Antimicrobial activity of essential oil from *Mimosa verrucosa* Benth. and *Illicium verum* Hook.f. against planktonic and biomass bacterial cells

Adriana Barbosa da Rocha[1]
Thereza Cristina da Costa Patricio[2]
Danniel Cosme Neves Grillo[3]
Mayara Gomes Oliveira[2]
Hosana dau Ferreira de Souza[2]
Nayana de Figueiredo Pereira[1]
Bianca Augusto de Souza[1]
Shana de Mattos de Oliveira Coelho[2]
Cristiano Jorge Riger[3]
Douglas Siqueira de Almeida Chaves[1]

[1]Laboratório de Farmacognosia e Bioativos Naturais, Instituto de Ciências Biológicas e da Saúde, Universidade Federal Rural do Rio de Janeiro, 23897-000, Seropédica, Brazil

[2]Microbiologia e Imunologia Veterinária, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, 23897-000, Seropédica, Brazil

[3]Laboratório de Estresse Oxidativo em Microrganismos, Instituto de Química, Universidade Federal Rural do Rio de Janeiro, 23897-000, Seropédica, Brazil

**Corresponding author: chavesdsa@ufrrj.br*

ABSTRACT

The development of biofilms is responsible for 80% of microbial infections, which are highly resistant to conventional antibiotics. The study aimed to assess the antimicrobial activity of essential oils of *Mimosa verrucosa* Benth. and *Illicium verum* Hook.f. against planktonic and sessile cells and evaluate toxicity. Thirty compounds were identified, the main ones being α-pinene (12.6%), β-pinene (16.7%) and (*E*)-caryophyllene (14.2%) for *M. verrucosa*, and estragole (4.2%) and anethole (86.8%) for *I. verum.* The minimum inhibitory concentrations of the EO of *I. verum* (29.40 µg/ml) and *M. verrucosa* (24.89 µg/ml) against planktonic cells showed 99% efficacy against all cells tested (sensitive and resistant *Staphylococcus aureus* and *Escherichia coli*). In sessile cells, essential oils of *I. verum* and *M. verrucosa* showed efficacy against sensitive *S. aureus*. The minimum bactericidal concentration (MBC) test revealed that *I. verum* caused cell death in sensitive *S. aureus* and *E. coli.* However, *M. verrucosa* only showed bactericidal activity against planktonic cells. Considering the expanding resistance to antimicrobials, the EOs tested represent an important therapeutic option, especially against *S. aureus* and *E. coli*, which can produce biofilms on various surfaces, becoming a serious public health problem.

Keywords: microbiological resistance, monoterpene, phenylpropanoids, volatile oils.

INTRODUCTION

Biofilms are complex microbial ecosystems characterized by one or more communities of microbial cells covered by an extracellular polymeric structure that adheres to substrates (Condò et al. 2020). This complex represents one of the main mechanisms of microbial resistance due to the evolutionary advantages it gives them, mainly protection against extreme conditions, such as lack of nutrients, pH and temperature changes, free radicals, and ultraviolet radiation (Cerca and Azevedo 2012).

Biofilm development is a severe health problem, given that persistent infections comprise around 80% or more of microbial infectious cases (Albano et al. 2019). Bacterial infections caused by biofilm proliferation, such as chronic, nosocomial, and medical device-associated infections, represent the highest incidence of resistance to conventional antibiotics compared to isolated infections (Khatoon et al. 2018).

Among the mechanisms of bacterial resistance, biofilms induce physiological changes in response to scarcity of nutrients, stimulate efflux pump expression, and provide an environment for interaction and genetic transfer, thus contributing to the spread of tolerance mechanisms against various drugs of clinical interest (Jiang et al. 2013). A pioneering report by the Global Antimicrobial Resistance and Use Surveillance System, which provides data on antimicrobial resistance (AMR) rates, showed that the global median levels of AMR

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were 42% (*E. coli*) and 35% (*S. aureus*) (WHO 2024).

Staphylococcal and *Escherichia coli* infections are caused by pathogens known for their diverse virulence factors. Staphylococcal infections are often associated with capsules, enterotoxins, toxic shock toxins, and leukocidin, all of which play crucial roles in pathogenesis, causing lysis of immune cells and triggering systemic inflammatory responses. On the other hand, *E. coli* has fimbriae, adhesins, and hemolysins as main virulence factors, facilitating adhesion, colonization, and cellular damage. Understanding these virulence factors is essential for developing effective therapeutic and preventive strategies against these infections (Ortega et al. 2010; Oogai et al. 2011).

Therefore, biofilms represent a source of infection and are associated with an increased mortality rate compared to isolates of the same species that do not form biofilms. In this context, the implementation of natural products with a therapeutic focus already used by the population is due to the high incidence of antimicrobial resistance (Wińska et al. 2019).

Essential oils and other natural products have attracted increasing interest as potential sources of antimicrobial activity. With growing concern regarding antimicrobial resistance, the search for therapeutic alternatives derived from natural sources has become a priority in pharmaceutical research. *Mimosa verrucosa* Benth. is an endemic plant of the Caatinga biome in Brazil, with antioxidant activity mainly associated with the chemical composition of its roots (Romanoski et al. 2017). At the same time, *Illicium verum* Hook.f. is endemic to southern China and some regions of Vietnam, and its essential oil is used topically for an antiseptic (Dos Santos et al. 2020).

This study aimed to evaluate the chemical composition and toxicity of the essential oils of *M. verrucosa* and *I. verum*, as well as to verify the antibacterial action of essential oils against the Gram+ bacteria MSSA (methicillin-sensitive *Staphylococcus aureus*), MRSA (methicillin-resistant *S. aureus*), *Streptococcus* spp, and Gram- *E. coli* CMY, sensitive *E. coli*, and *Pseudomonas* β-hemolytic, characterizing the minimum bactericidal concentration (MBC). To evaluate the toxicity of these essential oils on eukaryotic cells, tests were carried out using *Saccharomyces cerevisiae* yeast cells, a single-cell eukaryotic biological model widely used in cytotoxicity analyses of natural substances (Epifanio et al. 2020).

MATERIAL AND METHODS

Essential oil and chemical characterization

Essential oils from *M. verrucosa* (jurema) and *I. verum* (star anise) were obtained commercially by Empório Laszlo and Via Aroma brands, and their authenticity Chromatography-FID-MS verified their authenticity.

To separate, detect, and quantify the constituents, 1 µl of volatile oil samples diluted in dichloromethane (10 µl/ml) in the defined times was injected into a gas chromatography (GC) instrument. A Hewlett-Packard 5890 Series II (Palo Alto, USA), equipped with flame ionization detection and a split/splitless injector, in a split ratio 1:20, was used to separate and detect the constituents in the volatile oil. The compounds were separated with a fused silica capillary column (5% phenyl, 95% dimethylpolysiloxane), with 30 m x 0.25 mm (i.d.) x 0.25 µm (film thickness). Helium was used as the carrier gas at a 1 ml/min flow rate. The column temperature was programmed as follows: 60 °C for 2 min followed by heating at 5 °C/min to 110 °C, followed by heating at 3 °C/min to 150 °C and, finally, followed by heating at 15 °C/min until 290 °C and holding constant for 15 min.

The injector temperature was 220 °C, and the detector temperature was 290 °C. To separate and identify the substances, 1 µl of volatile oil samples diluted in dichloromethane (10 µl/ml), in the defined times, was injected in the gas chromatograph coupled to a mass spectrometer (GC-MS) QP-2010 Plus (Shimadzu, Japan). The flow of the helium gas carrier, the capillary column, and the temperature conditions for the GC-MS analysis were the same as described for the GC. The temperature of the injector was 220 °C, and the interface temperature was 250 °C. Mass spectra were obtained with a quadrupole detector operating at 70 eV, with a 40–400 *m/z* mass range and scanning rate equal to 0.5 scan/s. The identification of volatile compounds in the volatile oil has been based on linear retention indices (LRI) and mass spectra of the samples, compared with authentic standards injected under the same conditions, with the NIST database (2008) and the Linear retention index. The LRI was calculated based on the co-injection of the alkanes series (Adams 2007).

Essential oils and strains

Essential oils from *M. verrucosa* and *I. verum* were previously diluted in a 5% dimethylsulfoxide (DMSO) solution. Six bacterial strains were employed, including three standard strains: *S. aureus* ATCC 43300, *S. aureus* ATCC 25923, *E. coli* ATCC 25922, and three strains from the bacteria collection of the Bacteriology

Laboratory of the Federal Rural University of Rio de Janeiro: *E. coli* CMY, *Pseudomonas β*-hemolítica, and *Streptococcus* spp.

Biofilm formation

The strains were grown on Mueller-Hinton (MH) Agar at 37 °C for 24 h, and the bacterial suspension was prepared in sterile distilled water, adjusted to McFarland's 0.5 (5 x 10^5 CFU/ml). Afterward, 20 µl of the inoculums were added to 180 µl of TSB at 1% glucose, followed by incubation in a 96-well plate at the same temperature and for the period described. The planktonic cells were discarded, followed by two washes with 200 µl of 0.9% saline solution, then incubated at 60 °C for 1 h. 150 µl of 0.1% crystal violet was added and incubated at room temperature for 15 min. The dye was discarded, and the plate was washed with 150 µl of 0.9% saline solution. 150 µl of 92% ethanol was added, and after 30 min, the plate was read on a spectrophotometer at a wavelength of 630 nm. The biofilm was classified as weak, moderate, and strong, and only *S. aureus* and *E. coli* strains were used as positive biofilm control. The results were interpreted according to the standards established (Davey and O'toole 2000).

Minimum inhibitory concentration of planktonic and sessile cells

EOs of *M. verrucosa* and *I. verum* were tested in the respective concentration ranges 0.09 to 24.89 µg/ml and 0.11 to 29.40 µg/ml and diluted with 5% Tween 20 and DMSO. Planktonic cells were obtained from bacterial culture on MH agar at 37 °C for 24 h and adjusted to McFarland's 0.5 (5x10^5 CFU/ml). The sessile cells were obtained using the biofilm formation methodology; however, in a sterile falcon tube for greater inoculum availability, 500 µl of inoculum previously adjusted in 0.9% saline solution was inserted into 5,000 µl of TSB at 1% glucose after the incubation period already described, the medium was discarded, and two washing steps were carried out with 5,000 µl of 0.9% saline solution to remove the planktonic cells. The tubes were then sonicated at 50 W for 5 min, 500 µl of saline solution was added to each tube to make the inoculum, and the contents of the tube were aspirated and adjusted in saline solution until they reached 0.5 on the McFarland scale (5 x 10^5 CFU/ml). Both cells were then distributed in 96-well plates at the times and temperatures already described and at the concentrations of the respective EOs described. For the controls, only the strains MRSA ATCC 43300 was used for the positive control; negative controls were the strains and Imipenem, and the dilutions of the EOs in pure MH broth for the blank. UV spectroscopy at 630 nm measured the turbidity produced (Gajewska and Chajęcka-Wierzchowska 2020).

The minimum bactericidal concentration of planktonic and sessile cells

From the macroscopic observation of strain wells and EOs, an aliquot was sown in the absence of turbidity in the MH agar culture medium at 37 ºC. After 24 h, the MBC was defined as the lowest concentration of essential oil that could cause the growth of the inoculum to become unviable (BrCast 2023).

Culture media and growth conditions

Strain *Saccharomyces cerevisiae* used in this study has the code BY4741 and genotype *MATa; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0*. This strain was purchased from Euroscarf (Frankfurt, Germany). Stocks are maintained in PD containing 1% yeast extract (Difco/USA), 2% glucose (Sigma/Brazil), 2% peptone (Difco/USA), and 2% agar (HIMedia/Brazil). In the cytotoxicity and antioxidant potential assays of essential oils, cells were cultivated in liquid YPD medium, and kept in a shaker-type incubator, with controlled temperature and agitation (28 °C/160 rpm, IKA KS 130).

Cytotoxicity of essential oils in *S. cerevisiae* cells

After cell growth without treatment, the cells were collected, centrifuged, and resuspended in a fresh culture medium at 0.1 mg/ml. Cells were incubated with *M. verrucosa* and *I. verum* (0.17 and 0.20 µg/ml) oils, and the absorbance (DO_{570}) was measured in a spectrophotometer at 570 nm at times of 0, 2, 4, 6, 22, and 24 h. The cell suspension was maintained at temperature and agitation (28 °C/160 rpm). Cell growth curves in the presence of essential oils were compared with the cell growth rate without any treatment.

Intracellular oxidation levels

After cell growth, 3.0 mg of cells were incubated with *M. verrucosa* and *I. verum* (0.1 mg/ml for both) oils in sodium phosphate buffer solution (50 m*M*, pH 6.0) for 2 h. Then, the cells were collected, washed, and resuspended in potassium phosphate buffer solution (50 m*M*, pH 7.8), adding a solution of dichlorodihydrofluorescein diacetate (H_2DCF-DA) (20 µ*M*). The cell suspension was incubated at 28 °C/160 rpm for 15 min to allow the cells to absorb the fluorescent probe. Subsequently, the cells were exposed to oxidative stress with H_2O_2 (2.0 m*M*) for 1 h at 28 °C/160 rpm. The fluorescence of the cell suspension was measured in a microplate reader (Hidex) using 488 nm (excitation) and 520 nm (emission) filters. Data were expressed as

fluorescence intensity (De Souza et al. 2023).

Statistical analysis

GraphPad Prism 10 software was used for statistical analysis. The arithmetic means of triplicates analyzed the data for each concentration tested and then analyzed using a two-factor ANOVA (*p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001), comparing the values of each extract concentration, point by point, using the Tukey multiple comparisons within each row, compare columns test (simple effects within rows).

RESULTS AND DISCUSSION

Gas chromatography analysis led to the identification of 30 compounds in both EOs. The major compounds were α-pinene (12.6%), β-pinene (16.7%), and (*E*) caryophyllene (14.2%)

Table 1. Chemical composition of *Mimosa verrucosa* (MvEO) and *Illicium verum* (IvEO) essential oil.

Compounds	IRL_c	IRL_L	% MvEO	% IvEO
α-thujene	925	924	2.4	0
α-pinene	933	932	12.6	0.97
Sabinene	972	969	0.6	0
β-pinene	979	974	16.7	0
Myrcene	986	986	0.8	0
α-phellandrene	993	995	0	0.57
Carene	1003	1008	0	0.36
Limonene	1021	1024	1.6	0.9
β-phellandrene	1023	1025	2.7	0.43
1,8-cineole	1026	1026	1.5	0.42
β-ocimene	1032	1032	2.7	0
β-terpineol	1141	1140	0	0.5
Carquejol	1150	1150	0.4	0
Terpine-4-ol	1172	1174	1.3	0
Estragole	1189	1195	0	4.22
Neral	1231	1235	1.3	0
Anisaldehyde	1245	1247	0	1.4
(*Z*)-anethole	1249	1249	0	0.63
Geranial	1258	1264	1.1	0
(*E*)-anethole	1281	1282	0	86.84
Carquejyl acetate	1295	1293	19	0
Ylangene	1369	1373	0.9	0.31
Anisole	1373	1373	0	0.6
β-elemene	1389	1388	5.7	0
(*E*)-Caryophyllene	1412	1412	14.2	0
β-thujaplicin	1473	1475	1	0.39
α-humulene	1477	1477	3.1	0
γ-himachalene	1480	1481	5.4	1.2
Germacrene-D	1494	1494	5	0
Monoterpene hydrocarbons			35.4	3.23
Oxygenated monoterpenes			15.9	0.92
Sesquiterpene hydrocarbons			28.06	1.51
Oxygenated sesquiterpenes			20.0	0.39
Phenylpropanoid			0.0	93.09
Total			99.9	99.14

The chemical composition was analyzed by GC-MS and arranged in a table by order of elution. The concentration (%) was calculated based on the total peak area by GC-FID. IRL_L= Index Relative Linear Literature. IRL_c= Index Relative Linear calculated. MvEO = percentage of each component in the *Mimosa verrucosa* essential oil. IvEO = percentage of each element in the *Illicium verum* essential oil.

for *M. verrucosa*, estragole (4.2%) and anethole (86.8%) for *I. verum* (Table 1).

All strains were used for antibacterial activity. However, only *S. aureus* methicillin-resistant, *S. aureus* methicillin-sensitive, *E. coli*-resistant, and *E. coli*-sensitive essential oils were active. *Pseudomonas* β-hemolytic and *Streptococcus* spp, the essential oils, were not active.

Staphylococcus aureus strains stood out as solid biofilm formers (Figure 1). A community of microorganisms forms biofilm with an affinity for adhering to biotic and abiotic surfaces, which remain linked to each other and the substrate, with each cell surrounded by an extracellular matrix. This ability is consistent with its intrinsic pathogenic nature (Davey et al. 2000).

On the other hand, the *E. coli* CMY and ATCC 25922 strains showed varying levels of biofilm production, with categories ranging from weak to moderate (Figure 1). Characterized as Gram-negative bacteria, they use fimbriae and pili to adhere to solid surfaces. Type 1 and type P fimbriae are common in biofilm formation. This diversity in biofilm formation capacity highlights the phenotypic plasticity of these strains, which can adapt to different environmental conditions. The ability to produce biofilm is a multifactorial characteristic that can depend on several factors, including the presence of specific genes, the composition of the culture medium, and environmental conditions. The variation observed in the strains may be related to different genetic profiles, highlighting the complexity of this characteristic in bacterial populations (Gajewska and Chajęcka-Wierzchowska 2020).

Based on these classifications, strategies aimed at inhibiting biofilm formation can be developed, contributing to the effective management of pathogenic strains. The biofilms of both species undergo processes of maturation and structured development involving the production of an extracellular matrix. This structure protects the bacterial cells from the immune system and drugs. It is an emerging factor that significantly impacts public health and patient treatment, as it implies using antimicrobials, which are generally more expensive and toxic (Goetghebeur et al. 2007).

The robust evidence base in the scientific literature corroborates the efficacy of *M. verrucosa* and *I. verum* as therapeutic alternatives. Previous studies and systematic reviews highlight their promising potential antimicrobial activity, making these species innovative treatment options (Goetghebeur et al. 2007). Figures 2 and 3 show the absorbance values of the planktonic and sessile cell samples of the Gram-positive bacteria *S. aureus* resistant and sensitive to methicillin

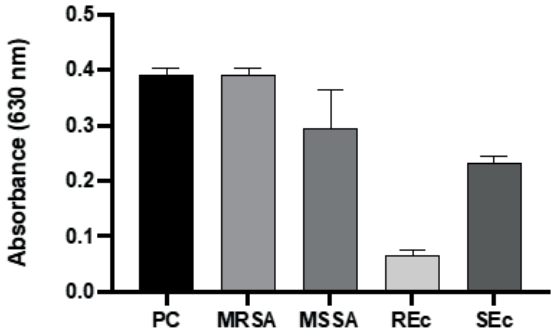

Figure 1. Capacity of biofilm formation - PC = positive control, MRSA = *Staphylococcus aureus* methicillin-resistant, MSSA = *Staphylococcus aureus* methicillin-sensitive, REc = *Escherichia coli* resistant, and SEc = *Escherichia coli* sensitive – by absorbance 630 nm growth. The vertical bars represent the standard deviation of the mean of three repetitions.

(ATCC 43300 and 25923) and Gram- *E. coli* (CMY and ATCC 25922) when treated with *M. verrucosa* and *I. verum* EO. The planktonic cells treated with *I. verum* EO (29.40 µg/ml) showed two samples with $p < 0.001$ (43300 and CMY) and the other two (25923 and 25922) with $p < 0.05$, showing respective confidence indices of 99.999% and 95% probability of antimicrobial action. These findings corroborate previous results where 0.031 µg/ml of this oil showed antibacterial action in both microdilution tests on MSSA strains (Outemsa et al. 2021).

In the tests of sessile cells against the treatment of *I. verum* EO (29.40 µg/ml), three samples showed $p < 0.01$ (43300, CMY, 25922), and one sample (25923) with $p < 0.0001$, confidence indexes of 99.99% and 99.9999% probability of antimicrobial action, highlighting the MSSA strain with a *p*-value expressive to the planktonic analytical findings, exhibiting highly significant efficacy. Interestingly, Ganesh et al. (2022), through GC-MS and molecular analysis on *S. aureus* biofilm, showed that the methanolic extract of *I. verum* inhibited sessile cells at 0.005 µg/ml; GC-MS analysis revealed anethole, *m*-methoxybenzaldehyde and 3-HBA as the major compounds. The extract inhibited the biofilm at the sub-inhibitory concentration of 0.002 mg/µl. Similarly, 3-HBA inhibited biofilm activity at the lower concentrations tested in this experiment, but the same concentrations tested on planktonic cells did not inhibit microbial growth, data that is compatible with our findings described in this article.

The planktonic cells treated with *M. verrucosa* EO (24.89 µg/ml) showed two samples

with $p < 0.05$ (43300 and CMY) and the other two (25923 and 25922) with $p < 0.01$, showing respective confidence indices of 95% and 99.99% probability of antimicrobial action. However, in the literature, there are few reports of the antimicrobial activity of this species. Still, within the mimosa genus, *M. tenuiflora* and *M. pudica* have already been analyzed for their antimicrobial activity. Padilha et al. (2010) evaluated thirty clinical isolates of *S. aureus*; the MIC values were 0.0002 µg/ml; when increasing the concentrations up to 4x MIC, a bacteriostatic effect was observed, but at 8x MIC, there was a rapid bactericidal effect. Abirami et al. (2014), using acetone extract of *M. pudica* in disk diffusion, observed a maximum zone of inhibition against *S. aureus*. These findings align with our results, which used a dose 100x higher than that described and observed antimicrobial activity of *M. verrucosa* EO.

The tests on sessile cells, when treated with *M. verrucosa* EO (24.89 µg/ml), showed values of $p < 0.05$ (43300 and CMY), $p < 0.01$ (25922) and one with $p < 0.001$ (25923), with respective confidence indices of 95%, 99.99% and 99.999% probability of antimicrobial action on cells from bacterial biofilm. The increased likelihood of antimicrobial action was also verified at this experiment stage. However, due to the scarcity of data in the literature addressing the antimicrobial activity of *M. verrucosa*, especially on sessile cells or biofilm, the results were correlated with findings of the specie significant class of compounds as sesquiterpenes and monoterpenes). According to Ghazal et al. (2022), monoterpenes showed moderate efflux pump inhibitory activity in MRSA strains; these contained *n*-hexane extract and showed inhibitory activity on biofilm formation under MRSA and *E. coli* strains.

The CBM was defined as the lowest concentration of essential oil capable of causing the growth of the inoculum to become unviable (BrCast 2023). The tests on planktonic cells showed that *I. verum* was able to induce cell death at the concentration tested (29.40 µg/ml) for the strains 25923, CMY, and 25922, while for sessile cells at the same concentration, it inhibited the strains 43300, 25923 and 25922. As for the tests on planktonic cells of *M. verrucosa*, the CBM at the concentration tested (24.89 µg/ml) caused cell death for all the strains tested; however, in the tests on sessile cells, there was no unviable growth of the strains tested. These results are compatible with the literature that a concentration of 0.589 µg/ml caused planktonic cells to become unviable for the *Helicobacter pylori* bacteria, a Gram-negative bacterium that colonizes the epithelial surface of the gastric mucosa (Dos Santos et al. 2020); 2 µg/ml of this same oil was also able to unviable the growth of *S. mitis*, a Gram-positive, facultative anaerobic bacteria that inhabit the oral cavity (Davey and O'toole 2000).

Antimicrobial activity was analyzed using nine antibiotic-resistant clinical isolates, including *S. aureus*, *P. aeruginosa,* and *Acinetobacter baumannii*. Among the samples tested, the *I. verum* leaves and twigs extracts exhibited bactericidal activities, with a CBM range of 0.0002-0.00045 µg/ml (Wang et al. 2007; Wang et al. 2011; Yang et al. 2021).

The toxicity of essential oils on eukaryotic cells was evaluated for 24 h. At these times, the absorbance of cell suspensions in the presence

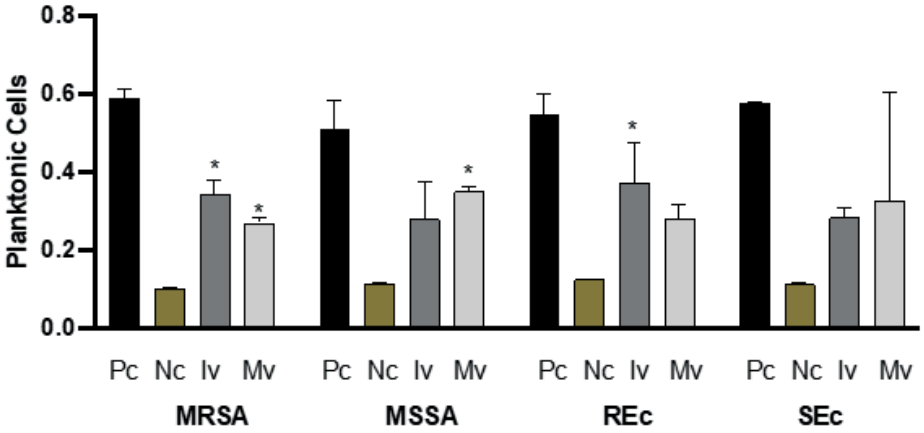

Figure 2. Essential oil of *Illicium verum* (29.40 mg/ml) and *Mimosa verrucosa* (24.89 mg/ml) against Gram-positive (*Staphylococcus aureus* methicillin-resistant – MRSA, and *Staphylococcus aureus* methicillin-sensitive – MSSA) and Gram-negative bacteria (*Escherichia coli* resistant – REc, and *Escherichia coli* sensitive – SEc) planktonic cells. *$p < 0.01$. Pc = Positive control; Nc = Negative control (imipenem); Iv= *Illicium verum* essential oil; Mv = *Mimosa verrucosa* essential oil. The vertical bars represent the standard deviation of the mean of three repetitions.

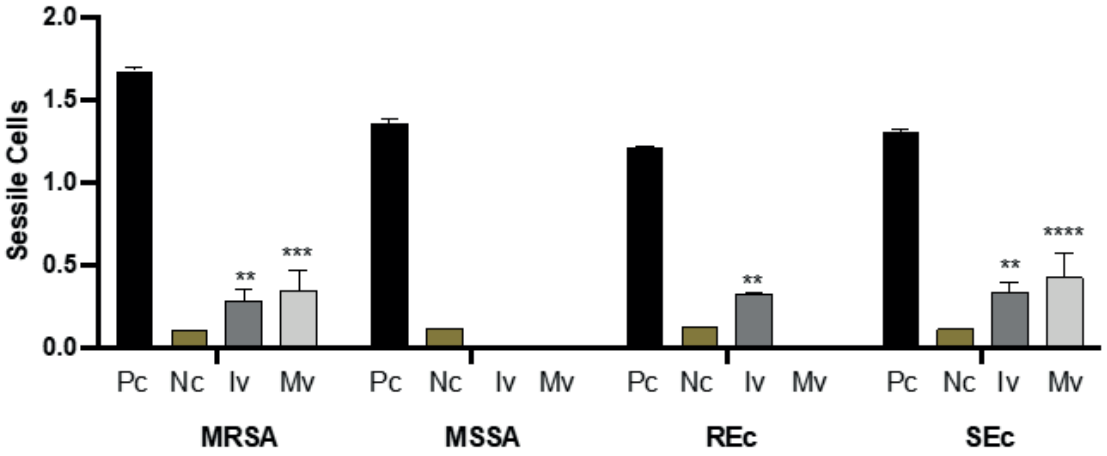

Figure 3. Essential oil of *Illicium verum* (29.40 mg/ml) and *Mimosa verrucosa* (24.89 mg/ml) against sessile cells Gram-positive (*Staphylococcus aureus* methicillin-resistant – MRSA, and *Staphylococcus aureus* methicillin-sensitive – MSSA) and Gram-negative bacteria (*Escherichia coli* resistant – REc, and *Escherichia coli* sensitive – SEc) sessile cells. ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. Pc = Positive control; Nc = Negative control (imipenem); Iv= *Illicium verum* essential oil; Mv = *Mimosa verrucosa* essential oil. The vertical bars represent the standard deviation of the mean of three repetitions.

of the *M. verrucosa* (0.17 µg/ml) and *I. verum* (0.20 µg/ml) essential oils was determined using a spectrophotometer. A control assay was carried out only with yeast cells without any treatment. In this context, results showed that both treatments did not interfere with cell growth, with results statistically equal to the control assay after 24 h (Figure 4).

It is known that toxicity analyses of essential oils use different cellular models involving plants of the *Mimosa* genus, such as *M. tenuiflora* (10 µg/ml) with growth inhibition for *S. aureus* and *E. coli* (De Souza Araujo et al. 2018), *M. pudica* (30, 60, 90, and 120 µl) with antimicrobial activity for *S. aureus* and *E. coli* (Abirami et al. 2014), *M. hamata* (500 mg/disc) with *in vitro* antimicrobial activity for *E. coli* (Jain et al. 2004), and *M. verrucosa* (250 and 500 µg/ml) with MIC for *S. aureus* and *E. coli* (Silva et al. 2020); with different extraction methodologies and solvents (hydroethanolic, ethanolic, methanolic extracts, aqueous fractions, and ethyl acetate) (Majeed et al. 2021).

A brine shrimp lethality assay (BSLA)

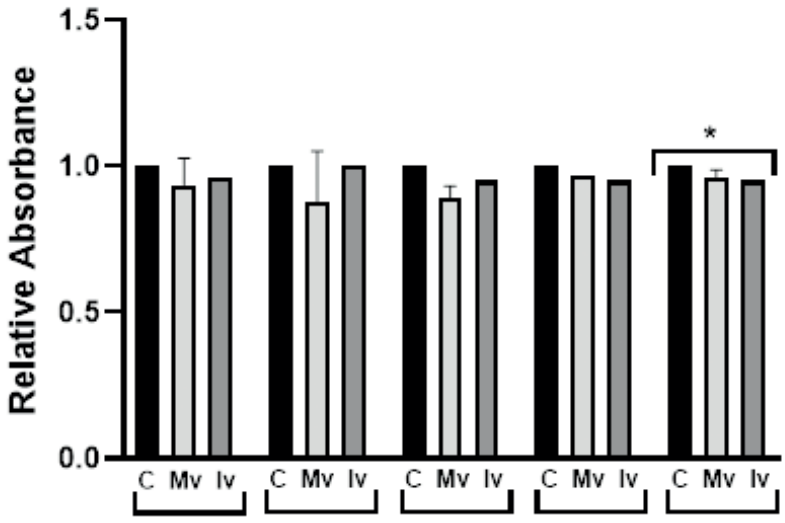

Figure 4. Analysis of the time-dependent cytotoxicity of *Mimosa verrucosa* (Mv) and *Illicium verum* (Iv) essential oils at 0.17 mg/µl and 0.20 mg/µl, respectively, on *S. cerevisiae* cells. Optical density (570 nm) was determined, and the absorbance relative to the control (untreated cells) was calculated. Asterisks represent significant statistical similarity between C = Control, Iv= *Illicium verum* essential oil and Mv = *Mimosa verrucosa* essential oil at 24 h. *$P < 0.0001$. The vertical bars represent the standard deviation of the mean of three repetitions.

study showed that *I. verum* EO's toxicity was high at concentrations above 10,000 mg/ml (Bussmann et al. 2011).

The *Mimosa* genus is already widely used in the literature as an excellent antimicrobial treatment option. Tests on *Bubalus bubalis* (dairy buffaloes) affected with clinical mastitis using the botanical extract of *M. tenuiflora* demonstrated antimicrobial activity against *S. aureus*, with relatively high toxicity. This same plant species showed antineoplastic activity in PC-3 human prostate cells and demonstrated low hemolytic activity, giving it low toxicity for normal cells (Ghazal et al. 2022; Yang et al. 2021).

However, other potential beneficial biological properties make these species attractive for therapeutic purposes. Studies have already demonstrated the antioxidant activities of *M. verrucosa* extract as a free radical scavenger. Furthermore, other plants of the Mimosa genus also showed antioxidant potential *in vitro*, such as *M. pudica* inactivating the DPPH radical (Franco et al. 2022), while *M. tenuiflora* required a very high concentration to present antioxidant activity (Villarreal et al. 2022).

Intracellular oxidation levels were determined in control cells (without treatment), cells under oxidative stress (H_2O_2 - 2.0 m*M*) for 1 h, and cells under oxidative stress with H_2O_2 for 1 h but pretreated with *M. verrucosa* and *I. verum* (0.17 and 0.20 µg/ml) EOs for 2 h (Figure 5). The results

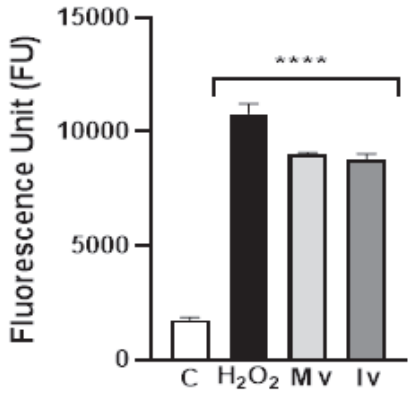

Figure 5. Intracellular oxidation levels were determined in (C) control cells (without treatment), cells under oxidative stress (H_2O_2) at concentration 2.0 m*M* for 1 h, and cells under oxidative stress with H_2O_2 for 1 h but pretreated with *Mimosa verrucosa* (Mv) and *Illicium verum* (Iv) (0.17 µg/ml e 0.20 µg/ml I, respectively) for 2 h. Asterisks represent statistical differences between Mv and Iv compared to cells under oxidative stress with ****$P < 0.001$. C = Control, Iv= *Illicium verum* essential oil, and Mv = *Mimosa verrucosa* essential oil. The vertical bars represent the standard deviation of the mean of three repetitions.

demonstrated that cells pre-treated with essential oils significantly reduced intracellular oxidizing environment levels.

These results suggest that *M. verrucosa* and *I. verum* essential oils have antioxidant properties, as they equally decrease the intracellular oxidizing environment caused by hydrogen peroxide stress. In addition, the action of essential oils is similar to stressed cells, reducing the intracellular oxidizing environment caused by hydrogen peroxide stress.

CONCLUSIONS

The results indicate that the EOs extracted from *M. verrucosa* and *I. verum* showed considerable antimicrobial activity on planktonic and sessile cells. This efficacy was particularly significant on Gram-positive strains MRSA (43300) and MSSA (25923), as well as Gram-negative *E. coli* (CMY) and *E. coli* (25922). However, no significant effects were observed against *Pseudomonas* β-hemolytic and *Streptococcus* spp. These results highlight the selectivity of essential oils, suggesting that antimicrobial action may vary between different types of bacteria depending on the treatment used.

The tested EOs' ability to act against sessile cells is particularly promising, indicating the potential to combat the formation of bacterial biofilms. In addition, *I. verum* demonstrated bactericidal activity on sessile cells, highlighting its therapeutic potential in effectively eradicating biofilms; concomitant to these promising results, they still present low toxicity to animal and human cells.

Considering the increasing resistance of antibiotics and the clinical importance of the strains evaluated, the essential oils of *M. verrucosa* and *I. verum* emerge as promising candidates for future development as antimicrobial agents. However, it is crucial to carry out additional studies to better understand the mechanisms of action, toxicity, and clinical applicability of these essential oils, with a view to their safe and effective use in treating bacterial infections, especially those associated with biofilm.

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AUTHORS' CONTRIBUTIONS

Conceptualization: ABR, SMOC, CJR, DSAC; methodology: ABR, TCCP, DCNG and MGO; formal analysis and investigation: ABR, TCCP, HFS, NFP; resources: ABR, TCCP, HFS, NFP, BAS; data analysis and curation: ABR, BAS; writing (original draft): ABR, SMOC, CJR, DSAC; writing (revision): SMOC, CJR, DSAC; supervision: DSAC; project administration: DSAC. All authors have read and approved the final manuscript.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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