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# *Plectranthus amboinicus* essential oil and carvacrol bioactive against planktonic and biofilm of oxacillin- and vancomycin-resistant *Staphylococcus aureus*

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## Abstract

**Background:** The emergence of multidrug-resistant bacteria is a worldwide concern and in order to find an alternative to this problem, the occurrence of antimicrobial compounds in *Plectranthus amboinicus* essential oil was investigated. Thus, this study aims to determine susceptibility of *Staphylococcus aureus* isolated from food to antibiotics, *P. amboinicus* essential oil (PAEO) and carvacrol.

**Methods:** Leaves and stem of *P. amboinicus* were used for extraction of essential oil (PAEO) by hydrodistillation technique and EO chemical analysis was performed by gas chromatography coupled to a mass spectrometer. *S. aureus* strains ( $n = 35$ ) isolated from food and *S. aureus* ATCC 6538 were used to evaluate the antimicrobial and antibiofilm activity of PAEO and carvacrol. All strains ( $n = 35$ ) were submitted to antimicrobial susceptibility profile by disk diffusion method. Determination of MIC and MBC was performed by microdilution technique and antibiofilm activity was determined by microtiter-plate technique with crystal violet assay and counting viable cells in Colony Forming Units (CFU).

**Results:** Carvacrol (88.17%) was the major component in the PAEO. Antibiotic resistance was detected in 28 *S. aureus* strains (80%) and 12 strains (34.3%) were oxacillin and vancomycin-resistant (OVRSA). From the 28 resistant strains, 7 (25%) showed resistance plasmid of 12,000 bp. All strains ( $n = 35$ ) were sensitive to PAEO and carvacrol, with inhibition zones ranging from 16 to 38 mm and 23 to 42 mm, respectively. The lowest MIC ( $0.25 \text{ mg mL}^{-1}$ ) and MBC ( $0.5 \text{ mg mL}^{-1}$ ) values were observed when carvacrol was used against OVRSA. When a  $0.5 \text{ mg mL}^{-1}$  concentration of PAEO and carvacrol was used, no viable cells were found on *S. aureus* biofilm.

**Conclusion:** The antibacterial effect of carvacrol and PAEO proves to be a possible alternative against planktonic forms and staphylococcal biofilm.

**Keywords:** *Staphylococcus aureus*, Drug-resistant bacteria, *Plectranthus amboinicus*

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## Background

*Staphylococcus aureus* is a Gram-positive cocci often associated with gastroenteritis acquired from contaminated foods such as milk [1] and shrimp. The occurrence of multidrug-resistant bacteria in food is a worldwide concern [2], and *S. aureus* drug-resistant to beta-lactams has been isolated from dairy products [1] and shrimp [3].

Some factors act for selecting drug-resistant bacteria pathogens, such as inappropriate use of antibiotics, and/or the inadequate disposal of antimicrobial drugs in the environment [4, 5]. Thus, the inappropriate use of antibiotics in aquaculture [6, 7] and livestock [8] has contributed to the selection of resistant microbial species, and, consequently, increased food contamination level of animal origin and derivatives.

As an alternative to mitigate the occurrence of multidrug-resistant bacteria, the study of antimicrobial compounds in phanerogams has been proposed [9]. In this context, the plants of genus *Plectranthus* - 3000 recognized species, spread along countries in Africa, South America, Asia and Australia – are widely recognized in folk and popular medicine, being employed in digestive treatments as well as in infectious, inflammatory and respiratory problems [10, 11]. Species of *Plectranthus* [12, 13], including *P. amboinicus* [14], has been studied due to its pharmacological properties in order to validate its popular use.

Bioactivity of *P. amboinicus* is related to the occurrence of 76 volatiles and 30 non-volatile compounds belonging to different classes of phytochemicals (monoterpenoids, diterpenoids, triterpenoids, sesquiterpenoids, phenolics, flavonoids, esters, alcohols and aldehydes) [15]. Studies about the pharmacological activities of *P. amboinicus* are conducted from extracts or essential oil, i.e., complex volatile compounds, synthesized naturally in different plant parts during the process of secondary metabolism with great potential in the field of biomedicine [16]. In this sense, *P. amboinicus* crude essential oils could be used as a tool for the developing novel and more efficacious antimicrobial agents against *S. epidermidis* and *Candida* species [17].

The susceptibility of methicillin-resistant *S. aureus* (MRSA) clinical isolates to extracts of *P. amboinicus* has been reported [18], however, studies about the action of this oil and its major component against planktonic and biofilm of drug-resistant *S. aureus* from food are still incipient.

Thus, this study aims to determine the susceptibility of drug-resistant *S. aureus* strains isolated from milk and shrimp to *P. amboinicus* essential oil (PAEO) and its major component - carvacrol.

## Methods

### Strains origin

Thirty five *Staphylococcus aureus* strains isolated from pasteurized milk ( $n = 12$ ) and shrimp ( $n = 23$ ) were

used. All strains are part of the bacterial catalogue available at Center for Applied Molecular Bioprospecting and Experimentation (NUBEM - INTA College) and had the following biochemical characteristics: Gram-positive cocci, catalase-positive, manitol-positive and coagulase-positive. The strains, maintained in Tryptone Soy Broth with 20% glycerol, were plated onto Mannitol Salt Agar (35 °C for 48 h) and isolated Tryptone Soy Agar for performing microbiological analyzes.

### Antibiogram

As screening for the selection of drug-resistant strain for Minimum Inhibitory Concentration (MIC) and minimum bactericidal concentration (MBC) and antibiofilm activity, all strains ( $n = 35$ ) were submitted to antimicrobial susceptibility profile by disk diffusion method using Mueller Hinton Agar [16]. The following antibiotics discs were used: oxacillin (1 µg), penicillin G (10 U), ampicillin (10 µg), cefotaxime (30 µg), ceftriaxone (30 µg), cefepime (30 µg), imipenem (10 µg), vancomycin (30 µg), gentamicin (10 µg), and chloramphenicol (30 µg). Antimicrobial susceptibility was detected by analyzing the inhibition zones, measured and compared according the standards set by the CLSI (2012) [19] which classify the strains as sensitive, intermediate or resistant.

### Plasmid DNA extraction

*S. aureus* resistant strains were inoculated in Brain Heart Infusion broth and cultured for 24 h at 35 °C. Then, 2.0 mL of each culture was retrieved for plasmid DNA extraction with a commercially available extraction kit (GeneJET Plasmid Miniprep Kit). A 3.0 µL aliquot of each sample of extracted DNA was submitted to 1.5% agarose gel electrophoresis in TBE buffer for 60 min at 120 V, 200 mA and 100 w. The plasmid DNA was viewed under UV light with a Spectrolinetransilluminator and photographed with a Kodak EDAS 290.

### Plant material

Leaves and stem of *P. amboinicus* were collected in October 2014, on a farm (3.49°52,16''S, 40.24°38,38''W) located in Cariré (Northeast Brazil). The botanical identification was made at Herbarium Professor Francisco José de Abreu Matos – (HUVA), Acaraú Valley State University (Sobral-CE). The dried specimens were deposited in the HUVA and registered under specific numbering 18,416.

### Extraction of *Plectranthus amboinicus* essential oil (PAEO)

Extraction of PAEO was made at the NUBEM (INTA College), using the hydrodistillation technique. Four extractions were performed, using 7.690 Kg of plant in order to obtain 5.2 mL essential oil, approximately. PAEO was transferred to sterile amber vial, which was covered

with aluminum foil and maintained at 2 to 8 °C. An aliquot of approximately 20 µL was used for chemical analysis.

#### Chemical analysis of the essential oil (PAEO)

The essential oil chemical analysis was performed by gas chromatography coupled to a mass spectrometer (GC-MS) using a gas chromatograph (Shimadzu QP2010 Plus), and helium (He) as carrier gas. A capillary column Factor Four/ VF-5 ms, 30 m length, 0.25 mm internal diameter, 0.25 mm film thickness was used. The carrier gas flow rate was 1 mL min<sup>-1</sup>. The initial oven temperature was 60 °C. After heating for 2 min, it increased at a constant rate of 2 °C per minute up to 110 °C; then 3 °C per minute to 150 °C; and 15 °C per minute to 290 °C to a final, isotherm 290 °C for 17 min. The injector and detector temperatures were respectively 250 °C and 310 °C. The mode of injection was split and the injection volume was 1 mL. Mass spectra were produced by electron impact (70 eV). Quantitative analysis of the essential oil composition was made on a gas chromatograph, coupled with HP5890 Series II ionization detector, using the same operating conditions and the same type of column as the CG/EM analysis (except for the injector and detector temperatures, which were of 220 °C and 250 °C, respectively). The percentage of each constituent is calculated by the integral of the area under the respective peaks to the total area of all the constituents of the sample. The various constituents of the essential oil were identified by visual comparison of their mass spectra with those in the literature [20] and with actual standards in Nist08 library computer system, as well as by comparing retention rates with those in the literature [20]. A standard solution of n-alkanes (C8-C20) was injected under the same chromatographic conditions used to provide samples and to get retention rates.

#### PAEO and carvacrol antimicrobial effect

Antimicrobial potential of OEPA and carvacrol was determined by: (1) antibiogram, (2) determination of minimum inhibitory concentration (MIC), (3) minimum bactericidal concentration (CBM) and (4) antibiofilm activity.

#### Antibiogram to PAEO and carvacrol

The antimicrobial activity of PAEO and its major component was performed from the disk diffusion test on Mueller Hinton Agar, as detailed on the Antibiogram item. Sterile paper discs (6 mm in diameter) were soaked with PAEO (20 µL) and 98% Carvacrol (20 µL) (Sigma). The antimicrobial effect was detected by the formation of inhibition zones around the disks soaked with PAEO and carvacrol. The halos were measured in

millimeters, labeled as bioactive those larger than 15 mm. As positive and negative control, tetracycline (30 µg) and disks soaked with 20 µL Tween 80 solution 3% were used, respectively.

#### Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

Determination of MIC and MBC was performed by microdilution technique in Tryptone Soy Broth (TSB) [19], using polystyrene plates of 96 wells and two strains: *S. aureus* ATCC 6538, and oxacillin- and vancomycin-resistant *S. aureus* (OVRSA). Concentration of both strains was adjusted in TSB to  $1.25 \times 10^7$  Colony Forming Units (CFU mL<sup>-1</sup>). 20 replicates of each strain were tested with PAEO and carvacrol in concentrations of 4 mg mL<sup>-1</sup>, 2 mg mL<sup>-1</sup>, 1 mg mL<sup>-1</sup>, 0.5 mg mL<sup>-1</sup>, 0.25 mg mL<sup>-1</sup>, 0.125 mg mL<sup>-1</sup> and 0.0625 mg mL<sup>-1</sup>. MIC was determined from visual reading and was considered the concentration able to inhibit microbial growth. In order to determine the MBC, a pool was made of 10 wells for each concentration, and then plating was performed in 10 µL in triplicate Tryptone Soy Agar. The concentration which was not verified microbial growth was considered CBM.

#### Antibiofilm activity

Antibiofilm activity was determined by microtiter-plate technique [21] with crystal violet (CV) assay, counting viable cells in Colony Forming Units (CFU) [22]. In order to determine the PAEO and carvacrol action in the biofilm, plates were subjected to optical density microplate reading (Molecular Devices -SpectraMaxParadigmMulti-Mode) at a wavelength of 570 nm. Activity was found in concentrations that failed to display the adhesion of crystal violet in any of the wells.

#### Controls

For CIM, CBM and antibiofilm activity, TSB containing the test substance (PAEO or carvacrol) in concentrations of 4 mg mL<sup>-1</sup>, 2 mg mL<sup>-1</sup>, 1 mg mL<sup>-1</sup>, 0.5 mg mL<sup>-1</sup>, 0.25 mg mL<sup>-1</sup>, 0.125 mg mL<sup>-1</sup> and 0.0625 mg mL<sup>-1</sup> was used as turbidity control. For contamination control, only the culture medium (TSB) in three wells on the plates we used. Negative control was done by inoculating 100 µL of strain ( $1.25 \times 10^7$  CFU mL<sup>-1</sup>) in wells containing culture medium (TSB). As positive control, tetracycline in the same concentration of test substances was used: 4 mg mL<sup>-1</sup>, 2 mg mL<sup>-1</sup>, 1 mg mL<sup>-1</sup>, 0.5 mg mL<sup>-1</sup>, 0.25 mg mL<sup>-1</sup>, 0.125 mg mL<sup>-1</sup> and 0.0625 mg mL<sup>-1</sup>.

#### Statistical analysis

Data analysis in GraphPad Prism 5 with ANOVA statistical, followed by a Student-Newman-Keuls test for significant variability comparisons ( $p < 0.001$ ) between different concentrations were used to conduct the analysis.

## Results

Antibiotic resistance was observed in 28 *S. aureus* strains (80%) and ten resistance profiles were observed: three profiles of monoresistance ( $n = 5$ , 17.8%), three cross-resistance to beta-lactams ( $n = 8$ ; 28.6%) and four multidrug resistant ( $n = 15$ ; 53.6%) (Table 1). From the 28 resistant strains, 7 (25%) showed plasmid of 12,000 bp (Fig. 1).

Carvacrol (88.17%) was the major component in the essential oil of *P. amboinicus*, followed by caryophyllene oxide, 1,8-cineole, 4-terpinenol, alpha-cisbergamoteno, p-cymene and gamma-terpinene (Table 2, Fig. 2).

All strains ( $n = 35$ ) of *S. aureus* were sensitive to PAEO and carvacrol with inhibition zones ranging from 16 to 38 mm and 23 to 42 mm, respectively (Table 3). The standard strain of *S. aureus* ATCC 6538 showed a inhibitory halo of 34 and 47, respectively, for PAEO and carvacrol.

MIC value of 0.25 mg mL<sup>-1</sup> was observed when PAEO were used against *S. aureus* ATCC 6538 and carvacrol was used against *S. aureus* ATCC 6538 and OVRSA. The lowest MBC value (0.5 mg mL<sup>-1</sup>) was detected when carvacrol was used against OVRSA (Table 4).

Biofilm activities against *S. aureus* ATCC 6538 and OVRSA were observed in concentrations of 0.5 mg mL<sup>-1</sup> (OEPA) and 0.25 mg mL<sup>-1</sup> (carvacrol) (Fig. 3). However, inhibition potential was detected in all tested concentrations of carvacrol and OEPA, in comparison to the negative control. Tetracycline inhibits biofilm formation (*S. aureus* ATCC 6538 and OVRSA) in all concentrations tested (0.062, 0.125, 0.25, 0.5, 1, 2 and 4 mg mL<sup>-1</sup>).

**Table 1** Antimicrobial resistance profile of 28 *Staphylococcus aureus* strains isolated from milk and shrimp

Profile	Drugs	N° of Isolates		Total (%)
		Shrimp	Milk	
Monoresistance	Pen	1	0	5 (17.8)
	Oxa	3	0	
	Cro	1	0	
Cross-resistance to beta-lactam	Pen + Amp	2	0	8 (28.6)
	Oxa + Amp	3	0	
	Pen + Amp + Oxa + Cro + Ctx + Cpm	3	0	
Multidrug resistance	Amp + Van	1	0	15 (53.6)
	Pen + Amp + Gen	1	0	
	Pen + Amp + Oxa + Van	1	11	
	Pen + Amp + Oxa + Ctx + Van	0	1	
	Total	16	12	

PEN penicillin G, OXA oxacillin, CRO Ceftriaxone, AMP ampicillin, Ctx cefotaxime, CPM cefepime, VAN vancomycin, GEN gentamicin

## Discussion

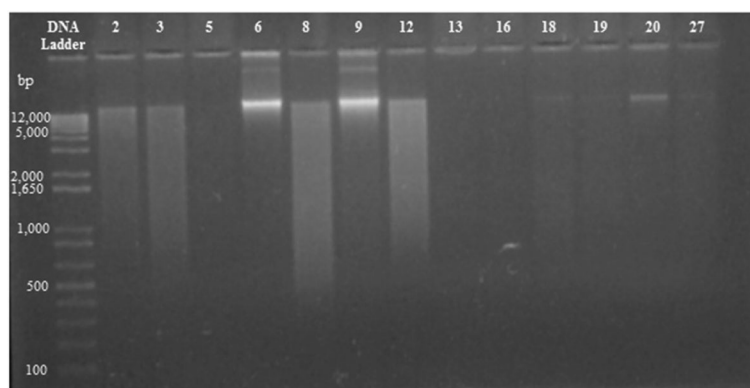
Multidrug-resistant profile was detected in 15 strains (53.6%) (Table 1). Some studies have described the incidence of multidrug-resistant strains of *S. aureus* in foods and in those who handle them, confirming the assertion that one of the main sources of food contamination by *S. aureus* is the handler [23, 24]. Contamination of foods by *S. aureus* multiresistant are related to care in handling, processing and transport of food, as well as correlation of bacterial resistance to the indiscriminate use of antimicrobials in agriculture and aquaculture [2, 25].

In addition to the considerable rate of penicillin resistance, it is valuable to mention the high incidence of strains resistant to oxacillin ( $n = 22$ ; 78.6%) (Table 1). Oxacillin resistance is associated of the *mecA* gene, which encodes a protein with low binding affinity to  $\beta$ -lactam. Thus, in vitro resistance to oxacillin indicated resistance to all other  $\beta$ -lactam drugs [26, 27].

In case of infection caused by strains resistant to oxacillin, the clinically chosen therapy is vancomycin. Thus, another fact worth mentioning in this study is that 14 (50%) of 28 all the resistant strains would persist against vancomycin (Table 1). The present research corroborate studies of Akindolire et al. (2015) [28] and Nunes et al. (2016) [29], that reporting a high incidence (60–100%) of *S. aureus* strains resistant to vancomycin in isolated samples of milk or its derivatives. Baumgartner et al. (2014) [23] reported the presence of *S. aureus* carriers of beta-lactam resistance genes, methicillin and vancomycin in foods, including dairy products.

Of the 28 resistant strains, 7 (25%) showed plasmid of 12,000 bp (Fig. 1) that are probably related to resistance, since these seven strains were isolated from milk and presented multi-drug resistance profile (Pen + Amp + Oxa + Van). For McCarthy and Lindsay (2012) [30], plasmids are responsible for the spread of virulence and resistance genes in *S. aureus* populations. The authors assigned a total of 39 plasmid groups of *Staphylococcus aureus* (pGSA) based on the combination of the *rep* genes each plasmid had and revealed 28 plasmid groups with large genomes (>15Kb), which carried a diverse range of genes.

Our findings corroborate several studies when reporting carvacrol as a major component of *Plectranthus amboinicus* essential oil. On the other hand, the concentration of carvacrol (88.17%) in the PAEO obtained in the present study (Table 2) was greater than the variation already described in literature - from 28 to 70%. Compounds such as  $\gamma$ -terpinene,  $\rho$ -ciment and caryophyllene oxide have been found in smaller amounts when compared to data from this study [17, 31, 32]. Phytocompounds variation in essential oils may be related to the time of botanical material collection,



**Fig. 1** DNA Plasmid electrophoresed on 1.5% agarose gel. Lane 1: DNA Ladder. DNA Plasmid 12,000 bp in strains 2 (Lane 2), 3 (Lane 3), 6 (Lane 5), 8 (Lane 6), 9 (Lane 7), 12 (Lane 8) and 20 (Lane 13)

region, soil and seasons [33]. Although detected in small quantities (Table 2), it is known that p-cymene boosts the pharmacological effects of carvacrol [34].

All strains ( $n = 35$ ) of *S. aureus* were sensitive to PAEO and carvacrol. Most strains isolated from milk showed sensitivity to tetracycline with halos of 21–25 mm ( $n = 11$ ; 91.6%) (Table 3). When subjected to the test with carvacrol, 100% ( $n = 12$ ) demonstrated halos size of an equal sensitivity or greater than those of positive control. Moreover, inhibition halos of an equal or greater than those of positive control were observed in 66.6% of the tested strains from milk with OEPA. *S. aureus* strains isolated from shrimp showed sensitivity to tetracycline and most of them presented halos between 31 to 40 mm ( $n = 13$ , 56.52%) in size (Table 3). When tested with carvacrol and OEPA, 39.13% ( $n = 9$ ) and 13.04% ( $n = 3$ ) strains showed, respectively, halos sizes greater than or equal to control. Thus, the carvacrol was also more effective when used against the strains of shrimp samples. In this study, the antimicrobial activity of PAEO seems to be related to the concentration of carvacrol

(88.17%), whereas all strains were susceptible to both compounds tested and the largest halos were observed when using carvacrol.

Providing support to the findings of this research, Ajitha et al. (2014) [35] found potential inhibition of the species *P. amboinicus* against *Staphylococcus* spp., *Escherichia coli*, *Pseudomonas* spp. and *Bacillus* spp. The authors describe halos measuring around 10 mm, i.e., values lower than those found in the present study.

Rodrigues et al. (2013) [36] evaluated the antimicrobial activity of OEPA containing 67.9% carvacrol, and found that the MIC against *S. aureus* ATCC 12692 ( $0.128 \text{ mg mL}^{-1}$ ) was higher than MIC against the strain of multidrug-resistant *S. aureus* ( $0.032 \text{ mg mL}^{-1}$ ). In the present study, it was observed that the MIC for OEPA OVRSA strain ( $0.5 \text{ mg mL}^{-1}$ ) was higher than the MIC for *S. aureus* ATCC 6538 ( $0.25 \text{ mg mL}^{-1}$ ). The difference between the two studies may be related to the chemical composition of the oils, since the OEPA obtained by Rodrigues et al., (2013) [36] had 13 compounds including thymol (1.3%) and thymol methyl ether (1.6%), while the OEPA used in the present study contained seven constituents, with a concentration of carvacrol (88.17%) higher.

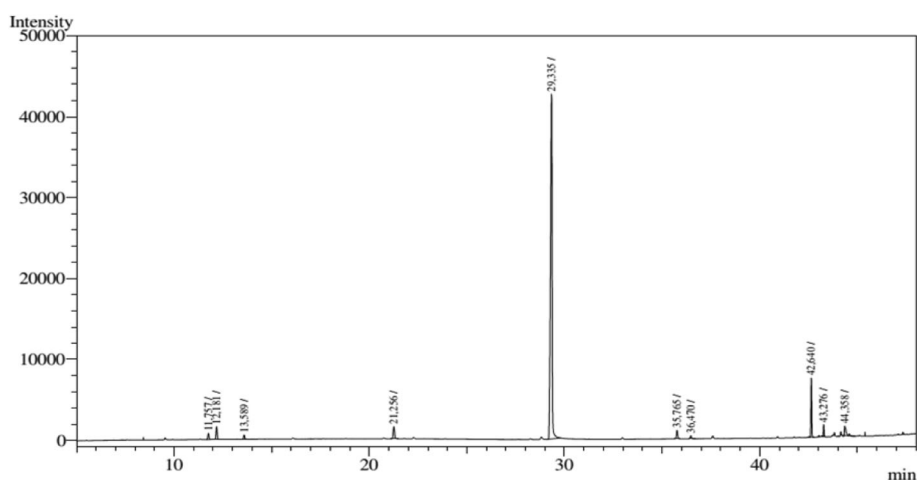
Regarding the CIM carvacrol, the same value ( $0.5 \text{ mg mL}^{-1}$ ) was observed when both strains (*S. aureus* ATCC 6538 and OVRSA) were tested (Table 4). Carvacrol and thymol present the ability to dissolve themselves in the cytoplasmic membrane, aligning the fatty acid chains and providing an increase in the passive permeability of the membrane.

Considering the values of CBM, OEPA and carvacrol showed better results against the OVRSA strain (Table 4). This finding is of utmost importance, since it demonstrates a promising potential of those two bioactive substances as antimicrobial agents tested against medically resistant strain. Hosseinkhani et al. (2016) [37] also obtained promising results when testing the antibacterial activity

**Table 2** Chemical characterization of the essential oil extracted from leaves of *Plectranthus amboinicus*

Components	CRI	RI <sub>LIT</sub>	RT <sub>CG/FID</sub>	%
p-cymene	1029	1026	11.757	0.72
1,8-cineol	1037	1031	12.181	2.01
Gamma-terpinene	1063	1059	13.589	0.45
4-terpineol	1184	1177	21.256	1.78
Carvacrol	1303	1299	29.335	88.17
Alpha-cisbergamoteno	1420	1412	35.765	0.84
NI	1435	–	36.470	0.17
Caryophyllene oxide	1591	1583	42.640	5.85
Total				99.99%

CRI calculated retention index, RI<sub>LIT</sub> retention index of the literature, RT retention time of CG-FID, NI unidentified



**Fig. 2** CG-MS chromatogram analysis of *Plectranthus amboinicus* essential oil

of an essential oil rich in monoterpenes against multi-drug resistant strains of *S. aureus*. According to the authors, the results may indicate the possibility of using monoterpenes incorporated into antimicrobial formulations in order to treat infections caused by multi-resistant bacteria.

In the present study, the isolated carvacrol demonstrated a bactericidal activity against both strains (ATCC and OVRSA) twice as effective when compared to OEPA. This fact may be explained considering that biological action of essential oils is probably related to the synergy between all its compounds, and not only to the action of their main constituent [38]. Sokovic et al. (2010) [39] tested several essential oils with therapeutic potential and also noted that the carvacrol compound had higher activity against *S. aureus* when compared with essential oils.

Souza et al. (2013) [40] investigated the influence of carvacrol on *S. aureus* strains isolated from food, and the monoterpenes strongly interfered in the permeability of the plasmic membrane, in halotolerance, in coagulase

activities, and in the production of enterotoxins. In addition, the authors report that the damage caused by carvacrol in the membrane is accompanied by important changes in the surface cells of *S. aureus*.

Figure 3 shows the biofilm reduction of two strains of *S. aureus* caused by the use of OEPA and carvacrol. All OEPA and carvacrol concentrations had inhibitory effect on the biofilm of ATCC strains, as a statistically significant difference ( $p < 0.001$ ) was observed when compared to the negative control. Regarding the OVRSA strain, all carvacrol concentrations showed an inhibitory effect on the biofilm, but complete inhibition was detected at a concentration of  $0.25 \text{ mg mL}^{-1}$ . The OEPA had inhibitory effect against biofilm OVRSA when used concentration of  $0.25 \text{ mg mL}^{-1}$ , but only completely inhibited biofilm at a concentration of  $0.5 \text{ mg mL}^{-1}$ . Studies often relate the effect of inhibiting biofilm formation OEPA and carvacrol to: (1) damage to the cell envelope, leading to cell death and to the ability to attach biofilms; and (2) a reduction in the expression of quorum sensing activation of genes, due to decreased bacterial density caused by a delay in the development of cell growth in planktonic cells [41, 42]. Yadav et al. (2015) [43] found activity of carvacrol in combination with eugenol against

**Table 3** Distribution of the *Staphylococcus aureus* strains according to inhibition zone size against the essential oil *Plectranthus amboinicus*, carvacrol and tetracycline

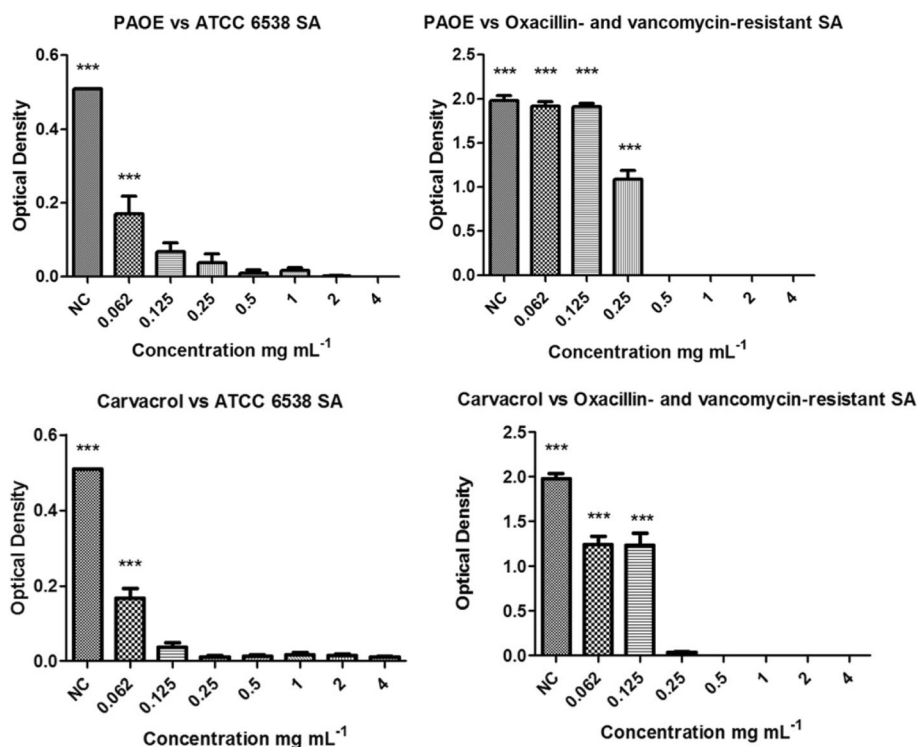
Inhibition halos interval (mm)	Shrimp (n = 23)			Milk (n = 12)		
	PAEO	Carvacrol	TCY	PAEO	Carvacrol	TCY
16–20	0	0	1	4	0	1
21–25	4	2	3	3	1	11
26–30	16	12	6	5	3	0
31–35	2	4	12	0	8	0
36–40	1	3	1	0	0	0
41–42	0	2	0	0	0	0

PAEO *Plectranthus amboinicus* essential oil. TCY tetracycline  $30 \text{ }\mu\text{g}$ . n number of bacterial strains. mm millimeters

**Table 4** Minimum Inhibitory Concentration (MIC  $\text{mg mL}^{-1}$ ) and Minimum Bactericidal Concentration (MBC  $\text{mg mL}^{-1}$ ) of *Plectranthus amboinicus* essential oil and carvacrol

Substances	Activity antimicrobial	<i>Staphylococcus aureus</i>	
		ATCC 6538	OVRSA
PAEO	MIC	0.25	0.5
	MBC	2	1
Carvacrol	MIC	0.25	0.25
	MBC	1	0.5

ATCC American Type Culture Collection, OVRSA Oxacillin and vancomycin-resistant *Staphylococcus aureus*



**Fig. 3** Optical density of bacterial biofilm in *S. aureus* ATCC 6538 and *S. aureus* isolated from food, treated with OEPA and carvacrol in varying concentrations (0.062, 0.125, 0.25, 0.5, 1, 2 and 4 mg mL<sup>-1</sup>). ANOVA, followed by Student-Newman-Keulstest \*\*\* $p < 0.001$  vs. all variables.\*SA: *Staphylococcus aureus*. NC: negative control

Methicillin-Resistant and Methicillin-Sensitive *Staphylococcus aureus* biofilm.

Arfa et al. (2006) [44] relate antibiofilm activity of carvacrol with the presence of free hydroxyl group, as carvacrol without this composition does not show antibacterial activity. In addition, the mixture of free hydroxyl with other chemical compounds enhances the effect against biofilm carvacrol [45]. Although its mechanism is not fully understood, evidences indicate that its antimicrobial activity is due to: (1) Increase the permeability of the cytoplasmic membrane, silted H<sup>+</sup> into the cell by binding to the hydroxyl group and (2) modification of fatty membrane acids [34].

## Conclusions

Our results show the antibacterial and antibiofilm potential of PAEO and carvacrol against the *S. aureus* standard strain and, especially, against oxacillin- and vancomycin-resistant *S. aureus*. Considering that *P. amboinicus* is a plant used in popular medicine practices in Brazil, more studies on the in vivo antibacterial and toxicity are recommended.

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## Availability of data and materials

All data has been provided in the paper.

## Authors' contributions

HMM collected the plants, was involved in the preparation of the essential oil. SECBV and TTAC were involved in the microbiological analysis as well writing of the manuscript. FEACJ and MGC were involved in the chemical analysis of the essential oil (PAEO). FGM and OVS were involved in the extraction and analysis of plasmids. RAC did the conception, supervised the work and corrected the final manuscript. All authors read and approved the final manuscript.

## Ethics approval and consent to participate

Not applicable.

## Consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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## References

- Ayele Y, Gutema FD, Edao BM, Girma R, Tufa TB, Beyene TJ, Tadesse F, Geloye M, Beyi AF. Assessment of *Staphylococcus aureus* along milk value chain and its public health importance in Sebeta, central Oromia, Ethiopia. *BMC Microbiol.* 2017;17:141.
- Song M, Bai Y, Xu J, Carter MQ, Shi C, Shi X. Genetic diversity and virulence potential of *Staphylococcus aureus* isolates from raw and processed food commodities in Shanghai. *Int J Food Microbiol.* 2015;195:1–8.
- Arfatahery N, Davoodabadi A, Abedimohasab T. Characterization of toxin genes and antimicrobial susceptibility of *Staphylococcus aureus* isolates in fishery products in Iran. *Sci Rep.* 2016;6:34216.
- Cheng VC, Wong SC, Ho PL, Yuen KY. Strategic measures for the control of surging antimicrobial resistance in Hong Kong and mainland of China. *Emerg Microbes Infect.* 2015;4:e8.
- Roca I, Akova M, Baquero F, Carlet J, Cavalieri M, Coenen S, Cohen J, Findlay D, Gyssens I, Heuer OE, Kahlmeter G, Kruse H, Laxminarayan R, Liébana E, López-Cerero L, MacGowan A, Martins M, Rodríguez-Baño J, Rolain JM, Segovia C, Sigafoos B, Tacconelli E, Wellington E, Vila J. The global threat of antimicrobial resistance: science for intervention. *New Microbes New Infect.* 2015;6:22–9.
- Di Cesare A, Luna GM, Vignaroli C, Pasquaroli S, Tota S, Paroncini P, Biavasco F. Aquaculture can promote the presence and spread of antibiotic-resistant Enterococci in marine sediments. *PlosOne.* 2013;8:e62838.
- Tajbakhsh E, Khamesipour F, Ranjbar R, Ugwu IC. Prevalence of class 1 and 2 Integrons in multi-drug resistant *Escherichia coli* isolated from aquaculture water in ChaharmahalVaBakhtiari province, Iran. *Ann Clin Microbiol Antimicrob.* 2015;14:27.
- Yang Q, Tian T, Niu T, Wang P. Molecular characterization of antibiotic resistance in cultivable multidrug-resistant bacteria from livestock manure. *Environ Pollut.* 2017;229:188–98.
- Atanasov AG, Waltenberger B, Pferschy-Wenzling EM, Linder T, Wawrosch C, Uhrin P, Temml V, Wang L, Schwaiger S, Heiss EH, Pollinger JM, Schuster D, Breuss JM, Bochkov V, Mihovilovic MD, Kopp B, Bauer R, Dirsch VM, Stuppner H. Discovery and resupply of pharmacologically active plant-derived natural products: a review. *Biotechnol Adv.* 2015;33:1582–614.
- Waldia S, Joshi BC, Pathak U, Joshi MC. The genus *Plectranthus* in India and its chemistry. *Chem Biodivers.* 2011;8:244–52.
- Daglia M. Polyphenols as antimicrobial agents. *Curr Opin Biotechnol.* 2012; 23:174–81.
- Kiraithe MN, Nguta JM, Mbaria JM, Kiama SG. Evaluation of the use of *Ocimum suave* Willd. (Lamiaceae), *Plectranthus barbatus* Andrews (Lamiaceae) and *Zanthoxylum chalybeum* Engl. (Rutaceae) as antimalarial remedies in Kenyan folk medicine. *J Ethnopharmacol.* 2016;178:266–71.
- Crevelin EJ, Caixeta SC, Dias HJ, Groppo M, Cunha WR, Martins CH, Crotti AE. Antimicrobial activity of the essential oil of *Plectranthus neochilus* against Cariogenic bacteria. *Evid Based Complement Alternat Med.* 2015;2015:102317.
- Swamy MK, Arumugam G, Kaur R, Ghasemzadeh A, Yusoff MM, Sinniah UR. GC-MS based metabolite profiling, antioxidant and antimicrobial properties of different solvent extracts of Malaysian *Plectranthus amboinicus* leaves. *Evid Based Complement Alternat Med.* 2017;2017:1517683.
- Arumugam G, Swamy MK, Sinniah UR. *Plectranthus amboinicus* (Lour.) Spreng: botanical, Phytochemical, pharmacological and nutritional significance. *Molecules.* 2016;21:369.
- Swamy MK, Akhtar MS, Sinniah UR. Antimicrobial properties of plant essential oils against human pathogens and their mode of action: an updated review. *Evid Based Complement Alternat Med.* 2016;3012462
- Santos NO, Mariane B, Lago JHG, Sartorelli P, Rosa W, Soares MG, Silva AM, Lorenzi H, Vallim MA, Pascon RC. Assessing the chemical composition and antimicrobial activity of essential oils from Brazilian plants—*Eremanthus erythropappus* (Asteraceae), *Plectranthus barbatus*, and *P. amboinicus* (Lamiaceae). *Molecules.* 2015;20:8440–52.
- Oliveira FF, Torres AF, Gonçalves TB, Santiago GM, Carvalho CB, Aguiar MB, Camara LM, Rabenhorst SH, Martins AM, Valença Junior JT, Nagao-Dias AT. Efficacy of *Plectranthus amboinicus* (Lour.) Spreng in a murine model of methicillin-resistant *Staphylococcus aureus* skin abscesses. *Evid Based Complement Alternat Med.* 2013;2013:291592.
- CLSI. Clinical and laboratory standards institute, Performance standards for antimicrobial susceptibility testing; twenty-fifth informational supplement, vol. M100-S25; 2012. p. 25–3.
- Adams RP. Identification of essential oils by gas chromatography/mass spectrometry. 4th ed. Carol Stream: Allured Publishing Corporation; 2007.
- Stepanovic S, Vukovic D, Dakic I, Savic B, Svabic-vlahovic M. A modified microtiter-plate test for quantification of staphylococcal biofilm formation. *J Microbiol Methods.* 2000;40:175–9.
- Cavalcante TT, Anderson Matias da Rocha B, Alves Carneiro V, Vassiliepe Sousa Arruda F, Fernandes do Nascimento AS, Cardoso Sá N, do Nascimento KS, Sousa Cavada B, Holanda Teixeira E. Effect of Lectins from *Diocleinae* Subtribe against oral *Streptococci*. *Molecules.* 2011;16:3530–43.
- Baumgartner A, Niederhauser I, Johler S. Virulence and resistance gene profiles of *Staphylococcus aureus* strains isolated from ready-to-eat foods. *J Food Prot.* 2014;77:1232–6.
- Patchanee P, Tadee P, Arjkumpa O, Love D, Chanachai K, Alter T, Hinjoy S, Tharavichitkul P. Occurrence and characterization of livestock-associated methicillin-resistant *Staphylococcus aureus* in pig industries of northern Thailand. *J Vet Sci.* 2014;15:529–36.
- Noor R, Hasan MF, Rahman MM. Molecular characterization of the virulent microorganisms along with their drug-resistance traits associated with the export quality frozen shrimps in Bangladesh. *Springerplus.* 2014;3:469.
- Dumitrescu O, Dauwalder O, Boisset S, Reverdy MÉ, Tristan A, Vandenesch F. Résistance aux antibiotiques chez *Staphylococcus aureus* les points-clés 2010. *Med Sci.* 2010;26:943–9.
- Spagnolo AM, Orlando P, Panatto D, Amicizia D, Perdelli F, Cristina ML. *Staphylococcus aureus* with reduced susceptibility to vancomycin in healthcare settings. *J Prev Med Hyg.* 2014;55:137–44.
- Akindolire MA, Babalola OO, Ateba CN. Detection of antibiotic resistant *Staphylococcus aureus* from milk: a public health implication. *Int J Environ Res Public Health.* 2015;12:10254–75.
- Nunes RS, Souza CP, Pereira KS, Del Aguilá EM, Paschoalin VM. Identification and molecular phylogeny of coagulase-negative staphylococci isolates from Minas Frescal cheese in southeastern Brazil: Superantigenic toxin production and antibiotic resistance. *J Dairy Sci.* 2016;16:00084–9.
- McCarthy AJ, Lindsay JA. The distribution of plasmids that carry virulence and resistance genes in *Staphylococcus aureus* is lineage associated. *BMC Microbiol.* 2012;12:104.
- Murthy PS, Ramalakshmi K, Srinivas P. Fungitoxic activity of Indian borage (*Plectranthus amboinicus*) volatiles. *Food Chem.* 2009;114:1014–8.
- Senthilkumar A, Venkatesalu V. Chemical composition and larvicidal activity of the essential oil of *Plectranthus amboinicus* (Lour.) Spreng against *Anopheles stephensi*: a malarial vector mosquito. *Parasitol Res.* 2010;107: 1275–8.
- Figueiredo AC, Barroso JG, Pedro LG, Scheffer JJC. Factors affecting secondary metabolite production in plants: volatile components and essential oils. *Flavour Fragr J.* 2008;23:213–26.
- Hyltdgaard M, Mygind T, Meyer RL. Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. *Front Microbiol.* 2012;3:12.
- Ajitha B, Ashok Kumar Reddy Y, Sreedhara Reddy P. Biosynthesis of silver nanoparticles using *Plectranthus amboinicus* leaf extract and its antimicrobial activity. *Spectrochim Acta A Mol Biomol Spectrosc.* 2014;128:257–62.
- Rodrigues FFG, Costa JGM, Rodrigues, FFG, Campos, AR. Study of the interference between *Plectranthus* species essential oils from Brazil and aminoglycosides. *J Evid Based Complementary Altern Med.* 2013;2013: 724161.
- Hosseinkhani F, Jabalameli F, Banar M, Abdellahi N, Taherikalani M, Leeuwen WB, Emaneini M. Monoterpene isolated from the essential oil of *Trachyspermum ammi* is cytotoxic to multidrug-resistant *Pseudomonas aeruginosa* and *Staphylococcus aureus* strains. *Rev Soc Bras Med Trop.* 2016;49:172–6.
- Yap PSX, Yiap BC, Ping HC, Lim SHE. Essential oils, a new horizon in combating bacterial antibiotic resistance. *Open Microbiol J.* 2014;8:6–14.
- Soković M, Glamočlija J, Marin PD, Brkić D, Griensven LJD. Antibacterial effects of the essential oils of commonly consumed medicinal herbs using an *in vitro* model. *Molecules.* 2010;15:7532–46.
- Souza EL, Oliveira CEV, Stamford TLM, Conceição ML, Gomes Neto NJ. Influence of carvacrol and thymol on the physiological attributes, enterotoxin production and surface characteristics of *Staphylococcus aureus* strains isolated from foods. *Braz J Microbiol.* 2013;44:29–35.



41. Gonçalves TB, Braga MA, Oliveira F, Santiago GMP, Carvalho CBM, Cabral PB, Santiago T, Sousa JS, Barros E, Nascimento RF. Effect of subinhibitory and inhibitory concentrations of *Plectranthus amboinicus* (Lour.) Spreng essential oil on *Klebsiella pneumoniae*. *Phytomedicine*. 2012;19:962–8.
42. Espina L, Pagán R, López D, García-Gonzalo D. Individual constituents from essential oils inhibit biofilm mass production by multi-drug resistant *Staphylococcus aureus*. *Molecules*. 2015;20:11357–72.
43. Yadav MK, Chae S-W, Im GJ, Chung J-W, Song J-J. Eugenol: a phyto-compound effective against methicillin-resistant and methicillin-sensitive *Staphylococcus aureus* clinical strain biofilms. *Plos One*. 2015;10(3):e0119564.
44. Arfa BA, Combes S, Preziosi-Belloy L, Gontard N, Chalier P. Antimicrobial activity of carvacrol related to its chemical structure. *Lett Appl Microbiol*. 2006;43:149–54.
45. Cacciatore I, Di Giulio M, Fornasari E, Di Stefano A, Cerasa LS, Marinelli L, et al. Carvacrol Codrugs: a new approach in the antimicrobial plan. *PLoS One*. 2015;10:e0120937.

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