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Modeling The Sensitivity Profile Of Different Genotypes Of Methacycline-Resistant Staphylococcus Aureus To The Essential Oils Of Cymbopogon Citratus And Lippia Origanoides

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Abstract:

Background*: Bacterial resistance to conventional antibiotics poses a growing threat to human and animal health, prompting increased research into alternative treatments. Essential oils have emerged as promising candidates due to their ability to disrupt bacterial structures and processes. Methicillin-resistant Staphylococcus aureus (MRSA) remains a major concern in both human and animal communities, particularly in cases of bovine mastitis, where its resistance to multiple drugs complicates treatment. Although essential oils like those from Cymbopogon citratus and Lippia origanoides have shown potential antimicrobial properties, there is still a need for more consistent methods to evaluate their effectiveness, especially across different MRSA genotypes. This study investigates the antimicrobial activity of these essential oils against various MRSA strains isolated from bovine mastitis and analyzes the sensitivity patterns using advanced techniques like multivariate curve clustering, addressing critical gaps in our current understanding of essential oil efficacy.*

Materials and Methods*: Nine MRSA strains were collected from cows with subclinical mastitis in northern Minas Gerais, Brazil. The strains were identified using PCR with the femA gene and classified into 10 genotypic profiles through RAPD-PCR. After being stored and reactivated, the strains were tested for antimicrobial susceptibility. Essential oils from C. citratus (OECC) and L. origanoides (OELO) were extracted by steam distillation, and their chemical composition was analyzed by gas chromatography-mass spectrometry. The antimicrobial activity of the oils was evaluated using the disk diffusion method, with varying concentrations applied to MRSA cultures. The diameter of inhibition zones was measured, and statistical analysis, including Tukey's test, quadratic regressions, and multivariate clustering, was used to compare the oils' effects across different MRSA genotypes and other variables.*

Results*: The main components of OECC were geranial (31.89%), β-myrcene (25.37%), and neral (24.62%), while OELO contained carvacrol (29.75%), o-cymene (15.33%), and γ-terpinene (11.03%). Both oils demonstrated dose-dependent antimicrobial activity against MRSA, with OECC effective at a concentration of 30 µl/ml and OELO at 60 µl/ml. Multivariate analysis revealed similar inhibition patterns across different MRSA genotypes, providing insight into how these oils' effects can be interpreted in quantitative terms. Notably, OELO's antimicrobial activity against MRSA has not been reported before, and the study highlights the novel use of both oils on MRSA strains isolated from bovine milk.*

Conclusion: *The study showed that C. citratus and L origanoides essential oils had varying effects on different MRSA genotypes, with the inhibition influenced by factors like genotypic profile and location. These results open the door for further research to explore how these oils affect genetically distinct strains and to uncover the mechanisms behind their antimicrobial action.*

***Key Word**: C. citratus; L origanoides; MRSA; Essential oils; Antibiotics; Resistance.*

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I. Introduction

The widespread bacterial resistance to conventional antimicrobials drives the search for new molecules that can help mitigate this serious problem in human and animal health. Studies have reported promising results with the use of essential oils (EOs) as antimicrobials. The antimicrobial action of essential oils is attributed to their ability to damage the bacterial cell wall and membrane and disrupt the cell's transport mechanisms[1]. The use of plant metabolites as antimicrobial agents against multidrug-resistant pathogens may occur through the modulation of natural metabolic pathways involved in antimicrobial resistance[2].

Staphylococcus aureus is a pathogen responsible for numerous severe diseases in humans and animals, with methicillin-resistant Staphylococcus aureus (MRSA) posing a global public health challenge[3]. The detection of MRSA colonizing or infecting animals, as well as its presence in animal-derived food products, highlights new reservoirs for these bacteria[4,5].

In Brazil, MRSA has been identified in bovine milk from mammary glands with mastitis, with genotypic profile variations observed among isolates from different herds[6]. This microorganism exhibits characteristics that enhance its persistence in the host, allowing it to evade the immune system. Additionally, its resistance to numerous antibiotics is a well-known survival mechanism within the host[7]. In this context, the widespread dissemination of MRSA and the challenges in establishing effective therapies have spurred the search for new molecules with antimicrobial activity against these bacteria.

Research reviewed by Zouhir et al.[3] and other studies have documented the inhibition of MRSA by essential oils (EOs), particularly the specific action of *Cymbopogon citratus* essential oil (OECC) against *S. aureus*[8,9]. The citral component in this oil is responsible for inhibiting efflux pumps, altering membrane permeability, and interfering with the bacterium's osmoregulatory mechanisms[9]. Similarly, the essential oil of *Lippia origanoides* (OELO) has demonstrated efficacy in modulating the action of aminoglycosides on MRSA, altering resistance phenotypes in *S. aureus*, possibly through the inhibition of efflux pumps[10].

Published studies evaluating the inhibitory activity of essential oils, including OECC and OELO, use varied methodologies and interpretations. While growth inhibition effects are often observed, there are no standardized quantitative parameters to infer the minimum effective doses for these oils. In disk-diffusion sensitivity tests, varying inhibition halo sizes are observed even when evaluating the same microbial species. Dose-response effect graphs are the most commonly used representation in published research; however, when a larger number of genotypes is evaluated, identifying similar patterns becomes challenging, leading to diverse representative curves. In similar dose-response studies in agricultural sciences, new statistical tools, such as multivariate curve clustering, have been applied, enabling clearer result presentations[11].

Although the antimicrobial effects of these essential oils against various pathogens have been confirmed, a gap remains in scientific research regarding their action on different MRSA genotypes isolated from bovine mastitis. MRSA isolates from animals such as pigs, cattle, or poultry have been increasingly reported, and the zoonotic risk of transmission to humans has already been demonstrated[12]. This underscores the importance of establishing genotypic markers sensitive to antimicrobial products as a strategic control measure against the microorganism.

The objective was to evaluate the antimicrobial effect of *C. citratus* and *L. origanoides* essential oils on different methicillin-resistant *S. aureus* genotypes isolated from bovine mastitis and analyze sensitivity patterns to these oils using multivariate curve clustering.

II. Material And Methods

Bacterial Isolates and MRSA Identification

Nine MRSA strains were obtained from the teats of cows with subclinical mastitis in nine dairy farms located across four municipalities in northern Minas Gerais, Brazil. The strains were identified using PCR with the *fem*A genetic marker and assessed for clonality through RAPD-PCR, revealing 10 genotypic profiles. Among these, nine genetic profiles exhibited different patterns of multidrug resistance to beta-lactams, including penicillin, amoxicillin, and oxacillin, while only one profile was sensitive to all these antimicrobials.

These MRSA isolates were stored at -20°C in BHI medium (Prodimol Biotechnology) containing 20% glycerol and were reactivated three times in TSB medium, incubated at 37°C for 24 hours, until vigorous growth was achieved. The purity of the cultures was confirmed by inoculation on 5% sheep blood agar and morphological analysis through Gram staining.

Plants and Essential Oil Extraction

Plants of *C. citratus* and *L. origanoides* were obtained from the Medicinal Garden of the Institute of Agricultural Sciences, UFMG, and herbarium specimens were deposited in the ICA/UFMG Herbarium. Plant collection and essential oil extraction via steam distillation were conducted according to Aquino et al.[13] for *C. citratus* oil and Andrade et al.,[14] for *L. origanoides* oil. Chromatographic analyses were performed using a gas chromatograph, Agilent Technologies (GC 7890A), coupled with a mass spectrometer (MS 5975C), as described by Almeida and collaborators[15].

Antimicrobial Susceptibility Test

The sensitivity of the strains under study to OECC and OELO was evaluated by the disk diffusion method on agar, as described by CLSI (2015) with adaptations. The concentrations of 30 μL mL^{-1}, 60 μL mL^{-1}, 120 μL mL^{-1}, and 240 μL mL^{-1} were defined for each oil. For control, a disc soaked in distilled water (considered the 0 μL concentration), another in Tween 80 (the solvent used to prepare the oil concentrations), and a disc containing the conventional antibiotic ciprofloxacin were used. After preparing the desired concentrations of the oils, 30 μL of each was applied to sterile 6 mm filter paper discs. After saturation, the discs were left to rest and dry.

The discs were placed on Mueller Hinton agar plates previously inoculated with the MRSA strains under study, standardized to 0.5 on the MacFarland scale. After incubation in a growth incubator at 37°C for 24 hours, the diameter of each inhibition zone was measured in millimeters (mm). *S. aureus* ATCC 6538 and *S. aureus* ATCC 4330 were used as standard strains for antimicrobial activity testing. All tests were performed in triplicates.

Statistical Analyses

The difference in activity between the oils was evaluated using the Tukey Test at a 5% significance level, comparing the means of the growth inhibition zone diameters at different concentrations for each oil. To study the effect of the doses on the zone diameter for each municipality, farm, and genotypic profile, quadratic regressions were adjusted. The coefficients of the fitted models and the coefficient of determination were subjected to multivariate analysis. To avoid the influence of different scales, the coefficient estimates were standardized for mean and standard deviation. Subsequently, the Euclidean dissimilarity matrix was estimated from the standardized coefficients using the dist function from the stat package in R software.

For access grouping, the Tocher optimization method was used with the tocher function from the biotools package. After the multivariate grouping of the curves, the treatments considered as equal were identified. For these treatments, new quadratic regressions were adjusted at the group mean level.

III. Result

The major compounds observed in the two oils under study are presented in Table no 1. Geranial (31.89%), β-myrcene (25.372%) and neral (24.62%) were the major compounds present in OECC. Regarding the composition of OELO, the major components were Carvacrol (29.75%), o-Cymene (15.33%), γ-Terpinene (11.03%).

Table no 1: Chromatogram of essential oils of *C. citratus* and *L. origanoides* RT- Retention time.

Nº	Compound	TR	% of ions detected in the chromatogram	
			C. citratus oil	*L. origanoides oil*
1	1R-α-Pinene	5.612	nd	1.01
2	Camphene	6.056	nd	1.25
3	β-Phellandrene	6.663	nd	0.25
4	β-Pinene	6.827	nd	0.26
5	6-methyl-5-heptene-2-one	6,978	0,693	nd
6	β-Myrcene	7,178	25,37	2,52
7	α-Phellandrene	7.677	nd	0.12
8	α-Terpinene	8.022	nd	2.47
9	o-Cimeno	8.289	nd	15.33
10	D-Limonene	8.436	nd	0.67
11	Eucalyptol	8.555	nd	0.68
12	β-trans-ocimene	8,667	0.903	nd
13	β-Ocimene	9.005	0.17	0,17
14	γ-Terpinene	9.463	nd	11,03
15	Terpinolene	10.457	nd	0,20
16	β-linalool	11.038	1.076	0.44
17	β-citral	12.775	1.526	nd
18	Camphor	12.894	nd	0.68
19	Verbanol isomer	13,529	3,898	nd
20	Borneol	13.916	nd	0.83
21	4-Terpineol	14.282	nd	0.42
22	Carane, 4-5-apoxy-,trans	14.328	6,002	nd

23	Methyl thymil ether	16.350	nd	9.64
24	β-citral	16.849	24,62	nd
25	trans-geraniol	17.302	1,268	nd
26	α-citral	18.175	31,899	nd
27	Acetate bornila	18.701	nd	1.88
28	Thymol	18.945	nd	3.94
29	2-undecanone	19,121	0,513	nd
30	Carvacrol	19.378	nd	29.75
31	β-Caryophyllene	24.315	nd	7.47
32	Caryophyllene	24,355	0,512	nd
33	α-Bergamotene	24.904	nd	0,80
34	α-Humulene	25.753	nd	1.18
35	ni	27.123	nd	1.10
36	ni	27.387	nd	1.40
37	β-Bisabolene	27.942	nd	1.36
38	Eudesma-3,7(11)-diene	28.298	nd	0.58
39	caryophyllene oxide	30.711	nd	0.29

ni - not identified; no - not detected

It was observed that, at a minimum concentration of 30 µl/ml, OECC was able to promote the formation of an inhibition halo in MRSA and that the activity was dose-dependent ($p<0.05$) (Table no 2). The antimicrobial activity of the oil obtained by this chemotype has not yet been analyzed, according to records in the literature and due to the difference between chemotypes and origin of the strains, it is difficult to compare the results, highlighting the novelty and pioneering nature of the present study. Considering other chemotypes and strains of different origins, the inhibitory action of OECC against MRSA and non-multiresistant *S. aureus* strains has already been confirmed; however, none of the studies evaluated isolates from bovine milk.

Regarding the results presented by OELO, inhibition halos were formed at a concentration of 60 µl/ml ($p<0.05$), and the inhibitory effect was dose-dependent. OELO activity on MRSA strains using disk diffusion methodology was not observed in the literature consulted.

Table no 2: Growth inhibition halo measures of multidrug-resistant *S. aureus* against different concentrations of *C. citratus* and *L. origanoides*.

Oil concentration	Inhibition halo averages	
	C. citratus oil	*L. origanoides* oil
240 µl	26.42^{a}	16.18^{a}
120 µl	17.73^{b}	10.55^{b}
60 µl	6.09^{c}	2.64^{c}
30 µl	4.27^{c}	0.97^{cd}
0 µl	0.00^{d}	0.00^{d}

Different letters in the column indicate difference in the 5% Tukey test

From the dissimilarity matrix, groups were formed using the Tocher method for municipalities, farms and genotypic profile (Table 3). There is a diversity between municipalities, farms and genotypic peripheries regarding the average size of the growth inhibition halo for OECC (FIGURE 1) and OELO (FIGURE 2).

Table no 3: Identification of clusters formed by the multivariate curve clustering methodology.

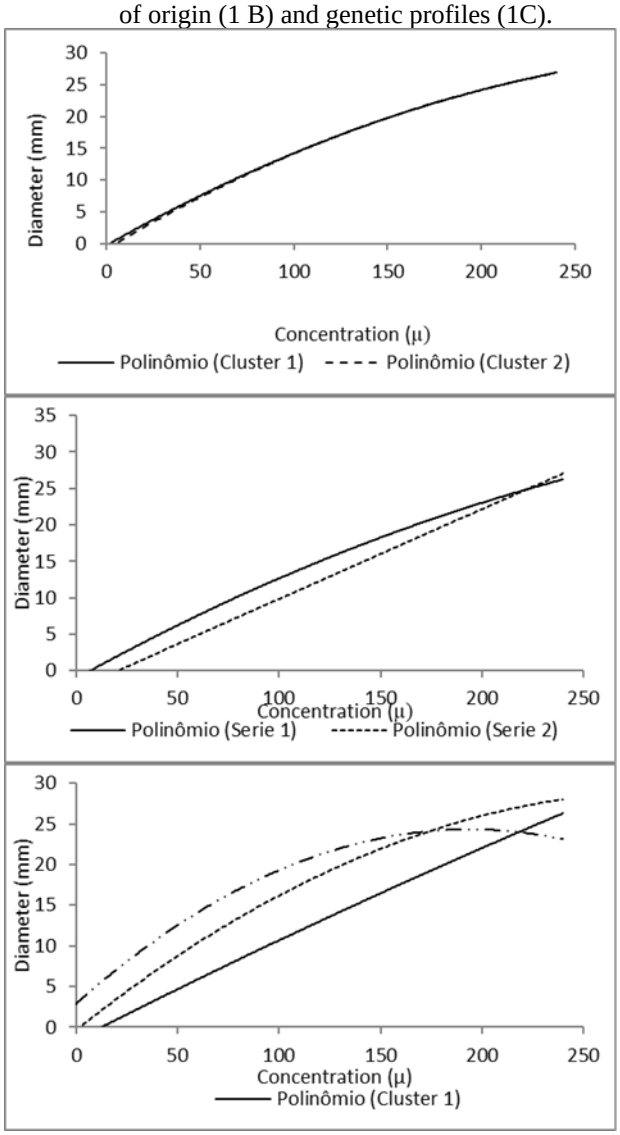
Identification of origin*	Cluster identification	
	C. citratus	*L. origanoides*
Municipalities		
Bocaiuva	1	1
Icarai_Minas	2	2
Janaúba	2	1
Porteirinha	1	1
Farms		
TR	1	1
CA1	1	1
CA2	1	1
GU	1	1
SL	3	1
VA	4	1
AG	2	2
NP	1	1
MU	2	2
Genotypic		
D	1	1
K	4	1
A	4	1

B	1	2
E	3	2
S	1	2
U	5	3
T	2	1
V	1	1
N	4	1

Municipality of Bocaiuva: farm TR and genotypic profiles D and K. Municipality of Icaraí de Minas: farms CA1, CA2, GU, SL and VA and genotypic profiles A, B, E, S and U, respectively. Municipality of Janauba: farms AG and NP and genotypic profiles V and T, respectively. Municipality of Porteirinha: Farm MU and genotypic profile N.

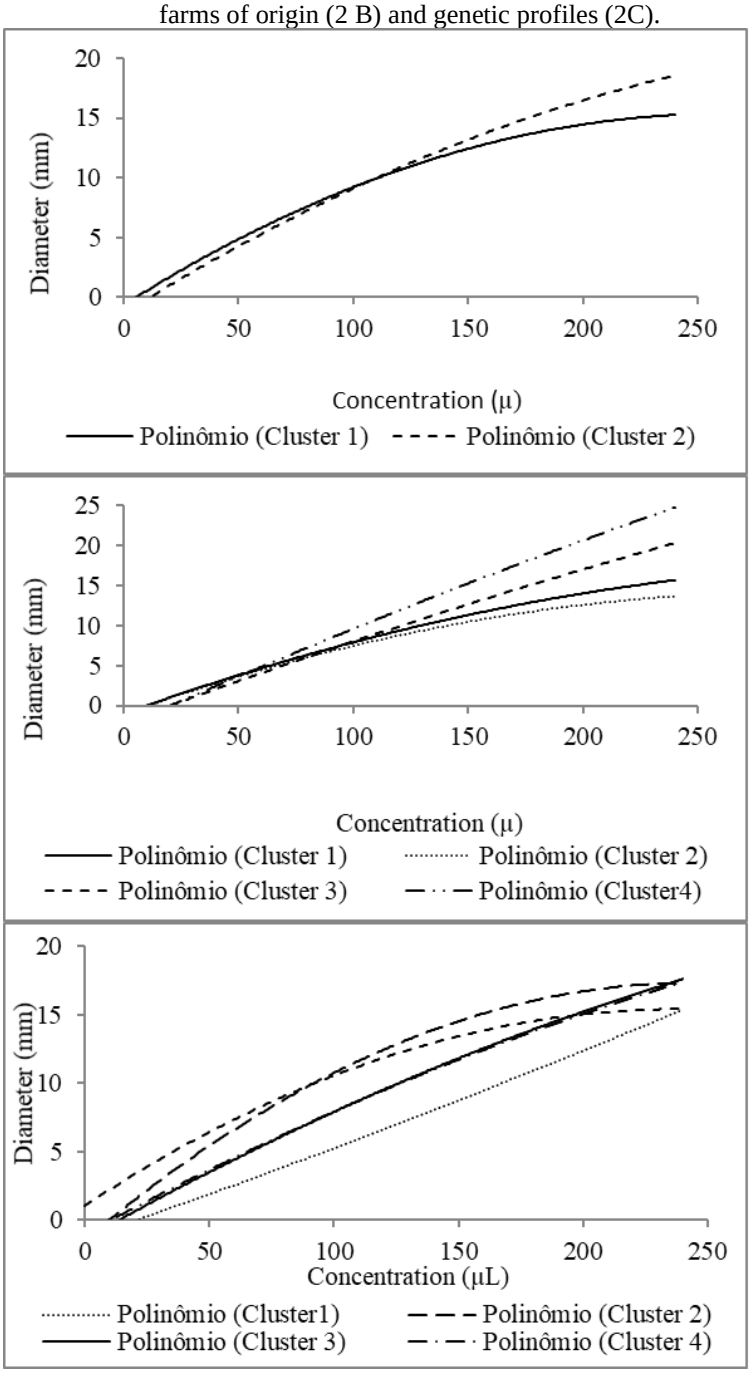
Strains from Bocaiuva and Porteirinha showed similar sensitivity to OECC considering the production of smaller average growth inhibition halos. (Figure 1A) However, Farms TR and MU, located in these municipalities, were grouped into different clusters (Figure 1B), but with similar sensitivity to oil at an estimated concentration between 180 µl ml-1 and 240 µl ml-1 and when compared to clusters 3 and 4, a behavior more distant from that observed between clusters 1 and 2 is observed. In agreement, genotypic profiles D (Farm TR) and N (Farm MU) presented smaller inhibition halos up to the concentration of 180 µl ml-1 (Figure 1C). When evaluating cluster 2 (Icaraí de Minas and Janaúba) (Figure 1 A), grouped into larger inhibition halos, this behavior refers to the Clusters of farm groupings (Figure 1B), as well as to the genotypic profiles that presented larger inhibition halos (FIGURE 1C).

Figure 1- Inhibitory effect of *C. citratus* essential oil on MRSA strains in relation to municipalities (1A), farms of origin (1 B) and genetic profiles (1C).



There was less variation in clusters for OELO when compared to OECC, although the response to the different oils was not comparable (Table 3). The municipality of Icarai de Minas was the only one grouped in cluster 2 and with larger growth inhibition halos (Figure 2A). When the farms were grouped, all those from this municipality (CA1, CA2, GU, SL and VA) were grouped in cluster 1 (Figure 2B), with larger growth inhibition halos up to estimated concentrations between 120 µl ml-1 and 180 µl ml-1, and then a sharp reduction in the size of the halos was observed, indicating that increasing the oil concentration did not promote greater growth inhibition. The genotypic profiles from these farms (A, B, E, S and U) were grouped in clusters 2 and 3, also with larger growth inhibition halos (Figure 2C).

Figure 2- Inhibitory effect of *L. origanoides* essential oil on MRSA strains in relation to municipalities (2A), farms of origin (2 B) and genetic profiles (2C).



The multivariate grouping of curves obtained from the study of growth inhibition of the strains by OECC and OELO made it possible to identify similar behaviors of different genotypes, indicating that means of inhibition halos can be grouped, which may help in the interpretation of the inhibitory effect of the oils in quantitative terms related to the size of the halos.

These same strains were studied by Xavier et al.,[6] and regarding their sensitivity to conventional antimicrobials, genotypic profile V was sensitive to all antimicrobials, and here, this was the only one grouped in cluster 5 for OECC (TABLE 3) Cluster 3 for OELO (Table no 3). Genotypic profiles D, K, U and S were resistant to Amoxicillin, Penicillin and Ampicillin, and however they were grouped in different clusters for both OECC and OELO (Table no 3).

The variation in methods used for plant cultivation and harvesting, oil supply, variation between genera and within the same bacterial species, and methodology for analyzing antimicrobial activity make it difficult to compare data, as demonstrated by several authors. In this sense, the proposal to use multivariate grouping of curves could be an important tool for this type of research.

Studies in the literature present different sizes of growth inhibitory halos for different species of microorganisms and different oils, as well as when using different methodologies. However, the results presented here refer to the same species obtained from clinical isolates tested using the same evaluation methodology.

The size of the inhibition zone for the oils does not allow us to infer that the strains are more sensitive, since standards that associate the inhibition zone measurements with inhibitory concentration and bactericidal concentration of the essential oils are not yet available, as is already the case for antimicrobials, making it difficult to interpret the results regarding a minimum effective concentration for each oil. However, it allows us to infer that there was greater inhibition of microbial growth.

The multivariate clustering methodology of the curves allowed us to infer that even with similar mean growth inhibition behaviors in the same cluster, variation is present for the two oils under study. The results observed here indicate that strains from the same municipality and farm presented different sensitivity to the same oil, as well as to the different oils. Therefore, it is suggested that essential oils are capable of causing different behaviors in bacterial strains depending on the genotypic profile and not on the origin. Studies that clarify the sensitivity of the different genotypic profiles to essential oils are necessary to establish effective control measures against MRSA and to elucidate the mechanisms of action of essential oils. Another fact that should be highlighted is that genotypic profile V, sensitive to all antimicrobials under study, presented smaller growth inhibition halos exerted by the two oils, which could be related to a more evident effect on sites of multidrug resistance to antimicrobials under study, as evidenced by previously published research.

IV. Discussion

The major compounds observed in OECC are compatible with those described in the literature describing the antimicrobial activity of this oil[16,17,18]. These compounds also indicate good oil quality. When relating the quality to the citral (3.7-dimethyl-2.6-octadienal) content of the oil, which was 56.51%, represented by what consists of a trans-isomer or geranial and a cis-isomer or neral, which are responsible due to the antimicrobial activity of the oil[19, 20,21,22,23].

The results obtained regarding the chemical composition of the oils obtained from the *C. citratus chemotypes* used in this study were similar to those obtained by several authors, but in different proportions[24,25,26,8]. Guimarães et al.,[24] and Millezi et al.,[25] observed higher citral content (69.31%; 82.03% respectively) in chemotypes from other regions of the state of Minas Gerais, and Ali et al.[26] observed lower values (34.8%) in a chemotype from Africa. Ambade and collaborators[8] used chemotypes from India and observed a citral content of 62%. In these studies, the chemical analysis was performed under similar conditions, but the oil was obtained by hydrodistillation in Clavenger, in addition to the different cultivation conditions of the plants.

Aquino et al.[13] used chemotype oil obtained from the same study area and the results regarding the major components were E-citral (43.69%) and Z-citral (34.05%), betamyrcene (15.11%), however, they performed the quantification of the essential oil components in a gas chromatograph with flame ionization detector (GC-FID). Avoshe et al.,[23] describe different chemotypes in Africa with Myrcene being the major compound. The chemical differences are attributed to biotic and abiotic factors, as well as plant collection processes, oil production and analysis of the major components[23,24,25,26,8].

In the analysis of the composition of OELO, the components are in agreement with those described in the literature, in analyses with chemotypes from the same area and the same methodology for obtaining and analyzing the oil[14,15]. Researchers describe that *L. origanoides* is classified in group VI of the verbenaceas and in a review of research in other countries they identified that p-cymene is present in greater quantities in the oils and carvacrol and thymol come in smaller quantities, confirming the difference in the chemotypes of plants of this species. In all studies, the authors discuss the influence of climatic factors on oil production, as well as the presence of the major compounds, which also explain the characteristic of the oil found in this study[27].

The quality of essential oils from different chemotypes also interferes with their biological activity, which can be evidenced in this work in the antimicrobial activity with different sizes of inhibition halo and/or different inhibitory and bactericidal concentrations of the same species.

The results obtained with MRSA strains are compatible with those described by Ambade et al.,[8] who used disk diffusion methodology and strains of different origins, isolated from skin wound secretions. An

inhibition diameter of 9.83 ± 0.28 mm was observed from a concentration of 3.25% OECC and they also observed dose-dependent behavior. Uzair and collaborators found inhibition halos of 12 ± 0.051 mm at a concentration of 125 µg ml^{-1} of OECC in MRSA obtained from clinical specimens in a hospital in Pakistan[28].

S. aureus strains using the same methodology and found satisfactory results. Ali et al.,[26] observed antibacterial activity in inhibition diameters greater than 38 mm and a dose-dependent effect. Millezi et al.,[25] observed halos of 5.0 mm from a concentration of 0.5% oil, but did not observe a dose-dependent effect.

Several authors also report results of OECC activity, but determining the minimum inhibitory concentration in tubes. Gupta et al.,[9] evaluated human clinical isolates and observed minimum inhibitory concentration ranging from 75 to 150 µg/ml^{1}. Salem observed a reduction in CFU of MRSA experimentally inoculated in beef in the presence of OECC. Ambade et al.,[8] observed minimum inhibitory concentration ranging from less than 1.5% to 12.5% of OECC concentration.

Although the activity of OELO on MRSA strains using disk diffusion methodology was not observed in the literature consulted, there are analyses using other methodologies. Authors observed the effect of OELO when associated with aminoglycosides. OELO at a concentration of 1024 µl/ml did not inhibit growth, however it considerably reduced the minimum inhibitory concentration of amikacin and neomycin, when tested in association with OELO. The antimicrobial activity of carvacrol, the major compound observed in the oil used in this study, on MRSA was described in the literature[10]. Other authors described modulation of the compound on MRSA strains isolated from the oral cavity, however they used a tube dilution test. The minimum inhibitory concentration ranged from 32 to 256 µl/ml and the minimum bactericidal concentration ranged from 64 to 512 µl/ml[29].

The effect of OELO was superior to that observed by Andrade et al.,[14] who used standard strains of *S. aureus* ATCC 25923 and essential oil from plants collected at the same location, but at different times. These authors observed dose-dependent activity and a significant inhibitory effect in the disk diffusion test at a concentration of 120 µl/ml, above that observed in this study.

Researchers observed the effect of *Salvia sclarea* EO on the expression of *mecA* genes in multidrug-resistant *Staphylococcus epidermidis*. They observed different levels of gene expression reduction in strains with different *SCC mec* types and different genetic backgrounds in *mec* and *bla* operons[30]. In addition, there are reports in the literature of 32 transcriptional alterations in *S. aureus* induced by *Melaleuca alternifolia* essential oil, related to cell membrane metabolism, among others[31].

The mechanism of action of plant derivatives is not restricted to the death of microorganisms, but also by acting on different pathways in pathogenic processes, altering the pathogenicity of microorganisms or by modulating gene transcription as described in research, mainly with an inhibitory effect on mechanisms such as efflux pumps [9,32]. Overexpression of efflux pumps may be the result of mutations in genes and/or in their expression regulators, making bacteria more resistant to antibiotic pressure, and EOs may act by affecting the expression of genes that encode efflux pumps[1,30] .

Current research seeks to clarify the action of essential oils on multidrug-resistant bacteria, and the inhibitory action of efflux pump mechanisms is described more clearly in MRSA, associating the action of oils on cell membranes and the resistance mechanism induced by the reduction of intracellular antibiotic concentration through changes in membrane permeability due to overexpression of efflux pumps, and in the case of multidrug-resistant strains, these would act on several classes of antibiotics[1,33,34]. In *S. aureus*, the pump called NorA is the predominant one and the search for blockers of this is stimulated[35,36].

V. Conclusion

Multivariate clustering of curves allowed grouping of different MRSA genotypes according to sensitivity to *C. citratus* essential oil and *L. origanoides* essential oil. The *C. citratus* essential oil and *L. origanoides* essential oil used in this study showed variation in their inhibitory effect on multiresistant *S. aureus* strains, observing the effect of genotypic profile, municipalities and farms on the inhibition of bacterial growth.

The results obtained here allow further research to evaluate the modulation of these oils on these genetically distinct isolates, as well as the modulation mechanism exerted by these oils. Further studies are needed to evaluate the efficacy and elucidate the exact mechanisms by which EOs from *L. origanoides* and *C. citratus* exhibit their antibacterial effects on genetically distinct strains.

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