










Environmental thecnology

Antibiofilm and antibacterial effects of essential oils extracted from plants on *Staphylococcus aureus*

Antibiofilme e efeitos antibacterianos de óleos essenciais extraídos de plantas sobre *Staphylococcus aureus*

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ABSTRACT

Staphylococcus aureus is a major pathogen among bacterial infections, also having the ability to produce biofilm, thus becoming tolerant and resistant to antibiotics and immune responses. Essential oil extracted from plants has shown broad-spectrum antibacterial and antibiofilm activities against bacteria. In this study it was evaluated whether rosemary, clove and cinnamon essential oil exert antibacterial and antibiofilm activities against *S. aureus* in vitro. The essential oils were purchased and their chemical composition was identified by gas chromatography and mass spectrometry (GC-MS). Antibiofilm and antibacterial effects were assessed by the 96-well microtiter plate adhesion assay. The greatest antimicrobial and antibiofilm activity were observed with 3% and 6% cinnamon essential oil, while clove and rosemary essential oils showed little or no inhibitory activity even at concentrations of 3% or 6%. These results support new antimicrobial therapies against *S. aureus* infections and biofilm formation, using cinnamon essential oil, thus contributing to both human and veterinary medicines.

Keywords: Vegetable oil; Antimicrobial; Biofilm; Antistaphylococcal

RESUMO

Staphylococcus aureus é um dos principais patógenos entre as infecções bacterianas, possuindo a capacidade de produzir biofilme, tornando-se, desta forma, tolerante e resistente aos antibióticos e às respostas imunológicas. O óleo essencial extraído de plantas tem mostrado atividades antibacterianas e antibiofilme de amplo espectro contra bactérias. Este estudo, avaliou se os óleos essenciais de alecrim, cravo-da-índia e canela exercem atividades antibacteriana e antibiofilme contra *S. aureus in vitro*. Os óleos essenciais foram adquiridos e sua composição química identificada por cromatografia gasosa e espectrometria de massa (CG-EM). Os efeitos antibiofilme e antibacteriano foram avaliados pelo ensaio de adesão em placa de microtitulação de 96 poços. As maiores atividades antimicrobiana e antibiofilme foram observadas com o óleo essencial de canela 3% e 6%, enquanto o óleo essencial de cravo e alecrim apresentou pouca ou nenhuma atividade inibitória, mesmo nas concentrações de 3% ou 6%. Esses resultados suportam novas terapias antimicrobianas contra infecções por *S. aureus* e formação de biofilme, utilizando óleo essencial de canela, contribuindo assim para medicamentos humanos e veterinários.

Palavras-chave: Óleo vegetal; Antimicrobiano; Biofilme; Antiestafilococos

1 INTRODUCTION

Bacterial infections represent, worldwide, an important public health problem, with *Staphylococcus aureus* standing out as the main pathogen (SANTOS *et al.* 2018). This microorganism is the main cause of mastitis in animals, being one of the biggest problems in dairy production, as it alters milk composition and increases the somatic cell count (LANGONI *et al.* 2017, PEIXOTO *et al.* 2015). In addition, strains of *S. aureus* can produce toxins in milk and milk products causing outbreaks of food poisoning (ACOSTA *et al.* 2017).

This species also has the capacity to produce biofilm (Peixoto *et al.* 2015). Biofilms are formed by microorganisms attached to a solid surface and are surrounded by an extracellular matrix of polysaccharides and proteins (SOUSA *et al.*, 2017). Under the protection of the biofilm, microbial cells become tolerant and resistant to antibiotics and immune responses, making the clinical treatment of infections difficult (WU *et al.* 2015).

In this condition, microorganisms are also resistant to the action of disinfectants and other adverse conditions, impairing the disinfection of medical instruments or equipment used in the food industry, thus increasing the possibility of cross infections (GENZ *et al.* 2017, PEIXOTO *et al.* 2015). Importantly,

studies have demonstrated the presence of biofilms both on abiotic surfaces, such as dental materials, prostheses, implants, endotracheal tubes, pacemakers and catheters; and on biotic surface, such as host tissues (CHAGAS *et al.* 2015, DAMASCENA *et al.* 2017, PEIXOTO *et al.* 2015, SOUSA *et al.* 2017, WU *et al.* 2015).

Natural products extracted from plants have been used by researchers with the objective of finding new active ingredients with diverse therapeutic properties. However, despite the use of several of these compounds being consolidated in human pharmacology and even introduced in the Unified Health System of Brazil, in veterinary medicine there is still little use of medicines formulated from plants (SANTOS *et al.* 2011).

Essential oils extracted from plants have been the subject of studies (ALMEIDA *et al.* 2013, ALMEIDA *et al.* 2016, ARAUJO & LONGO 2016, CASTRO *et al.* 2016), they constitute complex mixtures of volatile lipophilic substances in plant organs and are related to several functions necessary for plant survival, including defense against microorganisms (BAKKALI *et al.* 2008). Freire *et al.* (2014) shows that the essential oils of exotic basil (*Ocimum basilicum*), white thyme (*Thymus vulgaris*), and china cinnamon (*Cinnamomum cassia*), have antimicrobial activity on bacterial strains of *Streptococcus mutans* and *Staphylococcus aureus*. In addition, an antibiofilm activity of a number of essential oils have been demonstrated which can provide new alternatives for the control of biofilms (ALIBI *et al.* 2020, BUDRI *et al.* 2015, JARDAK *et al.* 2017).

Therefore, the infections caused by *S. aureus*, as well as the resistance of biofilms to antimicrobials, make this bacterium one of the main causes of healthcare-associated infections (HAIs), hence impacting the public health (BERNARDO *et al.* 2018, CARVALHO *et al.* 2017, CHAGAS *et al.* 2015, SOUSA *et al.* 2017). Thus, it is relevant the studies that seek to evaluate the capacity of biofilm production by this pathogen, as well as the use of natural products as alternatives

for the treatment of biofilm infections and new strategies aiming to control the biofilm formation on the surfaces of equipment and utensils.

The present work evaluated, *in vitro*, the capacity of biofilm formation by *S. aureus*, as well as the antibacterial and antibiofilm activity of the essential oils of rosemary (*Rosmarinus officinalis*), cinnamon (*Cinnamomum zeylanicum*) and clove (*Eugenia caryophyllus*).

2 MATERIALS AND METHODS

2.1 Obtaining essential oil

The essential oils were purchased from the Terra Flor Aromaterapia© online store (<http://terra-flor.com>) and are described in Table I. Terra Flor Aromaterapia© has Natural Ingredients certification from IBD and from SisOrg (Seal of the Brazilian Organic Conformity Assessment System) which certifies that the essential oils produced have their raw material grown without the use of pesticides, as well as their processing does not take any type of chemical additive. Oils were obtained as follow: *Rosmarinus officinalis* by steam distillation of the flowering plant; *Cinnamomum zeylanicum* by steam distillation of leaves and *Eugenia caryophyllus* by steam distillation of dry buds.

Table I - Essential oil information described by the supplier

Scientific name	Commercial name	Origin	Extraction	Composition
<i>Rosmarinus officinalis</i>	Rosemary - camphor	Spain	Steam distillation of flowering branches	<i>Rosmarinus officinalis</i> leaf oil
<i>Eugenia caryophyllus</i>	Clove - bud	Indonesia	Steam distillation of dry buds	<i>Eugenia caryophyllus</i> bud oil
<i>Cinnamomum zeylanicum</i>	Ceylon cinnamon - bark	India	Steam distillation of the bark	<i>Cinnamomum zeylanicum</i> bark oil

Source: Data obtained from the website <http://terra-flor.com>

2.2 Characterization of the compounds of essential oils

Gas chromatography with flame ionization detection (GC-FID) and coupled to mass spectrometry (GC-MS) were performed to identify the compounds. The GC-MS analysis of the oils was performed on a Shimadzu QP-2010 Ultra. The GC was equipped with a NST-01 column (30 m x 0.25 mm i.d., film thickness 0.25 µm, from NanoSeparation Technology). Helium was used as carrier gas at flow rate of 1.20 mL/min. The oven program started with an initial temperature of 50°C held for 5 min and then the oven temperature was heated at 8 °C/min to 280°C and finally held isothermally for 5 min. For GC-MS detection, an electron ionization system, with ionization energy of 70 eV was used. The characterization of the compounds was done by analyzing their fragmentation patterns in the mass spectra. The major substances of the oil were determined by analyzing their fragmentation profiles and comparing them with reference libraries (WILEY229, NIST147 e NIST27).

The GC-FID analysis of the oils was performed on a Shimadzu QP-2010 Ultra with flame ionization detection FID-2010 Plus. The GC was equipped with an NST-05 column (30 m x 0.25 mm i.d., film thickness 0.25 µm, from NanoSeparation Technology). Hydrogen was used as carrier gas at flow rate of 1.20 mL/min. The oven program started with an initial temperature of 50 °C held for 5 min and then the oven temperature was heated at 8 °C/min to 150 °C and finally heated at 20 °C/min to 280 °C. Retention indexes were calculated from the equation developed by Kováts (KOVÁTS, 1958) and modified by Vandendool & Kratz (1963). A solution of alkanes from C7 to C30 was used as a standard for the calculations. The values obtained were compared with the values available in the database of the National Institute of Standard Technology (NIST). The standards used in the analysis of the oils were cinnamaldehyde (Sigma-Aldrich, purity of 99%), for cinnamon essential oil, camphor (Sigma-Aldrich, purity of 98%), for rosemary essential oil and β-caryophyllene (Supelco/Sigma-Aldrich purity of 98%), for clove essential oil.

To quantify the constituents, methyleugenol (Supelco/Sigma-Aldrich purity of 98%), was used as standard in concentrations 0.1, 0.2, 0.5, 1.0 and 2.0 mg/mL in hexane. A 10.0 mg/mL stock solution was initially prepared by solubilizing 10 µL of methyleugenol in 990 µL of HPLC grade hexane, with diluted solutions being prepared from it. Component concentrations in essential oils were established using methyleugenol as an internal standard. The standard and the essential oil were co-jetted in a 0.5 mg/mL solution of methyl eugenol and 1 mg/mL of essential oil in hexane. The combination of analysis of fragmentation profiles, library information and retention index confirmed the structures of the compounds.

2.3 Microbiological culture

Sample of *Staphylococcus aureus subsp. aureus* ATCC® 25923™ (kindly provided by E. Rodrigues da Silva, UFAPE, Brazil) were applied to Brain Heart Infusion (BHI) broth and incubated at 37°C for 24 h and then plated on Müller Hinton agar to obtain isolated colonies. The adjustment of the bacterial inoculum was carried out through a dilution of the colonies in sterile 0.9% sodium chloride. Two hundred µL of this dilution were added to a 96-well flat-bottom plate and its optical density (OD) at 600 nm absorbance was determined using a microplate reader (Labtech LT-4000). The inoculum was considered adjusted when showing OD between 0.145 and 0.150 (~ 4 × 10⁷ cells/mL).

2.4 Biofilm production

To assess the potential for biofilm formation, it was used the 96-well plate adhesion assay proposed by Guimarães *et al.* (2012) with modifications. Müller Hinton broth (MH), MH broth with 1% glucose (MHG), BHI broth, BHI broth with 1% glucose (BHIG), tryptic soy broth (TSB), and TSB broth with 1% Glucose (TSBG) were employed to determine the best medium for bacterial growth and biofilm formation. After several experiments conducted to standardize the assay, the final

analysis of biofilm formation by *S. aureus* was performed once in triplicates, using 160 µL of the culture medium, 20 µL of saline and 20 µL of the adjusted inoculum. Then, an initial reading of the OD was performed at 600 nm and again after incubation at 37°C for 24 hours. For the biofilm biomass quantification, the crystal violet staining method was used according to the methodology described by Xu *et al.* (2016). Average OD readings at a wavelength of 570 nm of three different wells for each isolate (ODi) and the control (ODc) were performed. The isolates were classified as non-biofilm producers when $ODi \leq ODc$; weak biofilm producers when $ODc < ODi \leq 2 \times ODc$; moderate biofilm producers when $2 \times ODc < ODi \leq 4 \times ODc$ and strong biofilm producers when $4 \times ODc < ODi$ (Lima *et al.* 2017).

2.5 Antibiofilm and antibacterial activity

The antibiofilm and antibacterial activity of essential oils was assessed by the 96-well plate adhesion assay (GUIMARÃES *et al.* 2012) with modifications. The experiment was carried out with the two-culture media that provided the best biofilm formation by *S. aureus*. The essential oils were tested in concentrations of 3% and 6% (v/v). They were diluted in distilled water and 5% (v/v) dimethyl sulfoxide (DMSO) solution. All solutions were sterilized using a 0.22 µm milipore filter. In 96-well flat-bottom plates, 160 µL of culture medium, 20 µL of bacterial suspension and 20 µL of essential oil were added. The samples were tested once in triplicate and their initial OD reading was performed at 600 nm, then the plate was incubated at 37°C for 24 h, followed by a new reading of the OD and the quantification of the biofilm (LIMA *et al.* 2017, XU *et al.* 2016).

2.6 Statistical analysis

The Kruskal-Wallis (KW) test was initially used to verify whether there was a statistically significant difference ($p < 0.05$) in the biofilm formation by *S. aureus*

among the six-culture media described above. This test is a non-parametric alternative used to compare groups equivalent to the F test associated with ANOVA with a factor when the assumptions of normality and homogeneity of variances are not satisfied. Subsequently, the KW test was performed to independently compare the antimicrobial and antibiofilm activity of essential oils on *S. aureus* growing in BHIG and TSBG media. All statistical analyzes were performed using the R Development Core Team software (2020).

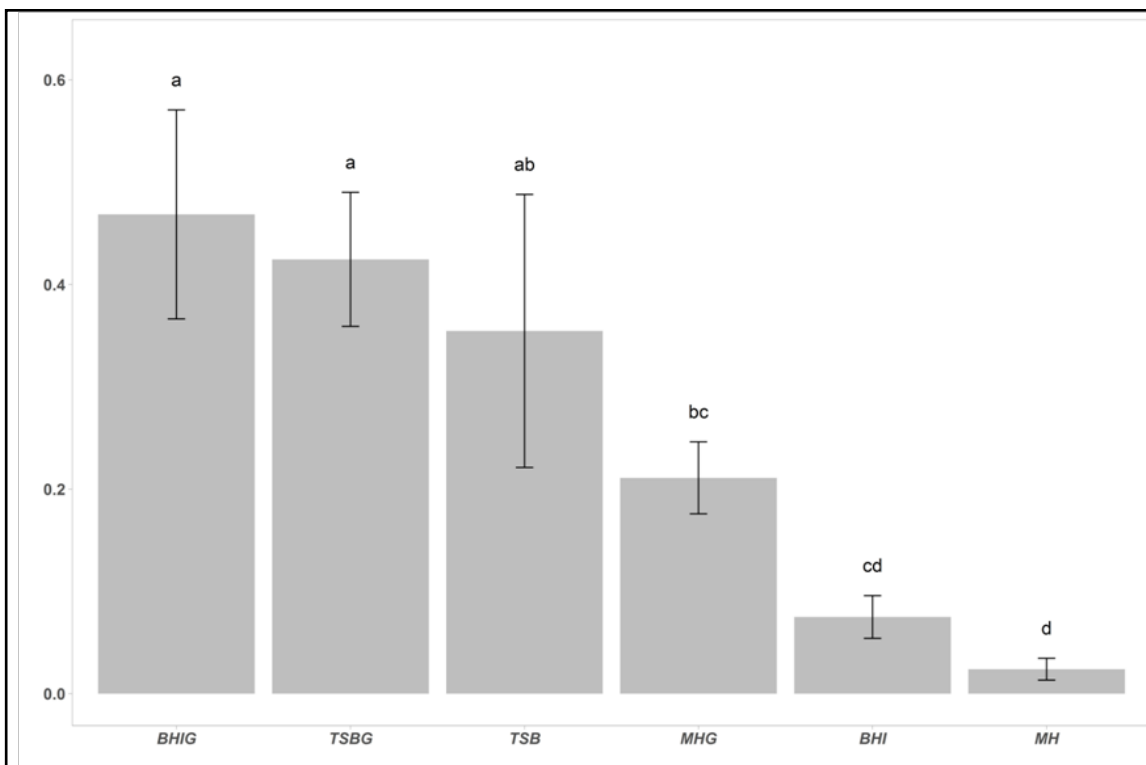
3 RESULTS AND DISCUSSION

The formation of biofilm by *S. aureus* in the different culture media was observed and the Kruskal-Wallis test statistic value was 18,201 (p-value of 0.002705), demonstrating that there is a significant statistical difference between the media used (Figure 1). According to the classification proposed by Lima *et al.* (2017), which takes into account the reading of the OD of each isolate in relation to the OD of the negative control, *S. aureus* in our study was classified as a weak biofilm producer in MH and BHI broths, moderate producer of biofilm in MHG and TSB broths, and strong producer of biofilm in BHIG and TSBG broths.

This way, it is demonstrated here that the capacity of biofilm formation by *S. aureus* is influenced by the type of medium in which the bacteria develop, and that the addition of glucose favored a greater production of biofilm. According to Stepanovic *et al.* (2007) the composition of the culture medium is probably the main factor that influences the ability of bacteria to produce biofilm. Marques *et al.* (2017) verified an increase in biofilm production by *S. aureus* with the addition of 0,24% glucose to the TSB broth while Cassenego *et al.* (2013) demonstrated that the addition of glucose to the culture medium increased the levels of *Enterococcus faecalis* classifying them as strong biofilm producers. Peixoto *et al.* (2015), using the plate adhesion method, determined that all the strains of *Staphylococcus spp* evaluated in their study were strong biofilm-forming. Friedriczewski¹ *et al.* (2018)

evaluated the biofilm formation by 20 strains of *S. aureus* isolated from mozzarella cheese made with buffalo milk, classifying two isolates as strong biofilm-forming, seven as moderate, ten as weak, and one non-formed biofilm. The production of biofilm by this and other species of bacteria increases their resistance to antimicrobials, making their eradication difficult. Chagas *et al.* (2015) demonstrated that *S. aureus* presented a greater resistance against cephalixin, amoxicillin and rifampicin when forming biofilm.

Figure 1 – Biofilm formation of by *S. aureus* in different culture media. Post hoc tests to identify which of the pairs of groups differ. Culture media indicated with the same letter do not differ statistically. Mueller Hinton broth (MH), MH broth with 1% glucose (MHG), BHI broth, BHI broth with 1% glucose (BHIG), Soy Trypticasein broth (TSB), and TSB broth with 1% glucose (TSBG)



The evaluation of the antimicrobial and antibiofilm activities of essential oils of rosemary, cinnamon and cloves in concentrations of 6% and 3% were made using BHIG and TSBG broths where *S. aureus* presented itself as a strong biofilm

producer. In BHIG broth, the statistical analysis of bacterial growth inhibition showed a KW test statistic value of 18.701 (p-value of 0.004699), while for antibiofilm activity the test statistic value was 19.164 (p-value of 0.003896); revealing a statistically significant difference among the oils used.

The greatest antimicrobial and also antibiofilm activity were observed with the cinnamon essential oil, with no statistical difference between the two concentrations tested whereas the use of clove essential oil, in both concentrations, stimulated both bacterial growth and biofilm formation. Rosemary oil at 3% demonstrated better antimicrobial effect than at 6%, while the reverse was observed in its antibiofilm activity (Figure 2). In this study, *S. aureus* was classified as a weak biofilm-forming in the presence of cinnamon oil at 3 and 6%, moderate biofilm-forming in rosemary oil at 6% and strong biofilm-forming in both rosemary oil at 3% and clove oil at 3 and 6%.

In the TSBG broth assay, the KW test statistic value showed a value of 18.494 (p-value of 0.00511) for antibiofilm activity and 15.924 (p-value of 0.01417) for antimicrobial activity, indicating significant statistical difference. The highest antimicrobial and antibiofilm activity were observed with cinnamon essential oil, with no statistical difference between the concentrations tested (Figure 3). Rosemary oil did not show antimicrobial and antibiofilm activities. The essential oil of clove, despite having antimicrobial action, it potentiated the formation of biofilm, demonstrating, in this case, that there was no direct correlation between the number of microorganisms in the medium and the amount of biofilm produced by them. In this TSBG trial *S. aureus* was revealed as a weak biofilm producer under the action of 3% and 6% cinnamon essential oil and a strong biofilm producer in the presence of essential clove oils and rosemary both at 3% and 6%. The use of DMSO was effective for solubilizing essential oils in water. DMSO by itself was used in the assays as a control to verify its interference with bacterial growth, showing no effects over the bacterial growth, biofilm formation or antibiofilm activity of the oils, in all tests performed.

Figure 2 – Antimicrobial (a) and antibiofilm (b) activity of essential oils on *S. aureus* in BHI medium with 1% glucose added. Post hoc tests to identify which of the pairs of groups differ. Essential oils (or concentrations) indicated with the same letter do not differ statistically. ROS3% (3% Rosemary), GC (growth control), ROS6% (6% Rosemary), CLO3% (3% Clove), CLO6% (6% Clove), CIN3% (3% Cinnamon), CIN6% (6% Cinnamon)

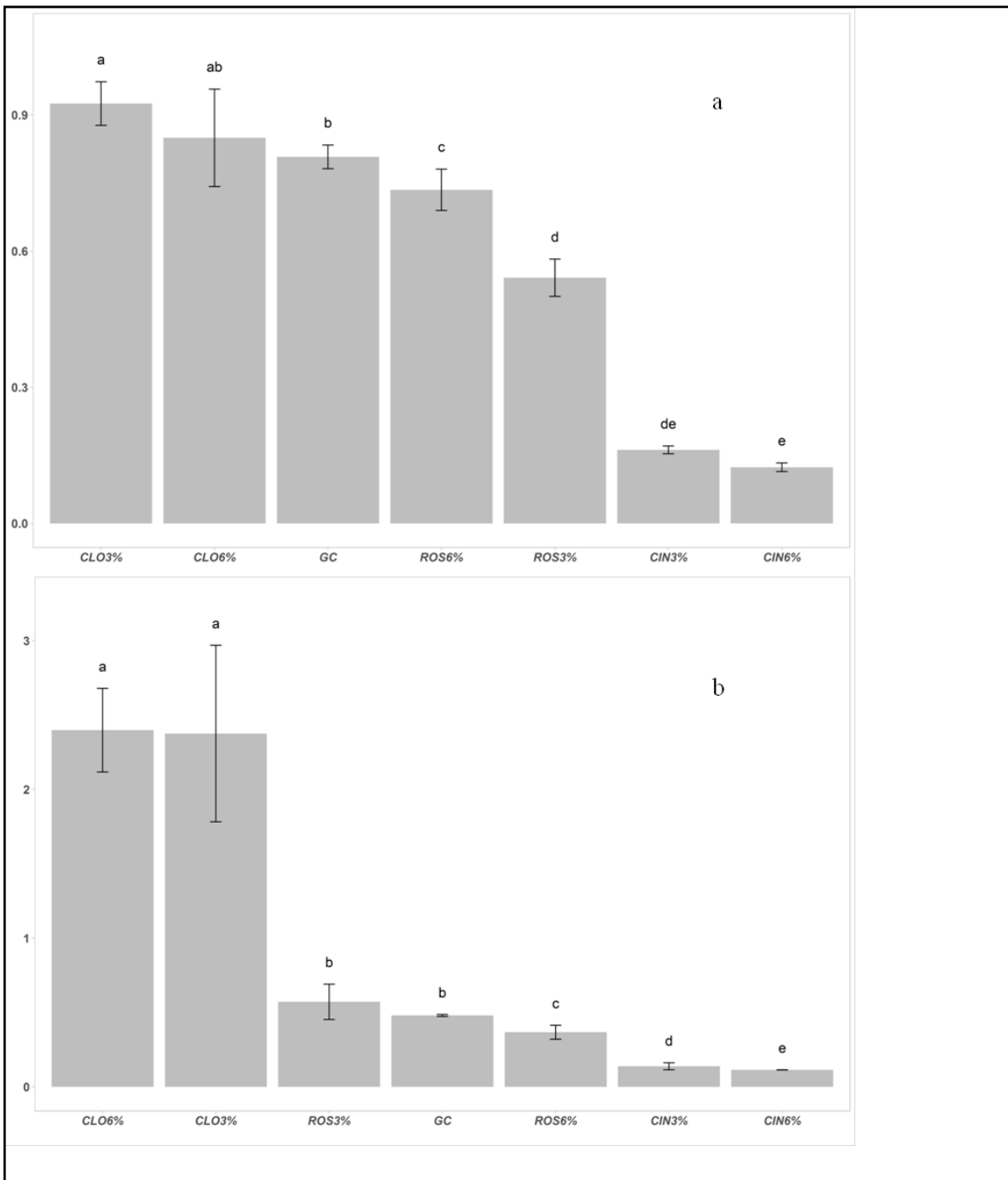
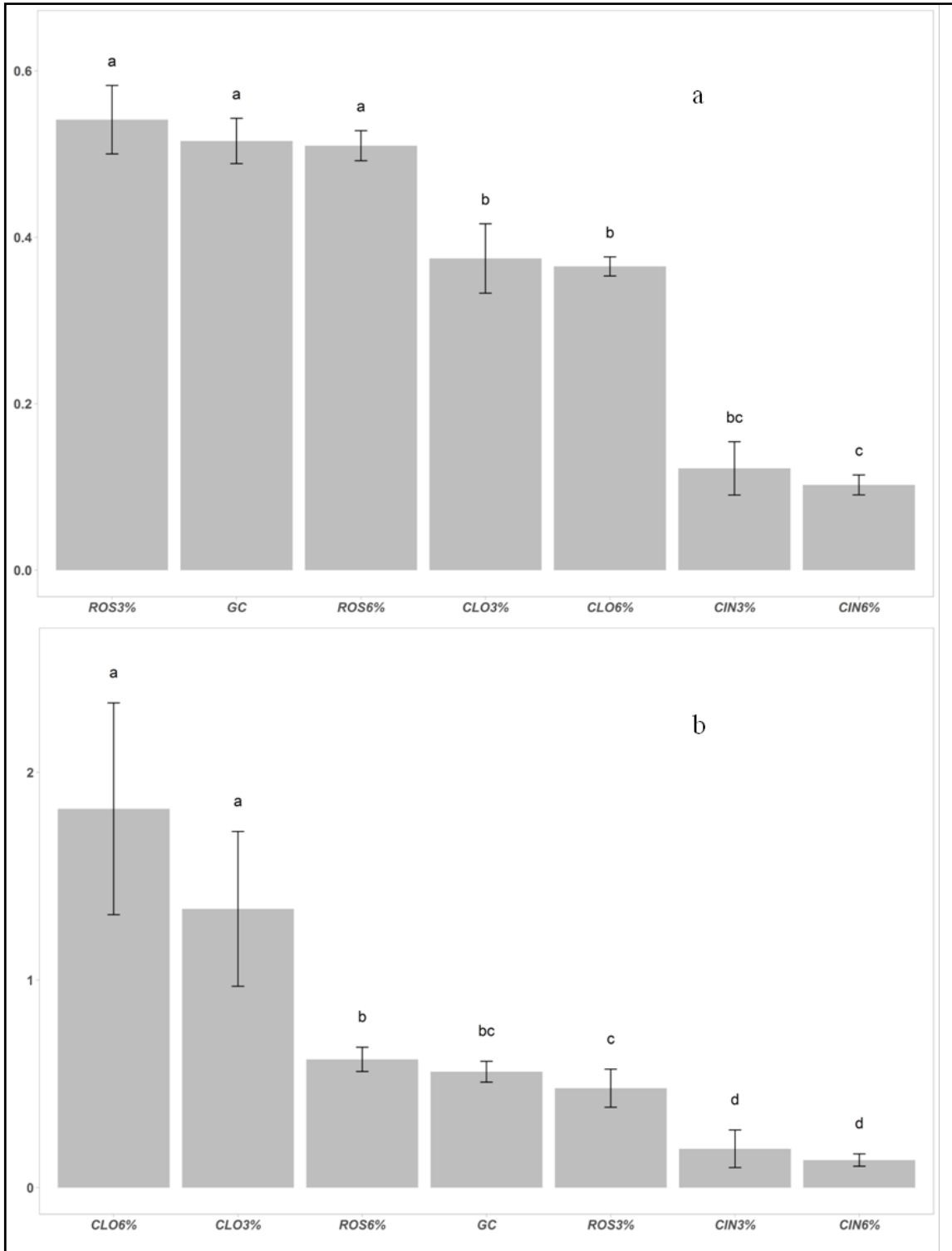


Figure 3 – Antimicrobial (a) and antibiofilm (b) activity of essential oils on *S. aureus* in TSB medium with 1% glucose added. Post hoc tests to identify which of the pairs of groups differ. Essential oils (or concentrations) indicated with the same letter do not differ statistically. ROS3% (3% Rosemary), GC (growth control), ROS6% (6% Rosemary), CLO3% (3% Clove), CLO6% (6% Clove), CIN3% (3% Cinnamon), CIN6% (6% Cinnamon)



Antimicrobial effects of plant originated products have been demonstrated. Almeida *et al.* (2013) determined the antimicrobial activity of essential oil of lemongrass (*Cymbopogon citratus*) on strains of *Staphylococcus* spp, *Streptococcus mutans* and *Candida* spp in both planktonic growth and in biofilm. Castro *et al.* (2014) described the antibacterial activity of essential oil and ethanolic extract of colony leaves (*Alpinia zerumbet*) on strains of *S. aureus* isolated from cows with subclinical mastitis. Mota *et al.* (2018) observed that the essential oils of cinnamon, pepper rosemary and mint showed high antibacterial potential against *Mycobacterium tuberculosis*. In the same way, other studies reveal essential oils as new alternatives to inhibit biofilm formation. Millezi *et al.* (2019) observed that the essential oil of *Cinnamomum zeylanicum* was effective in preventing the formation of biofilm by *S. aureus*. Budri *et al.* (2015) demonstrated that the essential oils of cloves and cinnamon caused a reduction in biofilm production by *S. aureus* isolated from milk of cows with subclinical mastitis, in polystyrene and stainless steel. In our study, however, essential oils of cloves and rosemary, at 3%, increased the formation of biofilm by *S. aureus*. Kwieciński *et al.* (2009) states that low concentrations of various essential oils can enhance the bacterial metabolic activity toward to the production of biofilm due to the environmental stress caused by the essential oil.

Table II shows the retention times and retention indices (KI) for the alkane standards used and table III shows the metabolite patterns present in essential oils. In order to quantify the compounds, present in essential oils, a calibration curve was constructed using five different concentrations of methyl eugenol, as shown in Table IV, obtaining the equation of the line $y = 1.77E + 06x - 2.62 E + 04$, $R^2 = 0.9968$.

Table II – Retention times of alkane standards and their respective retention rates (NST 05 Column)

Carbon Number	Retention Time	KI (standard)
7	2.310	700
8	3.970	800
9	7.147	900
10	10.130	1000
11	12.655	1100
12	14.858	1200
13	16.841	1300
14	18.536	1400
15	19.770	1500
16	20.754	1600
17	21.578	1700
18	22.297	1800
19	22.942	1900
20	23.532	2000
21	24.081	2100
22	24.599	2200
23	25.090	2300
24	25.591	2400
25	26.116	2500
26	26.685	2600
27	27.314	2700
28	28.031	2800
29	28.863	2900
30	29.843	3000

Table III – Retention times and indexes of the standards used (NST 05 Column)

Standards	Application	Retention Time	KI (NIST)	KI (found)
Camphor	Rosemary	13.595	1143	1143
Cinamaldehyde	Cinnamon	16.190	1268	1270
β -caryophyllene	Clave	18.924	1433	1431
Methyleugenol	Quantification	18.528	1401	1400

Table IV – Area values under the curve applied and corresponding concentrations, using methylphenol as standard

Concentration (mg / mL)	Area	Area	Area	Mean	SD
0.1	154949.7	193859	191699.4	180169.4	17854.78
0.2	333756.6	277608.5	321237.3	310867.5	24066.61
0.5	822430.2	741196,8	722252.1	761959.7	43452.94
1	1830889	1750522	1982535	1854648	96197.26
2	3651895	3298595	3466938	3472476	144287.5

The GC-MS analysis of the chemical composition of essential oils, as well as their concentrations calculated by co-injection with methyl eugenol is shown in Table V.

Table V – Chemical constituents detected in essential oils by GC-MS

Species	Peak	Chemical Compound ^a	Retention time (min.) ^b	RI (sample) ^c	RI (literature) ^d	Concentration (mg/mL of oil) ^e	Relative Area (%)
<i>Rosmarinus officinalis</i>	2	<i>Camphor</i>	13.569	1141	1143	64.06	74.06
	3	<i>Borneol</i>	14.068	1164	1165	100.75	11.65
	4	<i>4-Terpineol</i>	14.326	1176	1177	157.50	1.82
	5	<i>α-Terpineol</i>	14.598	1188	1189	42.89	4.96
	6	<i>Bicyclo [3.1.1] 4,6,6-trimethyl-hept-3-en-2-one</i>	14.965	1205	1204	44.28	5.12
	7	<i>Bornyl acetate</i>	16.545	1285	1285	0.91	0.10
	8	<i>trans-Caryophyllene</i>	18.914	1431	1433	1.01	0.12
	<i>Eugenia caryophyllus</i>	1	<i>Eugenol</i>	17.871	1359	1358	1862.97
3		<i>Copaene</i>	18.227	1382	1380	6.31	0.25
4		<i>trans-Caryophyllene</i>	18.926	1432	1433	518.70	20.28
6		<i>Eugenol acetate</i>	19.996	1523	1525	169.12	6.61
<i>Cinnamomum zeylanicum</i>	2	<i>2-Methoxybenzaldehyde</i>	15.685	1242	1242	1.94	0.33
	3	<i>trans-Cinnamaldehyde</i>	16.155	1268	1265	501.47	85.96
	5	<i>Copaene</i>	18.246	1383	1380	1.01	0.20
	7	<i>Cinnamyl acetate</i>	19.051	1440	1442	22.40	3.84
	8	<i>2-Methoxycinnamaldehyde</i>	20.049	f	1528	57.55	9.86

Compounds identified based on their mass spectra, comparison with libraries and retention indices

^b Retention times in column NST 05 (GC-FID)

^c NST 05 column retention indices, calculated from a series of linear alkane patterns from 7 to 30 carbons

^d Retention indices described in the literature for NST 05 column compounds or equivalent

^e Concentration calculated using methylphenol as an internal standard, with calibration curve

The essential oil of rosemary presented as major components the monoterpenes camphor (74%) and borneol (12%). A study with the oil of this plant grown in Tunisia showed that the main constituents were 1,8-cineole (24%), camphene (13%), camphor (13%) and β -pinene (12%), being this oil capable of inhibit the biofilm formation of *Staphylococcus epidermidis* by 57% at a concentration of 25 $\mu\text{L}/\text{mL}$ and to disrupt 68% of the preformed biofilm at a dose of 50 $\mu\text{L}/\text{mL}$ (JARDAK *et al.* 2017). Vieira *et al.* (2017) used rosemary essential oil extracted from plants cultivated in Portugal, showing as its main compounds β -myrcene (36%), 1,8-cineole (12%) e camphor (17%). This oil was able to inhibit biofilm formation by *S. aureus* and *Pseudomonas aeruginosa* at a dose of 10 mg/mL, and presented a minimum inhibitory concentration (MIC) value of 20 mg/mL against *S. aureus*. Borneol has been described as capable of increasing the penetration of molecules into cellular structures via the formation of pores in lipid membranes (Wang *et al.*, 2017), and its use has been proposed in the production of materials with antibacterial properties, such as polymers that prevent microbial adhesion (LUO *et al.*, 2014) and fabrics with antibacterial action (YANG *et al.*, 2020). Camphor, in turn, has shown potential for antimicrobial application in the form of camphorimine complexed with silver ions (COSTA *et al.*, 2021), as an adjuvant by increasing the antimicrobial activity of an essential oil by synergism (KARACA *et al.*, 2020) or even in the synthesis of antimicrobial compounds with a better activity profile, by conferring an increase in the lipophilicity of the new compounds in relation to the original substances (PERAMAN *et al.*, 2018).

It has previously been shown that the chemical composition of rosemary essential oil varies according to geographic location (LOUASTÉ *et al.* 2019), genetic trait (LI *et al.* 2016), extraction process (GRABER *et al.* 2010) and sample preparation (ZHELJAZKOV *et al.* 2015), thus explaining the difference in chemical composition between the oil in the present work and the others cited. However, despite the oil's chemical variability, the presence of camphor is a constant, and in all oils being the monoterpenes are the major constituents.

There is some uncertainty regarding the scientific name of the clove, and it is possible to find studies with at least four scientific names for the same species: *Eugenia caryophyllus*, *E. caryophyllata*, *Caryophyllus aromaticus* and *Syzygium aromaticum*, the latter being more common (ITIS, 2020). The GC-MS analysis of the clove essential oil obtained from the plant specified in this study as *E. caryophyllus*, identified as major components the phenylpropanoid eugenol (73%) and the sesquiterpene *trans*-caryophyllene (20%). Mayaud *et al.* (2008) by using a clove essential oil made available via a Belgian company, reported eugenol (76%) and eugenyl acetate (12%), as its major composition. This oil presented a MIC value of 0.6 % (v/v) against *S. aureus*.

Working with the essential oil of Moroccan origin, obtained from the plant identified as *E. caryophyllata*, it was described that it was mainly composed of eugenol (79%) and β -caryophyllene (16%), presenting a MIC value of 0.6 μ L/mL against *Streptococcus oralis* (HSAINE *et al.* 2017). Alibi *et al.* (2020) demonstrated that essential oil extracted from *E. caryophyllata* was able to produce inhibition zones diameter greater than 20 mm when administered in a dose of 10 μ L and reductions above 90% in biofilm formation against clinical isolates of antimicrobial resistant *S. aureus*. Eugenol is widely recognized as an antimicrobial and inhibitor of biofilm formation when used as the pure compound (ASHRAFUDOULLA *et al.*, 2020; CAZELLI *et al.*, 2017; MARCHESE *et al.*, 2017) or functionalized as in the case of eugenol Fe₂O₃ magnetite which showed greater biofilm inhibition efficacy than pure eugenol (BRUCKMANN *et al.*, 2022). Its antimicrobial activity is related in part to the ability to cause membrane damage (Walsh *et al.*, 2003) and also with the advantage that, even in situations of repetitive exposure, it does not induce an antimicrobial resistance response (APOLONIO *et al.*, 2014). Beta-caryophyllene also had its antimicrobial activity demonstrated, being able to kill bacteria and fungi (SELESTINO NETA *et al.*, 2017; SCHMIDT *et al.*, 2010).

Syzygium aromaticum oil (90% eugenol) was shown to reduce infestation and biofilm formation by *S. aureus* isolated from bovine mastitis, presenting a MIC value of 0.237 mg/mL and reducing the formation of biofilm in polystyrene by 69% and in

stainless steel by 64%, both at the dose of 0.01% (BUDRI *et al.* 2015). A study with the oil of Hungarian origin (89% eugenol) showed a MIC value of 0.1 mg/mL against methicillin-resistant *Staphylococcus aureus* (MRSA) (ÁCS *et al.* 2016). The essential oil of Indian origin obtained by extraction in supercritical fluid showed as main components eugenol (46%), α -phellandrene (12%), o-cymene (12%), γ -terpinene (15%) and cumic aldehyde (26 %), being able to inhibit biofilm formation by *S. aureus* (WALMIKI & RAI 2017). Razafimamonjison *et al.* (2013) described that the composition of this oil varies according to the maturation stage of the bud, with the mature bud rich in eugenol (95%) and low in eugenyl acetate (2%) and the immature one richer in eugenyl acetate (56 %) than in eugenol (40%). Despite differences in chemical composition, eugenol is a common constituent in different studies, being implicated with antimicrobial/antibiofilm activity (YADAV *et al.* 2013).

The analysis by GC-MS in our study showed that the essential oil of cinnamon had as major compound cinnamaldehyde (86%). In work using the bark of the plant commercially available in the state of São Paulo, the researchers identified cinameldehyde (86%) as the main compound, with the essential oil having a MIC_{90%} value of 0.25 mg/mL against *S. aureus*, both resistant and sensitive to methicillin (Barbosa *et al.* 2015). The essential oil of Moroccan origin showed mainly cinnamaldehyde (74%) and exhibited an MIC value of 1.25 μ L/mL against *S. oralis* (HSAINE *et al.* 2017). Budri *et al.* (2015) using essential oil composed mainly of cinnamaldehyde (86%) demonstrated a MIC value of 0.243 mg/mL (0.199 mg/mL for isolated cinnamaldehyde) against *S. aureus* and ability to reduce biofilm formation in polystyrene and stainless steel by 75% and 45%, respectively, at the dose of 0.01% (69% and 45% for cinnamaldehyde isolated at the same dosage). Although we have not found studies on factors that affect the chemical composition of the essential oil of *C. zeylanicum*, we can assume that its chemical composition suffers the influences already described for the other essential oils. Moreover, it was observed that the presence of cinnamaldehyde is a constant among the different origins of the oil, which may be related to the biological activity presented. The antimicrobial activity of

cinnamaldehyde has been demonstrated in several studies (BURT, 2004; CHANG *et al.*, 2001; OOI *et al.*, 2006), being this compound capable of interfering with the fatty acid composition of the microbial cell membrane (DI PASQUA *et al.*, 2007).

3 CONCLUSIONS

The present study showed that there was a significant difference in the formation of biofilm by *S. aureus* in the different culture media, and that the addition of glucose to the medium increased the production of biofilm by *S. aureus*, which was classified as a strong biofilm-forming in BHI and TSB broths both added with 1% glucose. The essential oil of cinnamon showed antimicrobial and antibiofilm activity while the essential oil of clove and rosemary did not present satisfactory results. The analysis of the composition of the oils using GC-MS demonstrated that the predominant compounds in the essential oils were camphor (rosemary), eugenol (clove) and cinnamaldehyde (cinnamon). The data obtained have the potential to collaborate for the formulation of new antimicrobial therapies that have efficacy, greater accessibility and reduction of adverse effects, contributing to both human and veterinary medicines. The last could be improved in an important way since the use of medicines for animals formulated from plants are still scarce.

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