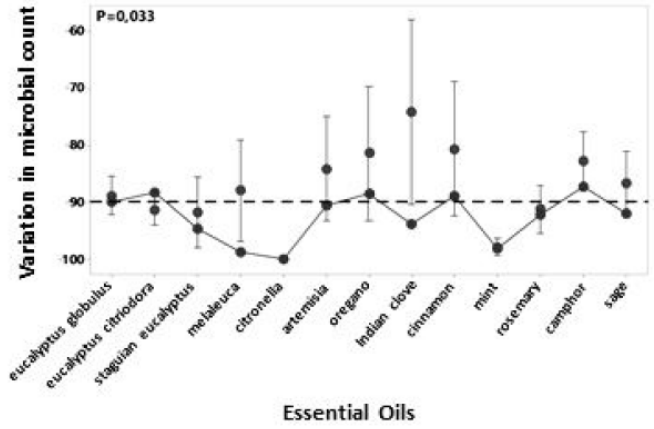

Figure 1: Percentage variation of the microbial count of the essential oils studied.

Note: Blue dots refer to the mean and red dots to the median.

The high dispersion of microbial load variation data determined the need for a non-parametric test, which addresses the comparison between the medians of the data distributions, since this statistic is not influenced by outliers that, for in turn, significantly influence the distribution mean, making it impossible to apply it as a test statistic. If a parametric test, which uses the mean as test statistic, were used in this case, the result would be mistaken, since the dispersion of data for some essential oils is significant, influencing the mean of the distribution.

Figure **2** shows an overview of the microbial count variations per essential oil evaluated. It was possible to observe that none of the essential oils presented positive variation, that is, in no case did an increase in the microbial count occur. Figure **3** shows the results of the microbial count variation for the most effective essential oils (citronella, mint and tea tree). It was verified that the variation in all the cases was negative, being lower than -60%, evidencing a greater decrease of the microbial activity. The microbial count was zero at concentrations above 0.78% for citronella essential oil (more effective), concentrations above 3.12% for mint essential oil and above 6.25% for essential oil of tea tree.

Table **4** shows the means of the distributions of the *Acinetobacter baumannii* count per time of exposure to the minimum bactericidal concentration (MBC). The results indicate that the essential oils of tea tree, oregano and clove, in their minimum bactericidal concentrations, showed greater efficacy, since they canceled the microbial load in up to 60 minutes. The

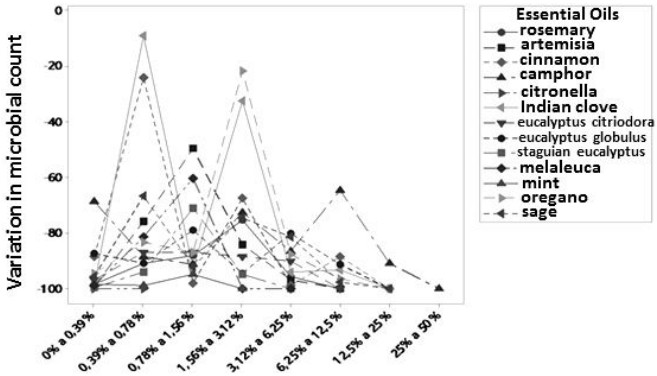

Figure 2: Percentage variation of the microbial count of the essential oils used in the control of *Acinetobacter baumannii.*

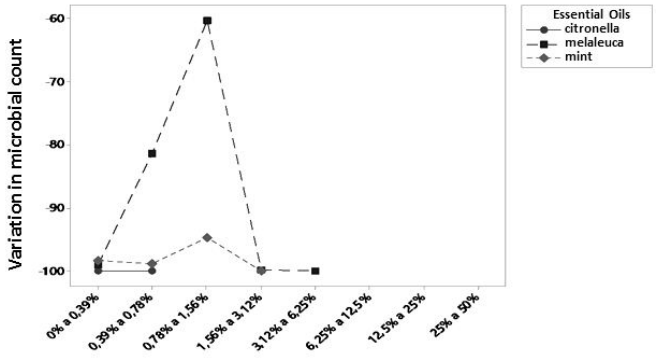

Figure 3: Variation of the microbial count for the most effective essential oils in the control of *Acinetobacter baumannii.*

essential oils of camphor, *Eucalyptus globulus* and sage were less effective in their respective minimum bactericidal concentrations, since they canceled the microbial load in 180, 140 and 130 minutes, respectively.

Table **5** shows the descriptive statistics of the percentage variation of the microbial count in relation to the analysis interval considered for each essential oil. The results indicate the presence of significant differences in the variations of the microbial count when the essential oils were compared ($p < 0.001$). It is possible to assume that the oils of tea tree, oregano, citronella*, Eucalyptus staigerian* and clove presented negative variations that exceeded 90% of reduction, showing a significant decrease in the microbial count when the minimum bactericidal concentration was used. The efficacy of *Eucalyptus globulus* and camphor oils was lower, resulting in a lower negative variation, assuming a lower reduction in microbial counts. It is worth mentioning that camphor essential oil, despite the high dispersion of the data, presented a positive mean variation, indicating growth of the microorganism over the time observed in the analysis.

Table 4: Means of the *Acinetobacter baumannii* Counts for the Times Evaluated Using the MBC of each Essential Oil

Times (min)	Rosemary (6,25%)	Artemísia (6,25%)	Cinnamon (6.25%)	Camphor (25%)	Citronella (0,078%)	Clove (12,5%)	*Eucalyptus citriodora* (6,25%)	*Eucalyptus globulus* (12,5%)	*Eucalyptus staigeriana* (3,12%)	Tea Tree (6,25%)	Mint (3,12%)	Oregano (12,5%)	Sage (12,5%)
0	$1,2.10^6$	$1,0.10^6$	$1,1.10^6$	$1,0.10^6$	$1,6.10^6$	$1,3.10^6$	$1,0.10^6$	$1,2.10^6$	$1,0.10^6$	$1,1.10^6$	$1,0.10^6$	$1,1.10^6$	$1,2.10^6$
10	$2,0.10^4$	$2,0.10^4$	$2,3.10^4$	$1,1.10^5$	$5,9.10^4$	$1,3.10^5$	$3,2.10^5$	$2,0.10^4$	$7,6.10^4$	$1,6.10^3$	$8,1.10^5$	$3,2.10^3$	$9,3.10^3$
20	$3,3.10^3$	$3,3.10^3$	$5,1.10^3$	$8,1.10^4$	$4,1.10^3$	$1,4.10^4$	$6,0.10^4$	$5,4.10^4$	$4,0.10^3$	$6,0.10^1$	$1,1.10^5$	$6,2.10^2$	$3,4.10^3$
30	$5,5.10^3$	$5,5.10^3$	$2,1.10^3$	$2,1.10^4$	$3,0.10^2$	$8,0.10^2$	$4,3.10^4$	$4,3.10^3$	$5,3.10^2$	3,3	$7,5.10^4$	$2,0.10^1$	$8,5.10^2$
40	$6,3.10^2$	$5,0.10^2$	$2,0.10^2$	$1,0.10^3$	$8,3.10^2$	$5,3.10^1$	$6,3.10^3$	$6,7.10^3$	$8,0.10^1$	0,0	$2,3.10^3$	$2,1.10^1$	$6,3.10^2$
50	$6,2.10^1$	$2,3.10^2$	$6,0.10^2$	$7,2.10^3$	$1,3.10^1$	8,6	$2,3.10^2$	$3,8.10^3$	$2,5.10^1$	0,0	$1,8.10^3$	0,0	$3,3.10^2$
60	$4,5.10^1$	$8,2.10^2$	$1,6.10^2$	$3,2.10^3$	$1,3.10^1$	0,0	$7,1.10^1$	$5,6.10^2$	1,0	0,0	$5,5.10^2$	0,0	$1,5.10^2$
70	8,6	$1,0.10^2$	$2,5.10^1$	$3,0.10^2$	0,0	0,0	$1,0.10^1$	$5,2.10^2$	0,0	0,0	$2,3.10^1$	0,0	$3,0.10^1$
80	0,0	$7,7.10^1$	5,0	$8,1.10^2$	0,0	0,0	0,0	$2,7.10^2$	0,0	0,0	$5,1.10^1$	0,0	$6,3.10^1$
90	0,0	$1,0.10^1$	0,0	$5,5.10^2$	0,0	0,0	0,0	$1,5.10^2$	0,0	0,0	$2,2.10^1$	0,0	$4,8.10^1$
100	0,0	2,0	0,0	$2,2.10^2$	0,0	0,0	0,0	$1,0.10^1$	0,0	0,0	2,3	0,0	$2,1.10^1$
110	0,0	0,0	0,0	$9,6.10^1$	0,0	0,0	0,0	$7,7.10^1$	0,0	0,0	0,0	0,0	8,3
120	0,0	0,0	0,0	$3,0.10^1$	0,0	0,0	0,0	$2,6.10^1$	0,0	0,0	0,0	0,0	3,0
130	0,0	0,0	0,0	$8,8.10^1$	0,0	0,0	0,0	3,6	0,0	0,0	0,0	0,0	0,0
140	0,0	0,0	0,0	$6,3.10^1$	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
150	0,0	0,0	0,0	$3,0.10^1$	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
160	0,0	0,0	0,0	$1,3.10^1$	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
170	0,0	0,0	0,0	6,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
180	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0

Table 5: Descriptive Statistics of the Percentage Variation (%) of the *A. baumannii* Count in Relation to Times of Analysis

Essential oil	N	Mean±SD	Median (Md)[2]	Value p[1]
Rosemary	24	-40.80±138.60	-83.10	
Artemisia	33	-24.10±154.20	-85.90	
Cinnamon	27	-43.70±127.10	-80.00	
Camphor	54	2.50±171.80	-56.00	
Citronella	21	-37.70±113.40	-93.20	
Clove	18	-91.71±5.30	-92.02	
Eucalyptus citriodora	24	-76.96±21.49	-83.16	<0,001
Eucalyptus globulus	42	-31.10±95.30	-46.40	
Eucalyptus staigeriana	21	-89.01±10.10	-92.86	
Tea tree	12	-97.63±2.51	-98.04	
Mint	33	-47.30±68.60	-69.40	
Oregano	15	-69.40±58.70	-96.80	
Sage	39	-49.18±51.76	-62.37	

[1]Value p regarding the Kruskal-Wallis test P<0,05.

4. DISCUSSION

Although *Acinetobacter baumannii* is common in nature and considered human skin and respiratory tract commensal, it has also been associated to serious infectious diseases, such as pneumonia, urinary tract infection, endocarditis, wound infection, meningitis and septicemia. This pathogen is found in many health care settings and is a very effective human colonizer in hospitals. Its wide range of antibiotic resistance makes it a successful nosocomial pathogen, threatening the current era of the antibiotic. The emergence of resistant strains of bacterial pathogens is a source of high morbidity, mortality and cost increase, making their treatment much more difficult [34,35].

Given the spread of multidrug resistance, the low number of antibiotics in development, and the lack of antibiotics in the management of infections caused by multidrug-resistant *A. baumannii*, interest in treatments and discovery of new antibacterial strategies has increased. One of these strategies is to use extracts of essential oils that are a potential reservoir of effective antibacterial molecules [24].

Essential oils are natural, volatile and complex compounds, characterized by a strong odor. They are known for their medicinal properties and bactericidal and fungicidal action, and can be used in food preservation and as antimicrobials, analgesics, sedatives and anti-inflammatories. They are very complex natural mixtures that can contain about 20 to 60 components at very different concentrations and are characterized by having two or three major components in very higher concentrations (20-70%) compared to the other components. Generally, these major components determine the biological properties of the essential oil [18].

As the sensitivity and resistance of the microorganisms to antimicrobial agents is changeable, it becomes necessary to analyze the patterns of susceptibility of the organisms against conventional antimicrobial agents and to evaluate possible alternatives. Thus, the lower concentration of an antimicrobial agent that inhibits the growth of the organism is known as the minimal inhibitory concentration (MIC). Assays for determining MIC may be performed on agar or in liquid medium. The traditional method of determining the MIC by means of the liquid dilution technique where dilutions of the test agent are incorporated into the culture medium into microplate wells or culture tubes. Each tube or well contains a different antimicrobial concentration and is inoculated with the organism being tested. After adequate incubation, the lowest concentration that does not show visible growth to the naked eye is considered as the MIC [29]. Using the plate microdilution method, in the present study it was possible to determine the MIC of thirteen essential oils (Table **2**). It was found that MIC varied from 0.78% to 25%, and citronella oil (*Cymbopogon winterianus*) was highly effective in controlling *A. baumannii* with MIC of

0.78%, while camphor oil (*Cinnamomum camphora*) had a lower effect, MIC of 25%. Hammer *et al*. [22] evaluated the citronella oil by the agar diffusion method and found inhibition of growth of *A. baumannii* at the concentration of 0.25%. These results corroborate those obtained in the present research, evidencing the antimicrobial potential of this essential oil.

The efficacy of citronella oil and citral oil (main component) was verified by Adukwu *et al*. [17], who obtained MICs of 0.25 and 1% for oil, whereas for citral it was between 0.06 and 0.25%. These authors state that the ability of the essential oil and citral to inhibit and kill multidrug-resistant *A. baumannii* highlights its potential for use in the treatment of infections caused by this bacterium. However, they emphasize that *in vitro* cytotoxicity suggests that further testing is required prior to human exposure *in vivo* or *ex vivo*.

The important characteristics responsible for the antimicrobial action of the essential oils include hydrophobic components that allow the lipidic participation of the bacterial cell membrane, which alters the cellular structures and makes them more permeable [21]. According to Silva and Fernandes Júnior [16] different concentrations of eugenol can inhibit the production of amylase and protease, promote cell wall degradation and cell lysis in some bacteria. In the present study, clove oil from India, whose main component is eugenol, showed antimicrobial activity at a concentration of 12.5% (Table **2**).

The antimicrobial activity of eucalyptus essential oil may be associated with the presence of 1,8-cineol, linalool, pinocarol and limonene. Other components present in lower amounts, such as terpineol and terpinen-4-ol, may also contribute to the antimicrobial activity of the oil [35,36]. The efficacy of *Eucalyptus staigerian* oil in the control of *A. baumannii* was verified in the concentration of 1.56% (MIC) and MBC 3,12 (Table **2**). This result may be associated with limonene, the main component of the oil used in the research (Table **1**).

Eucalyptus globulus and *Eucalyptus citriodora* oils presented MICs of 12.5% and 6.25%, respectively, considered to be less efficient (Table **2**). Damjanović-Vratnica *et al*. [23] also verified antimicrobial activity of *Eucalyptus* essential oil, especially against *Streptococcus pyogenes*, *Escherichia coli*, *Candida albicans*, *Staphylococcus aureus*, *Acinetobacter baumannii* and *Klebsiella pneumoniae*.

Study performed by Nickavar *et al*. [37] shows that compounds of some species of *Mentha* (*M. longifolia* (L.) Huds., *M. piperita* L., *M. pulegium* L., *M. rotundifolia* (L.) Huds., and *M. spicata* L.) have antioxidant properties, which may be due to their phytochemical compositions. In the present study, high antimicrobial activity of *Menha piperita* L. essential oil was observed, presenting a 3.12% MIC (Table **2**), whose main components were l-menthol (33%); Mentone (30%); Methyl acetate (4%); Eucalyptol (6%).

Sienkiewicz *et al*. [25] determined the antibacterial properties of cinnamon, lavender and geranium essential oils against bacteria of the genus *Acinetobacter* isolated from various clinical and hospital materials and found that cinnamon oil was the most active against the clinical and environmental strains of *Acinetobacter baumannii* with MIC values ranging from 0.5 to 2.5 µL/mL. The antimicrobial activity of cinnamon oil was considered intermediate, MIC of 12.5% (Table **2**). The effectiveness of cinnamon oil may be associated with cinnamic aldehyde (81%, Table **1**), which according to Mayaud *et al*. [38] shows high antimicrobial activity against Gram-positive and Gram-negative clinical strains.

According to Boukhraz *et al*. [15], the effect of an antimicrobial agent can also be expressed as the death rate for a given concentration of agent under controlled conditions (bactericidal kinetics of essential oils). This rate is determined by measuring the number of viable bacteria at different time intervals. The resulting graphical representation is known as the "death curve." By using the bactericidal kinetics of essential oils, it was found that the essential oils of tea tree (MBC 6.25%), oregano (MBC 12.5%) and clove (MBC 12.5%), in their minimum bactericidal concentrations, were more efficient, since they annulled the microbial load of *A. baumannii* in up to 60 minutes. The essential oils of camphor, *Eucalyptus globulus* and sage were less effective in their respective minimum bactericidal concentrations, since they canceled the microbial load in 180, 140 and 130 minutes, respectively (Tables **4** and **5**). The antimicrobial activity of oregano oil is probably related to some of the major components, such as carvacrol, whose concentration was 71%, associated with thymol, gamma-terpinene, para-cymene present in lesser amounts (Table **1**).

The results of several studies have indicated that the antioxidant and antimicrobial effects of oregano may be related to the major components, carvacrol, thymol, linalool, gamma-terpinene, para-cymene and

terpinene-4-ol [13,39-41]. These authors state that oregano essential oil has potent antimicrobial activity against multidrug resistant *A. baumannii* when used alone or in combination with antibiotics.

Sakkas *et al*. [42] evaluated camomile, oregano, tea tree, thyme and basil oils on *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, and found that the oils had a variable antibacterial effect, whereas the camomile oil did not show antibacterial activity, oregano thyme and basil were ineffective on isolated *P. aeruginosa*. The minimum values of inhibitory concentration (MIC) and minimum bactericidal concentration ranged from 0.12% to 1.50% (v / v) for the tea tree oil, 0.25-0.37% (v / v) for the oil of oregano and thyme, 0.50% to 1.25% For basil oil > 4% for chamomile oil when *A. baumannii* was tested. These authors state that the antimicrobial activities of the essential oils are influenced by the origin of the strain (wild, reference, sensitive to drugs or resistant) which should be taken into account when investigating the potential of plants for the development of new antimicrobials.

Antibacterial activity was observed against multiresistant bacteria using Indian cloves and rosemary was observed by Abdullah *et al*. [43]. Clove oil was more active against *Acinetobacter baumannii* and *Enterococcus faecalis* isolates at concentrations of 0.312%, while rosemary oil was active against *E. faecalis* as 5% or more. *A. baumannii* was sensitive to concentrations of 0.312%. These authors concluded that clove oil is more potent than rosemary oil, yet both exhibited high antibacterial activity and may be employed in the treatment of infections caused by multidrug resistant bacteria.

Sienkiewicz *et al*. [25] suggest that some essential oils could be used in the fight against infections caused by bacteria of the genus *Acinetobacter* and as components of formulations for the hygiene and disinfection of the hospital environment. However, Yap *et al*. [44] suggest that although oils should be used in dilute forms, especially when applied directly to the skin, their antimicrobial properties are as effective as those of chemical antibacterial agents. In addition, it is important that microorganisms do not acquire resistance to essential oils or their components. However, if essential oils are used for food preservation or medicinal purposes, safety and toxicity issues will need to be addressed, as well as the standardization of methods used for *in vitro* testing [22].

5. CONCLUSION

Based on the results obtained and the methodology used, it can be concluded that:

- All the oils performed antibacterial activity against *Acinetobacter baumannii*.
- Citronella, *Eucalyptus staigeriana* and mint oils presented lower minimum inhibitory concentration and minimum bactericidal concentration.
- Oregano, tea tree and Indian clove oils presented higher bacterial death rate, and they canceled the microbial load of *A. baumannii* in up to 60 minutes.

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DECLARATION OF POTENTIAL CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

[1] Peleg AY, Seifert H, Paterson DL. *Acinetobacter baumannii*: emergence of a successful pathogen. Clin Microbiol Ver 2008; 21: 538-82. https://doi.org/10.1128/CMR.00058-07

[2] Tiwari V, Tiwari M. Quantitative proteomics to study carbapenem resistance in *Acinetobacter baumannii*. Front Microbiol 2014; 5: 512. https://doi.org/10.3389/fmicb.2014.00512

[3] Fregolino E, Gargiulo V, Lanzetta R, Parrilli M, Holst O, Castro CD. Identification and structural determination of the capsular polysaccharides from two *Acinetobacter baumannii* clinical isolates, MG1 and SMAL. Carbohydr Res 2011; 346: 973-77. https://doi.org/10.1016/j.carres.2011.03.024

[4] Gentile V, Frangipani E, Bonchi C, Minandri F, Runci F, Visca P. Iron and *Acinetobacter baumannii* biofilm formation. Pathogens 2014; 3: 704-19. https://doi.org/10.3390/pathogens3030704

[5] Lee HW, Koh YM, Kim J, *et al*. Capacity of multidrug-resistant clinical isolates of *Acinetobacter baumannii* to form biofilm and adhere to epithelial cell surfaces. Clin Microbiol Infect 2008; 14: 49-54. https://doi.org/10.1111/j.1469-0691.2007.01842.x

[6] McConnell MJ, Luis Actis L, Pachón J. *Acinetobacter baumannii*: human infections, factors contributing to pathogenesis and animal models. FEMS Microbiol Rev 2013; 37: 130-55. https://doi.org/10.1111/j.1574-6976.2012.00344.x

[7] Pérez-Llarena FJ, Bou G. Proteomics as a tool for studying bacterial virulence and antimicrobial resistance. Front Microbiol 2016; 7: 1-21. https://doi.org/10.3389/fmicb.2016.00410

[8] Tiwari V, Tiwari M. Phosphoproteomics a san emerging we a ponto develop new antibiotics against carbapenem resistants strain of *Acinetobacter baumannii*. J Proteomics 2015; 112: 336-38. https://doi.org/10.1016/j.jprot.2014.09.008

[9] Dijkshoorn L, van Aken L, Shunburne L, *et al*. Prevalence of *Acinetobacter baumannii* and other acinetobacter spp in faecal samples from non-hospitalised individuals. Clin Microbiol Infect 2005; 11: 329-32. https://doi.org/10.1111/j.1469-0691.2005.01093.x

[10] Playford E, Craig J, Iredell J. Carbapenem-resistant *Acinetobacter baumannii* in intensive care unit patients: risk factors for acquisition, infection and their consequences. J Hosp Infect 2007; 65: 204-11. https://doi.org/10.1016/j.jhin.2006.11.010

[11] Tuon FF, Penteado-Filho SR, Amarante D, Andrade MA, Borba LA. Mortality rate in patients with nosocomial Acinetobacter meningitis from a Brazilian hospital. Braz J Infect Dis 2010; 14: 437-40. https://doi.org/10.1590/S1413-86702010000500003

[12] Gonzalez-Villoria AM, Valverde-Garduno V. Antibiotic-resistant *Acinetobacter baumannii* increasing success remains a challenge as a nosocomial pathogen. J Pathog 2016; 2016: 1-10. https://doi.org/10.1155/2016/7318075

[13] Miyasaki Y, Rabenstein JD, Rhea J, *et al*. Isolation and characterization of antimicrobial compounds in plant extracts against multidrug-resistant *Acinetobacter baumannii*. PLoS ONE 2013; 8: e61594. https://doi.org/10.1371/journal.pone.0061594

[14] Qureshi WK, PAlayekar V, Dayan E, Mack JP, Rojtman A. Combating the antibiotic resistance threat. Int J Pharm Pharm Sci 2015; 7: 68-72.

[15] Boukhraz A, Elhartiti H, Barrahi M, *et al*. Evaluation of the bacteriostatic and bactericidal activity of essential oil of Thymus Satureioides. Int J Res Studies Sci Eng Tech 2016; 3: 24-8.

[16] Silva NCC, Fernandes Júnior A. Biological properties of medicinal plants: a review of their antimicrobial activity. J Venom Anim Toxins 2010; 16: 402-13. https://doi.org/10.1590/S1678-91992010000300006

[17] Adukwu EC, Bowles M, Edwards-Jones V, Bone H. Antimicrobial activity, cytotoxicity and chemical analysis of lemongrass essential oil (Cymbopogon flexuosus) and pure citral. Appl Microbiol Biotechnol 2016; 100: 9619-27. https://doi.org/10.1007/s00253-016-7807-y

[18] Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils-a review. Food Chem Toxicol 2008; 46: 446-75. https://doi.org/10.1016/j.fct.2007.09.106

[19] Langeveld WT, Veldhuizen EJ, Burt SA. Synergy between essential oil components and antibiotics: a review. Crit Rev Microbiol 2014; 40: 76-94. https://doi.org/10.3109/1040841X.2013.763219

[20] Saviuc C, Gheorghe M, Coban S, *et al*. Rosmarinus officinalis essential oil and eucalyptol act as efflux pumps inhibitors and increase ciprofloxacin efficiency against *Pseudomonas aeruginosa* and *Acinetobacter baumannii* MDR strains. Rom Biotechnol Lett 2016; 21: 11782-790.

[21] Burt S. Essential oils: their antibacterial properties and potential applications in foods – a review. Int J Food Microbiol 2004; 94: 233-53. https://doi.org/10.1016/j.ijfoodmicro.2004.03.022

[22] Hammer KA, Carson CF, Riley TV. Antimicrobial activity of essential oils and other plant extracts. J Appl Microbiol 1999; 86: 985-90. https://doi.org/10.1046/j.1365-2672.1999.00780.x

[23] Damjanović-Vratnica B, Đakov T, Šuković D, Damjanović J. Antimicrobial effect of essential oil isolated from eucalyptus globulus labill. from Montenegro. Czech J Food Sci 2011; 29: 277-84. https://doi.org/10.17221/114/2009-CJFS

[24] Montagu A, Saulnier P, Cassissa V, Rossines E, Eveillard M, Joly-Guillou ML. Aromatic and terpenic compounds loaded in lipidic nanocapsules: activity against multi-drug resistant *Acinetobacter baumannii* assessed *in vitro* and in a murine model of sepsis. J Nanomed Nanotechnol 2014; 5: 206. https://doi.org/10.4172/2157-7439.1000206

[25] Sienkiewicz M, Głowacka A, Kowalczyk E, Wiktorowska-Owczarek A, Jóźwiak-Bębenista M, Łysakowska M. The biological activities of cinnamon, geranium and lavender essential oils. Molecules 2014; 19: 20929-40. https://doi.org/10.3390/molecules191220929

[26] Tiwari V, Roy R, Tiwari M. Antimicrobial active herbal compounds against *Acinetobacter baumannii* and other pathogens. Front Microbiol 2015; 6: 1-11. https://doi.org/10.3389/fmicb.2015.00618

[27] Taherkhani, M. Chemical investigation and protective effects of bioactive phytochemicals from Artemisia ciniformis. J Chem Chem Eng 2016; 35: 471-81.

[28] Tutar U, Çelik C, Karaman I, Ataş M, Hepokur C. Anti-biofilm and antimicrobial activity of mentha pulegium L essential oil against multidrug-resistant *Acinetobacter baumannii*. Trop J Pharm Res 2016; 15: 1039-46 https://doi.org/10.4314/tjpr.v15i5.20

[29] CLSI. Performance standards for antimicrobial susceptibility testing. 27th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2017.

[30] Ogbebor NO, Adekunle AT, Enobakhare DA. Inhibition of colletotrichum gloeosporioides (Penz) Sacc. causal organism of rubber (Hevea brasiliensis Muell. Arg.) leaf spot using plant extracts. Afr J Biotechnol 2007; 6: 213-18.

[31] Allahghadri T, Rasooll I, Owlia P, *et al*. Antimicrobial property, antioxidant capacity and cytotoxicity of essential oil from cumin produced in Iran. J Food Sci 2010; 75: H54-H61. https://doi.org/10.1111/j.1750-3841.2009.01467.x

[32] Sylvester PW. Optimization of the tetrazolium dye (MTT) colorimetric assay for cellular growth and viability. Meth Mol Biol 2011; 716: 157-68. https://doi.org/10.1007/978-1-61779-012-6_9

[33] Favre B, Hofbauer B, Hildering K, Ryder NS. Comparison of *in vitro* activities of 17 antifungal drugs against a panel of 20 dermatophytes by using a microdilution assay. J Clin Microbiol 2003; 17: 41-8. https://doi.org/10.1128/JCM.41.10.4817-4819.2003

[34] Morgan DJ, Liang SY, Smith CL, *et al*. Frequent multidrug-resistant *Acinetobacter baumannii* contamination of gloves, gowns, and hands of healthcare workers. Infect Control Hosp Epidemiol 2010; 31: 716-21. https://doi.org/10.1086/653201

[35] Aggarwal KK, Khanuja SPS, Ahmad A, Kumar TRS, Gupta VK, Kumar S. Antimicrobial activity profiles of the two enantiomers of limonene and carvone isolated from the oils of Mentha spicita and Anethum sowa. Flav Frag J 2020; 17: 59-63. https://doi.org/10.1002/ffj.1040

[36] Viljoen A, Vuuren SV, Ernst E, *et al*. Osmitopsis asteriscoides (Asteraceae) – the antimicrobial and essential oil composition of a Cape-Dutch remedy. J Ethnopharmacol 2003; 88: 137-43. https://doi.org/10.1016/S0378-8741(03)00191-0

[37] Nickavar B, Alinaghi A, Kamalinejad M. Evaluation of the antioxidant properties of five Mentha species. Iran J Pharm 2008; 7: 203-09.

[38] Mayaud L, Carricajo A, Zhiri A, Aubert G. Comparison of bacteriostatic and bactericidal activity of 13 essential oils against strains with varying sensitivity to antibiotics. Lett Appl Microbiol 2008; 47: 167-73. https://doi.org/10.1111/j.1472-765X.2008.02406.x

[39] Baser KH. Biological and pharmacological activities of carvacrol and carvacrol bearing essential oils. Curr Pharm Des 2008; 14: 3106-19. https://doi.org/10.2174/138161208786404227

[40] Al-Janabi KW, Alazawi FN, Ibrahim Mohammed M, Kadhum AA, Mohamad AB. Chlorophenols in tigris river and drinking water of baghdad, Iraq. Bull Environ Contam Toxicol 2011; 87: 106-12. https://doi.org/10.1007/s00128-011-0315-y

[41] Saghi H, Bahador A, Dastjerdi FA, *et al*. Antibacterial effects of origanum vulgare essence against multidrug-resistant *Acinetobacter baumannii* isolated from selected hospitals of Tehran, Iran. Avicenna J Clin Microb Infec 2015; 2: e22982. https://doi.org/10.17795/ajcmi-22982

[42] Sakkas H, Gousia P, Economou V, Sakkas V, Petsios S, Papadopoulou C. *In vitro* antimicrobial activity of five essential oils on multidrug resistant Gram-negative clinical isolates. J Intercult Ethnopharmacol 2016; 5: 212-18. https://doi.org/10.5455/jice.20160331064446

[43] Abdullah BH, Hatem SF, Jumaa W. A Comparative study of the antibacterial activity of clove and rosemary essential oils on multidrug resistant bacteria. UK J Pharm Biosci 2015; 3: 19-22. https://doi.org/10.20510/ukjpb/3/i1/89220

[44] Yap PSX, Yiap BC, Ping HC, Lim SHE. Essential oils, a new horizon in combating bacterial antibiotic resistance. Open Microbiol J 2014; 8: 6-14. https://doi.org/10.2174/1874285801408010006

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