

## ***Antibacterial and anti-biofilm potential of Plectranthus amboinicus (Lour.) Spreng essential oil and Carvacrol against Staphylococcus aureus and Escherichia coli***

Potencial antibacteriano e anti-biofilme do óleo essencial de *Plectranthus amboinicus* (Lour.) Spreng e carvacrol frente a *Staphylococcus aureus* e *Escherichia coli*

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### **Abstract**

**Introduction:** *Staphylococcus aureus* and *Escherichia coli* are pathogens that cause chronic infections due to antibiotic resistance mechanisms and their ability to adhere to surfaces and to form biofilms. The search for new agents from natural resources to counter microbial biofilms is an urgent priority in healthcare. **Objective:** chemical composition, antibacterial and anti-biofilm activity of *Plectranthus amboinicus* essential oil (PAEO) and carvacrol were investigated against *E. coli* and *S. aureus*. **Methodology:** PAEO was chemically analyzed using gas chromatography coupled to a mass spectrometer. Antimicrobial activity was assessed by the disc diffusion method and broth microdilution method to determine minimum inhibitory concentrations (MICs). Antibiofilm activity was investigated using 96-well plates with a crystal violet assay. **Results:** carvacrol (85.25%) was the major component of PAEO. The disc diffusion test confirmed the ability of PAEO and carvacrol in inhibiting bacteria in their planktonic form. The MICs of PAEO against *S. aureus* and *E. coli* were 0.31 and 1.25%, respectively, with bactericidal effect. Carvacrol demonstrated a significant antibacterial property (MIC = 0.31%), exhibiting bacteriostatic effects against *S. aureus* and bactericidal effects against *E. coli*. Carvacrol considerably inhibited *E. coli* biofilm formations (58.9%). Moreover, carvacrol inactivated the mature biofilms. **Conclusion:** the data obtained are promising, and facilitates the development of new therapeutic alternatives. These results indicate the potential of carvacrol in treating diseases caused by *E. coli* and *S. aureus*.

**Keywords:** Biofilm. Volatile oil. Natural product.

### **Resumo**

**Introdução:** *Escherichia coli* e *Staphylococcus aureus* são patógenos que podem causar infecções persistentes devido aos mecanismos de resistência e à sua capacidade de aderir e formar biofilme. A busca por novos agentes a partir de recursos naturais para combater o biofilme é uma prioridade na área da saúde. **Objetivo:** a composição química, a atividade antibacteriana e anti-biofilme do óleo essencial de *Plectranthus amboinicus* (PAEO) e do carvacrol foram investigadas frente a *S. aureus* e *E. coli*. **Metodologia:** a análise química do PAEO foi realizada por cromatografia gasosa acoplada a espectrômetro de massa. A atividade antimicrobiana foi avaliada por difusão em disco e microdiluição em caldo para determinar as concentrações inibitórias mínimas (CIMs). As atividades do anti-biofilme foram investigadas usando placas de 96 poços pelo ensaio de cristal violeta. **Resultados:** o carvacrol (85,25%) foi o principal componente do PAEO. A capacidade do PAEO e do carvacrol para inibir bactérias planctônica foi confirmada por difusão em disco. A CIM do PAEO para *S. aureus* e *E. coli* foi de 0,31 e 1,25%, respectivamente, com efeito bactericida. O carvacrol demonstrou uma propriedade antibacteriana significativa (CIM=0,31%), exibindo efeito bacteriostático frente a *S. aureus* e efeito bactericida para *E. coli*. O carvacrol inibiu significativamente a formação de biofilme de *E. coli* (58,9%) e promoveu a desestabilização do biofilme maduro. **Conclusão:** os dados obtidos são promissores, possibilitando o desenvolvimento de novas alternativas terapêuticas e revelam a potencialidade do carvacrol como fonte para o tratamento de doenças causadas por *E. coli* e *S. aureus*.

**Palavras-chave:** Biofilme. Óleo volátil. Produto natural.

### **INTRODUCTION**

*Microbial infections are considered a major public health issue and are responsible for approximately 20% of deaths worldwide (WHO, 2020). Escherichia coli and Staphylococcus aureus are important pathogens that cause clinical and epidemiological problems in health care-*

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associated infections (HAIs), such as community-acquired infections, in both hospitalized and immunocompromised patients. *S. aureus* and *E. coli* can survive in the presence of limiting environmental factors and can endure the hostile conditions imposed by the host because of their ability to adapt to the physiological state and cellular activities via biofilm formation and antimicrobial resistance (ANSARI et al., 2019).

Biofilms consist of complex communities that are embedded in a matrix composed of extracellular polymeric substances secreted by microorganisms. In clinical practices, biofilms can form on the outer surface of catheters and implanted medical devices, and chronic or recurrent infections because of ineffective treatments. In addition, biofilm bacteria can survive in planktonic form at concentrations above lethal levels (SURESH; BISWAS; BISWAS, 2019). According to Ansari et al. (2019), 25% of infections are associated with the biofilm producing capacity. Thus, there is an urgent need to develop an integrative approach to optimize the use of antimicrobial therapy and control resistant microorganisms, without affecting the health of plants, animals, and humans (RESENDE; SILVA; DINIZ, 2020).

The Lamiaceae family contains important aromatic plants that produce essential oils (EOs), which are used in traditional and modern medicine, and in food and pharmaceutical industries (NIETO, 2017). EOs exhibit antimicrobial activity against strains from clinical and environmental origin (CORREA et al., 2019). *Plectranthus amboinicus*, an important species of the family Lamiaceae demonstrates antibacterial, antifungal, anti-inflammatory and analgesic properties (CHIU et al., 2012). However, studies on the action of the oil and its major components on biofilm-forming cells are still scarce.

The field of antimicrobials has been observing an increasing interest in the use of natural compounds. Thus, the present study aimed to determine the chemical composition of *P. amboinicus* EO (PAEO), and the antibacterial activity of this EO and its major component, carvacrol, against planktonic and biofilm cells of *E. coli* and *S. aureus*.

## Methodology

### Plant materials

Leaves of *P. amboinicus* (Lour.) Spreng were collected in the morning in Alegre (ES, Brazil), with geographical coordinates of 20° 44' 49" S, 41° 27' 58" W and an altitude of 250 m. The exsiccate (n° 21590) was deposited in the herbarium at the Federal University of Espírito Santo (UFES).

### Essential oil: Extraction and composition

EO was extracted from the leaves of *P. amboinicus*. Approximately 600 g of fresh leaves were cut into small

pieces and placed in a 5-liter round-bottom flask coupled to a Clevenger. The flask was half-filled with distilled water and hydrodistillation was performed for 3 h. The hydrolate (250 mL) was collected and subsequently subjected to liquid-liquid extraction using pentane (3 × 50 mL). The organic phase was dried with anhydrous sodium sulfate and filtered. The filtrate was concentrated in a rotary evaporator under reduced pressure to obtain EO, which was stored in an amber glass at -5 °C.

PAEO components were determined and quantified according to the methodology described by Pinheiro et al. (2015). PAEO (10 mg) was diluted in 1 mL ethanol, and 1 µL of this mixture was injected into the gas chromatograph coupled to a mass spectrometer (GC-MS, QP-PLUS-2010, Shimadzu). A Rtx-5MS capillary column of fused silica (30 m long, 0.25 mm internal diameter) was used with helium as the carrier gas. The temperature of the injector and the detector was set at 220 °C and 300 °C, respectively. The initial column temperature was set at 60 °C, and programmed to increase by 3 °C per minute to attain a maximum temperature of 240 °C.

To determine the chemical constituents of PAEO, the obtained mass spectra were compared with the reference data obtained from the equipment database, using data from other sources and retention indices (RI). The retention indices (RI) were calculated by injecting, a mixture of linear alkanes (C9–C26) into the chromatograph under the same experimental conditions.

For component quantification, 1 µL of PAEO-ethanol solution (10 mg mL<sup>-1</sup>) was injected into a gas chromatograph equipped with a flame ionization detector (CG-DIC, CG-2010 Plus, Shimadzu). The capillary column Rtx-5MS (30 m length and 0.25 mm internal diameter) was used as the stationary phase. Nitrogen was used as carrier gas. Temperature was programmed similarly to that previously reported for the GC-MS analysis. The temperatures of the injector and detector were 240 °C and 250 °C, respectively (PINHEIRO et al., 2015).

### Antibacterial activity assays

The organisms used in this study were *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923). Antimicrobial susceptibility tests were performed using the disk diffusion method, following the standard method (CLSI, 2019).

Strains were suspended in sterile 0.9% NaCl solution and the turbidity was adjusted to obtain 1.5 × 10<sup>8</sup> colony-forming units per milliliter (CFU mL<sup>-1</sup>) (McFarland standard, 0.5). The inoculums were spread on solidified Mueller-Hinton Agar (MHA) plates (90 mm diameter) by swabbing. Sterile paper discs (Whatman disc 6 mm diameter) were impregnated with 10 µL of PAEO or carvacrol and placed on the MHA plates, and incubated at 35 ± 2 °C for 18-24 hours. Ampicillin (10 µg) and chloramphenicol (30 µg) were used as positive controls. The inhibition zones around the filter paper were measured in millimeters and the mean and standard deviation (SD) were calculated. All

tests were performed in triplicates in two independent assays.

Carvacrol (5-Isopropyl-2-methylphenol) was obtained commercially from Sigma-Aldrich (W224511).

#### **Determination of minimum inhibitory concentration (MIC)**

The MIC of PAEO against bacterial strains was determined by broth microdilution method using 96-well microtiter plates, according to the standard method (CLSI, 2015), with some modifications.

Cell suspensions of the strains were prepared in Mueller-Hinton broth (MHB) and their concentrations were adjusted to obtain  $10^6$  CFU mL<sup>-1</sup>. PAEO or carvacrol solutions were prepared in MHB supplemented with 0.1% Tween-80. Two-fold serially diluted PAEO or carvacrol were added to each well, with concentration ranging from 1.5 to 100 mg mL<sup>-1</sup> (0.15-10%). Aliquots (50 µL) of the standard bacterial suspension were added to the well to obtain a final reaction volume of 100 µL ( $5 \times 10^4$  CFU/well). The microtiter plates were incubated at  $35 \pm 2$  °C for 24 hours. The controls used were as follows: MHB medium supplemented with kanamycin solution (4 µg mL<sup>-1</sup>) (positive control), MHB medium with PAEO or carvacrol, MHB medium with each bacterium, MHB medium with solvents (0.1% Tween-80) and only MHB medium (media control). All controls were subjected to the same experimental conditions.

The assay was considered valid when the growth control either showed a pellet  $\geq 2$  mm at the bottom of the well or was turbid, and the sterility control test showed the absence of turbidity. MIC is defined as the lowest concentration of the test substance that inhibited bacterial growth after 24 hours of incubation at  $35 \pm 2$  °C. Microbial growth was determined at intervals of 0, 2, 3, 4, 6, 8, 12, 20, and 24 hours, and the absorbance was recorded at 630 nm by spectrophotometry (Multiskan™ Sky; Thermo Fisher Scientific, Japan). All experiments were performed in triplicate, and the mean  $\pm$  SD was recorded.

#### **Inhibition of bacterial growth: Bactericidal or bacteriostatic action**

The contents in the MIC wells were streaked onto nutrient agar plates. The plates were incubated at  $35 \pm 2$  °C for 18-24 hours to check the growth and to classify whether the essential oil demonstrated a bacteriostatic (presence of growth) or bactericidal (absence of growth) effect.

#### **Biofilm production inhibition assay**

The effect of carvacrol on biofilm-forming ability was assessed using the method described by Jadhav et al. (2013), with modifications. Cell suspensions of the strains were prepared in MHB and their concentrations were adjusted to obtain  $10^6$  CFU mL<sup>-1</sup>. Carvacrol solutions

were prepared in MHB supplemented with 5% DMSO. The assays were performed in triplicate.

Aliquots of 100 µL of carvacrol solutions (0.5 MIC or 1 MIC) and 100 µL of bacterial inoculum ( $5 \times 10^4$  CFU/well) was added to each well of a 96-well microtiter plate and incubated at  $35 \pm 2$  °C for 24 hours. The following controls were added: positive control, bacterial inoculum (100 µL) and MHB supplemented with 5% DMSO (100 µL); and two negative controls, I) MHB (200 µL); II) MHB (100 µL) and MIC concentration of carvacrol in 5% DMSO (100 µL).

Biofilm formation was quantified by measuring the absorbance of the crystal violet (CV) stained biofilm at 590 nm. After 24 hours of exposure, the medium was discarded and each well was washed thrice with sterile saline (0.9%) to remove non-adherent cells. Adherent cells were fixed by adding 150 µL methanol (96%) for 15 minutes. The contents of the wells were then aspirated, and 200 µL of CV (1%) was added to the wells and incubated for 15 minutes. Excess stain was removed with sterile saline, and the plates were air-dried at  $25 \pm 2$  °C. To measure the absorbance of the adherent cells, CV was re-solubilized in 200 µL ethanol acetone solution (8:2), and the absorbance was measured at OD590 nm using a Multiskan Spectrum (Multiskan™ Sky; Thermo Fisher Scientific, Japan).

The percentage of biofilm inhibition at different carvacrol concentrations was calculated from the ratio of the OD590 values of samples with carvacrol to that of without carvacrol.

The phenotypic characterization of biofilm formation was performed by comparing the absorbance of the biofilm (Absb) with the absorbance of the negative control (Absc) (STEPANOVIĆ et al., 2000). The biofilm was considered non-adherent when  $Absb \leq Absc$ ; poorly adherent, when  $Absb \leq 2 \times Absc$ ; moderately adherent, when  $2 \times Absc < Absb \leq 4 \times Absc$ ; extremely adherent, when  $Absb > 4 \times Absc$ .

#### **Effect of carvacrol on disarticulation of mature biofilm**

Biofilms were produced by aseptically adding the bacterial cultures in MHB medium to the wells of a 96-well microtiter plate and incubating at 37 °C for 24 hours. After biofilm formation, the medium was aspirated gently, and non-adherent cells were removed, following which 200 µL carvacrol solutions (0.5 MIC or 1 MIC) was added to each well. The following controls were added, positive control: bacterial inoculum (100 µL) and MHB supplemented with 5% DMSO (100 µL); two negative controls: I) MHB (200 µL); II) MHB (100 µL) and MIC concentration of carvacrol in 5% DMSO (100 µL). After incubating for 60 minutes at  $25 \pm 2$  °C mature biofilm inhibition was confirmed using crystal violet staining by measuring the absorbance at 590 nm, as described above. The percentages of reduction in biofilm structures in the presence of different concentrations of carvacrol was calculated.

### Statistical analysis

Data analysis was performed using GraphPad Prism 5 with ANOVA, followed by a post hoc Tukey's test for multiple comparisons. Differences between groups were considered significant at  $p < 0.05$ .

## RESULTS AND DISCUSSION

### Essential oil

The PAEO yield compared with that of the plant fresh mass was 0.11% ( $w w^{-1}$ ), which was probably due to the high moisture content in the leaves. A similar result was reported by Pinheiro et al. (2015). Bandeira et al. (2011) and Arumugam et al. (2020) reported 0.43% ( $w w^{-1}$ ) and 0.48% ( $w w^{-1}$ ) of EO, respectively, in the leaves of this species.

The GC-MS results are presented in Table 1. Five compounds were discovered in PAEO, representing 98.46% of the total oil. The major compounds identified in this study was carvacrol (85.25%), which was also identified in studies by Pinheiro et al. (2015) (88.61%) and Vasconcelos et al. (2017) (88.17%). The PAEO composition varies depending on factors such as plant age and developmental stage, climatic and geographic conditions, and the EO extraction method (REYES-JURADO et al., 2020). *P. amboinicus* is a perennial plant distributed throughout tropical Africa, Asia, Australia, and South Americas, including Brazil (LUKHOBBA; SIMMONDS; PATON, 2006).

**Table 1** – Chemical composition (%) of essential oil from the leaves of *Plectranthus amboinicus*.

Essential oil <i>Plectranthus amboinicus</i>	RI	Compound	(%)
	1305	Carvacrol	85.25
	1399	Beta-caryophyllene	5.06
	1360	Eugenol (3-allyl-2-methoxyphenol)	3.31
	1430	Alpha-bergamotene	2.31
	1570	Caryophyllene oxide	2.53
		NI	1.54
		Total	98.46

RI: retention index compared to linear alkanes. Components listed in the elution order in the Rtx-5MS column. NI: not identified.

Source: Research data, 2021

### Antimicrobial activity of PAEO and carvacrol

The antimicrobial activity of PAEO was confirmed for *S. aureus* ( $35.3 \pm 1.2$  mm) and *E. coli* ( $32.7 \pm 3.1$  mm) (Table 2), with no significant difference ( $p = 0.25$ ), and is supported by a previous study (VASCONCELOS et al., 2017).

Carvacrol inhibited the growth of *S. aureus* ( $31.0 \pm 2.3$  mm) and *E. coli* ( $33.0 \pm 1.3$  mm), with no significant difference ( $p = 0.48$ ). Furthermore, no significant difference was observed between PAEO and carvacrol inhibition zone diameters ( $p = 0.49$ ) (Table 2). This result suggests that carvacrol majorly contributes to the antibacterial effect of PAEO. The antimicrobial activity of EO is determined by its major components (ČABARKAPA et al., 2019; NOSTRO et al., 2007). In contrast, the major components may not be the only factor responsible for the activity of the EO, thereby suggesting the importance of the interactions between the major and the minor constituents in the EO (CHOUHAN; SHARMA; GULERIA, 2017).

Different pharmacological activities of carvacrol, including antibacterial and antifungal effects have been previously studied (ČABARKAPA et al., 2019; VASCONCELOS et al., 2017). For the large-scale production of EO, access to a large amount of plant biomass is necessary (LOPES et al., 2020), which may present another obstacle in the application of PAEO. Thus, considering the large-scale production, it is important to evaluate the antimicrobial properties of the major components of the EO, since such compounds can be obtained synthetically and can majorly contribute to the biological activity of the oils. In addition, once the antimicrobial activity of the major component has been proven, new semi-synthetic molecules with enhanced antimicrobial activity can be created by performing small chemical modification in its structure (synthesis of biomolecules).

**Table 2** – Antibacterial activity of *P. amboinicus* essential oil and carvacrol.

Substance tested	Inhibition zones (mm) *	
	<i>S. aureus</i>	<i>E. coli</i>
PAEO	$35.3 \pm 1.2$	$32.7 \pm 3.1$
Carvacrol	$31.0 \pm 2.3$	$33.0 \pm 1.3$
Ampicillin 10µg	$35.3 \pm 8.1$ (S)	$24.0 \pm 4.0$ (S)
Chloramphenicol 30µg	$30.0 \pm 3.5$ (S)	$31.3 \pm 6.1$ (S)

\* Data are presented as mean  $\pm$  SD of inhibition zones. The tests are carried out in triplicate in two independent tests, under the same conditions. S= susceptible, size of the halo is in accordance with that recommended by CLSI (2019). PAEO= *P. amboinicus* essential oil.

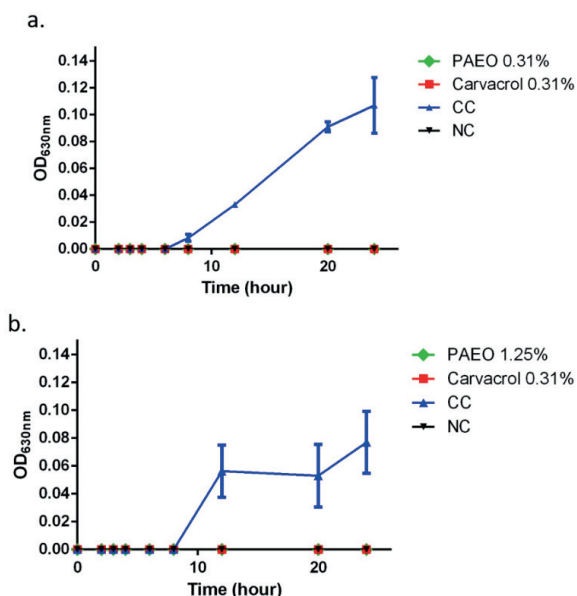
Source: Research data, 2021

### Determination of the minimum inhibitory concentration (MIC) of PAEO and carvacrol against planktonic cells

PAEO displayed a bactericidal effect on *S. aureus* and *E. coli* growth, with MICs of 0.31% ( $3.1 \text{ mg mL}^{-1}$ ) and 1.25% ( $12.5 \text{ mg mL}^{-1}$ ), respectively (Figure 1). A four-fold higher concentration of PAEO was required to inhibit the growth of *E. coli* compared to that of *S. aureus*. Gram-negative

bacteria have higher MIC values for EO, which can be attributed to their outer membrane structure that is more resistant to lipophilic molecules (LOPES et al., 2020).

**Figure 1** – MIC of *P. amboinicus* essential oil and carvacrol. a) Inhibition of *S. aureus* growth. b) Inhibition of *E. coli* growth. The data are presented as the mean  $\pm$  SD of the OD<sub>630nm</sub> after 24 h of incubation. All tests are performed in triplicates. PAEO = *P. amboinicus* essential oil; CC = growth control (culture medium + 0.1% Tween-80 + inoculum); NC = negative control (sterile culture medium).



Source: Research data, 2021

Carvacrol exhibited a bacteriostatic action on *S. aureus* (MIC 0.31%, 3.1 mg mL<sup>-1</sup>) and was bactericidal (MIC 0.31%, 3.1 mg mL<sup>-1</sup>) toward *E. coli*. Its bactericidal activity against *E. coli* was four times more effective than that of PAEO. Sokovic et al. (2010) also demonstrated higher inhibitory activity of carvacrol against *E. coli* compared to that of *Thymus vulgaris* EO. Carvacrol increased the passive permeability of the microbial cytoplasmic membrane, which can be attributed to its ability to align with fatty acids, resulting in distortion of the physical and functional structure of the cytoplasmic membrane (ČABARKAPA et al., 2019; VASCONCELOS et al., 2017). This monoterpene can also interact with membrane proteins and intracellular targets, causing interference in reactions that are essential for the survival of microorganisms (ČABARKAPA et al., 2019). Our results show that the action of an isolated component may not be identical to that of the EO because of the possible interactions that may occur between the other components. The effectiveness of the action can occur through the synergistic interaction of the constituents, including those at lower concentrations (LOPES et al., 2020; VASCONCELOS et al., 2017).

### Effect of carvacrol on bacterial biofilm

Under the conditions tested, the *E. coli* strain was classified as a highly adherent biofilm, whereas *S. aureus* was moderately adherent. The dose-dependent effect of carvacrol on the formation of bacterial biofilms was evaluated. Our results indicated that carvacrol was capable of inhibiting the initial cell adhesion of *E. coli*, thereby significantly ( $p = 0.0005$ ) reducing biofilm formation at both tested concentrations (average inhibition of 58.9%). A dose-dependent effect was not observed; the inhibition varied from 60.5% to 57.2% for concentrations of 0.5 MIC and 1 MIC, respectively, without significant difference (Figure 2a). In contrast, carvacrol showed no inhibitory activity on the formation of *S. aureus* biofilms. The addition of carvacrol to *S. aureus* culture attenuated the formation of bacterial biofilms by 23.6% and 20.9%, when tested at concentrations of 0.5 MIC and 1 MIC, respectively. However, the inhibition was not statistically different when compared to the untreated culture ( $p = 0.17$ ). The bacteriostatic effect of carvacrol was probably inadequate to significantly reduce the biofilm formation by *S. aureus* (Figure 2a).

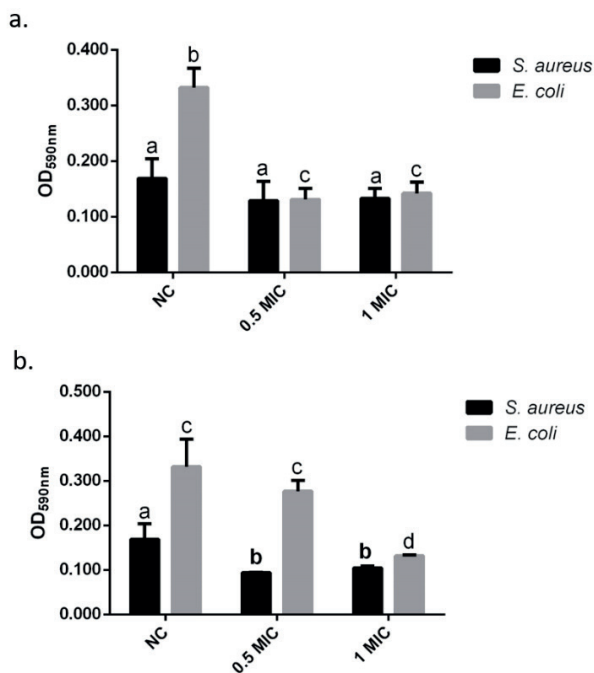
The classification of the biofilm changed when carvacrol was added to the cultures before the initial cell adhesion; both were reclassified as poorly adherent under the tested conditions. Carvacrol inhibits the initial phase of cell adhesion and matrix synthesis, consequently interfering with the quorum sensing mechanism and biofilm (ČABARKAPA et al., 2019). Nostro et al. (2007) reported that carvacrol can destabilize the polysaccharide matrix of the biofilm due to its intrinsic antimicrobial properties. Knowles et al. (2005) suggested that exposure to carvacrol alters the cell surface and partially compromises the initial phase of bacterial cell binding to microtiter plates, which results in the stagnation of biofilm development in the microcolony stage. Nostro et al. (2007) explained that carvacrol penetrates the polysaccharide layer of the biofilm matrix due to its hydrophilic properties, thereby destabilizing the biofilm.

The effect of carvacrol on the disarticulation of *E. coli* and *S. aureus* biofilms is shown in Figure 2b. Compared with the negative control (NC), the biofilm destabilized in a dose-dependent manner, when *E. coli* culture was treated in vitro with carvacrol ( $p = 0.0071$ ). The addition of 1 MIC destabilized 60.3% of biofilm, while the addition of 0.5 MIC resulted in the destabilization of 16.6%. For *S. aureus*, carvacrol destabilized the biofilm at both concentrations of 0.5 MIC (44%) and 1 MIC (37.5%), without any statistical difference.

In order to disarticulate the mature *E. coli* biofilm, it was necessary to double the concentration of carvacrol compared with that used during the initial phase on cell adhesion (Figure 2a). Similar results have been reported by Čabarkapa et al. (2019), who demonstrated that the delayed contact between bacterial cells and carvacrol reduced the inhibitory effect on biofilm when compared

to the culture treatment in the initial phase of cell adhesion, which displayed a weak effect of carvacrol on mature biofilms. The reduced effect of carvacrol on mature biofilms may be related to the presence of an already established extracellular matrix, which acts as a barrier and prevents the diffusion of carvacrol (ČABARKAPA et al., 2019).

**Figure 2** – Effect of carvacrol on the biofilm of *S. aureus* and *E. coli*. a) Effect of carvacrol on bacterial biofilm formation. The data are presented as the mean  $\pm$  SD. b) Effect of carvacrol on established bacterial biofilm. NC= culture not treated with carvacrol; 0.5 MIC = culture treated with 0.5 MIC of carvacrol; 1 MIC = culture treated with 1 MIC of carvacrol. Different letters within the bacterial groups present a significant difference.



Source: Research data, 2021

## CONCLUSION

In this study, PAEO and its major compound, carvacrol, demonstrated significant antibacterial activity on the biofilm and planktonic cells of *E. coli* and *S. aureus*. The data showed that carvacrol inhibited the formation and destabilized the mature *E. coli* biofilms. Owing to the complex epidemiological framework, controlling bacterial infections necessitates the implementation of simultaneous actions in several sectors. Thus, the antimicrobial and anti-biofilm properties described for carvacrol in this study may present new avenues for its application in products for regulating bacteria in various industries.

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