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Evaluation of antibacterial and toxicological activities of essential oil of *Ocimum gratissimum* L. and its major constituent eugenol

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A R T I C L E I N F O

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A B S T R A C T

The present study aimed to comparatively analyze the antibacterial activity, combined with antibiotics, and toxicological activity of the essential oil of *O. gratissimum* L. and its major compound, eugenol. The phytochemical analysis of the essential oil was performed by gas chromatography coupled with mass spectrometry. The minimum inhibitory concentration and combined antibiotic activity were performed by microdilution. *Drosophila melanogaster* was the model organism chosen to evaluate the toxicological response of substances. The chromatographic assay showed eugenol as the major compound of the essential oil of *O. gratissimum* L. The microbiological assays showed that in terms of antibacterial activity, both products showed similar activity, quite significant in Gram-negatives, such as those of strains of *Pseudomonas aeruginosa*. Similar results were verified in the modulatory activity, allowing the verification that the bioactivity performed by the essential oil of *O. gratissimum* L. is mainly influenced by its major constituent. Although the EC50 of the compounds varied subtly, the essential oil was less toxic compared to eugenol. The information obtained in the present study demonstrates that the essential oil of *O. gratissimum* L. is a promising antibacterial agent and better tolerated than its majority compound alone, in the model organism *D. melanogaster*.

1. Introduction

Medicinal plants have always been used in traditional medicine practices since the prehistoric period, becoming a centuries-old habit linked to many cultures (Badke et al.., 2011). The usefulness offered by medicinal plants in the treatment of diseases is associated with their secondary metabolites and their biological effects, which have been studied in recent years (Rimawi *et al.*, 2020).

The discovery of new drugs for the treatment of bacterial infections has ensured the extension of human life expectancy, minimizing the number of deaths (HE et al., 2020). Research for new drugs is essential, given the obsolescence of many drugs and their common side effects (Al Zuhairi et al., 2020). Thus, studies with essential oils, as well as the screening of phytochemicals and isolated compounds, have grown in recent years, due to the increase in bacterial resistance to antibiotics. (Achmit et al., 2021; Kuhn et al., 2019).

Essential oils are volatile compounds of different combinations and abundant in flowers, leaves and bark of different species (SWAMY et al., 2020), In addition, they can contain different concentrations of substances, such as monoterpenes, sesquiterpenes and phenylpropanoids,

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applicable in the industry, pharmacy and food (Kucukbay et al., 2014).

In this context, the Lamiaceae family has gained prominence due to its medicinal and cosmetic properties. It is estimated that this family has a total of 200 genera and more than 6900 species distributed throughout the world (Mohammadhosseini, 2017). Among these species, *Ocimum gratissimum* L. is an aromatic herb that has been introduced extensively in tropical and subtropical regions, originating in the African continent and subspontaneously in Brazil, mainly in the Northeast region (Sharma et al., 2011).

Borges et al. (2012) distinguished eugenol as the major constituent of *Ocimum gratissimum* L. This phenolic, aromatic and lipophilic compound showed substantial biological activities, including antibacterial effects (Zhang et al., 2018). The widespread use of medicinal plants and their chemical components can generate toxic responses (Cunha et al., 2015). Therefore, the *Drosophila melanogaster* (fruit fly) model is important in the evaluation of the pharmacological and toxicological effects offered by metabolites derived from medicinal plants (Zemolin et al., 2014).

Thus, this study characterized the chemical profile of the essential oil of *Ocimum gratissimum* L., evaluating its antibacterial effect and its activity associated with antibiotics. A similar analysis was performed for eugenol alone, comparing its effect with those presented for the essential oil. In addition, both substances were analyzed for their effects on inducing mortality or interfering with the negative geotaxis mechanism of fruit flies.

2. Materials and methods

2.1. Botanical material

Two hundred and 10 g (210 g) of *Ocimum gratissimum* L. leaves were collected in July 2020 at the Crato Municipal Nursery, located at the Chico Mendes Institute for Biodiversity Conservation – ICMBio, according to coordinates 7°14′27.7″S 39°25′01.4″W. The leaves were collected at 12:00 p.m., eugenol peak time, as stipulated by Borges and collaborators (2012). An excicata of the specimen was deposited at the Herbarium Dardano de Andrade de Lima – HCDAL of the Regional University of Cariri - URCA, Crato/CE, under number 14.389.

2.2. Essential oil extraction and obtaining the eugenol

The extraction of the essential oil of *Ocimum gratissimum* L. was carried out by the hydrodistillation method, using the Clevenger-type apparatus. The leaves were crushed, placed in a 5.0 L glass flask and placed in contact with 2.5 L of distilled water for subsequent boiling for 2 h. The oil was separated by adding sodium sulfate (Na_2SO_4) anhydrous and preserved in refrigeration (−4 °C) until its use. Eugenol (Sigma-Aldrich) was kindly provided by Dr. Francisco Assis Bezerra da Cunha from the Semi-Arid Bioprospecting and Alternative Methods Laboratory - LABSEMA, Regional University of Cariri (URCA), Crato/CE.

2.3. Chemical analysis of the essential oil of Ocimum gratissimum L

Analyzes in CG/MS were performed on the chromatograph (GCMS-QP2010 SE, AOC-5000 auto injector from SHIMADZU), following the following analysis conditions: Injector 250 °C; initial oven temperature of 50 °C, with a first heating ramp of 5 °C/min to 180 °C, remaining for 4 min; a second ramp of 10 °C/min to 260 °C, remaining for 10 min; Split ratio of 1:100; interface temperature: 250 °C and 250 °C source; mass range from 50 to 400 Da; ionization by electron impacts, 70 eV. For component chromatography, a DB5-MS (Agilent) column, 30 m × 0.25 mm with an inner film thickness of 0.25 μm and a stationary phase of diphenyl dimethylpolysiloxane and helium as carrier gas was used. The identification was carried out by comparing the mass spectra with those of the Nist08 libraries and comparison with the base records of the literature.

2.4. Preparation of the essential oil of Ocimum gratissimum L. And eugenol

In a test tube 10 mg of essential oil and 500 μL of DMSO were added. This solution was transferred to another tube and diluted in 9265 mL of sterile distilled water, resulting in a solution with a final concentration of 1,024 μg/mL that was used in all tests. The same process was repeated for eugenol.

2.5. Determination of the minimum inhibitory concentration (MIC)

The bacterial strains *Escherichia coli* ATCC 25922, *Escherichia coli* 06, *Klebsiella pneumoniae* ATCC 4352, *Pseudomonas aeruginosa* ATCC 9027, *Pseudomonas aeruginosa* 24, *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* 10 and *Streptococcus mutans* ATCC *0446* were seeded in Petri dishes containing HIA culture medium and incubated at 37 °C for 24 h.

Then, a sample of each culture was dragged and sterile dilution in test tubes containing saline, in triplicate. After this procedure, the turbidity was adjusted according to the McFarland 0.5 scale, by which the intensity of bacterial multiplication in liquid culture medium is determined, established by an increase in the particles that oppose the passage of light, causing opacity in the medium, which will increase as the bacteria grow, and the analysis is performed against a standard scale, which ranges from 0.5 to 10. A 100 μL aliquot of each bacterial inoculum (referring to 10% of the total solution) was transferred to a tube containing 900 μL of a 10% Brain and Heart Infusion Broth (BHI) solution.

Each well of a 96-well microdilution plate was filled with 100 μL of the solution formed, and then the essential oil was microdiluted in a 1:1 ratio, the concentrations ranged from 512 μg/mL to 8 μg/mL. A well with no essential oil added was used as a positive control of bacterial growth. The same process performed for the essential oil was repeated with eugenol. The reading of the Minimum Inhibitory Concentration (MIC) was performed by colorimetric method after 24 h of incubation at 37 °C in a bacteriological oven. Bacterial growth was analyzed by adding 20 μL of resazurin (0.4 mg/ml) to each well. After the addition of resazurin, the plates were incubated for 1 h at room temperature and after this period the colorimetric variation was observed, since there was no bacterial growth in the wells that remained blue, and there was bacterial growth in the wells that changed from blue to pink coloring (Gallucci et al., 2009.). The tests were performed in triplicate.

2.5.1. Evaluation of modulating antibiotic activity

To evaluate the activity associated with antibiotics, was used the method described by Coutinho and collaborators et al. (2010). The substances (chlorpromazine, eugenol and essential oil) were tested in their sub-inhibitory concentrations (MIC/8). Each substance was diluted in tubes containing 900 μL of BHI 10%, where 100 μL of the bacterial inoculum of the strains *Escherichia coli* 06, *Pseudomonas aeruginosa* 24 and *Staphylococcus aureus* 10 were added separately. 100 μL of the obtained concentration were placed in each well of the microdilution plate. Microdilutions were performed with antibiotics amikacin, ampicillin, norfloxacin and penicillin and the enzymatic inhibitor sulbactam (associated with Ampicillin), all of them with an initial concentration of 1,024 μL/mL. The last well of each plate was used as a growth control. The plates were incubated in an oven at 37 °C for 24 h and the minimum inhibitory concentrations of each tested substance were determined by colorimetric method, through the addition of resazurin (Gallucci et al., 2009). The tests were performed in triplicate.

2.6. Toxicity assay

2.6.1. Breeding and stocking of Drosophila melanogaster

Drosophila melanogaster (Harwich strain) was obtained from the National Species Stock Center, Bowling Green, OH. The flies were grown according to methodology described by Cunha et al. (2015) in 340 mL

glass containers and grown in medium containing: 83% corn mass, 4% sugar, 4% lyophilized milk, 4% soybean, 4% wheat or oat bran and 1% salt). When cooking the mixture, 1 g of Nipagin (Methylparaben) was added. After the mixture cooled in the growth flasks, 1 mL of a solution containing *Saccharomyces cerevisiae* was added.

The flies were grown in BOD type incubator at a temperature of 25 °C ± 1 °C, with light-dark cycles 12:12 h and a relative humidity of 60%.

2.6.2. Mortality assay

Mortality tests were performed in accordance with the methodology of Cunha et al. (2015). Adult flies, male and female, were placed in 130 mL containers (6 cm high and 6.5 cm in diameter). The pots were divided into control and test groups. One milliliter (1 mL) of 20% sucrose solution in distilled water was placed at the bottom of all control pots. The test groups consisted of pots that contained eugenol or essential oil of *Ocimum gratissimum* L. The fumigation methodology was adopted, where the compounds are added on a filter paper in the tube cap and thus are volatilized and absorbed. The concentrations of 1, 5 and 10 μL/mL of eugenol and essential oil of *O. gratissimum* were used, with a counting time of 1, 2, 3, 6, 9, 12, 24 and 48 h. The temperature of 25 °C ± 1 °C was maintained throughout the test, as well as the relative humidity of 60% in a BOD type incubator with a light-dark cycles 12: 12 h. The tests were performed in sextuplicate, with each pot containing a total of 20 flies.

2.6.3. Negative geotaxis assay

Damage to the locomotor system was determined by the negative geotaxis test, as described by Cunha et al. (2015). Live flies, after exposure to eugenol or essential oil of *O. gratissimum* at predetermined times, were taken to the bottom of the pots. After 1 min, the flies that reached 4 cm in height in the containers were counted and this procedure was repeated after the 1-min interval.

2.7. Statistical analysis

The antibacterial assays were performed in triplicates and the results were expressed as the geometric mean. Statistical hypothesis analysis was performed for the antibacterial assays using a Two-Way ANOVA followed by Bonferroni's post hoc test, using the GraphPad Prism 7.0 software. To analyze the toxicity data, a two-way ANOVA followed by a Tukey's multiple comparisons test were performed. No statistical differences using the same concentration as a function of time were observed.

3. Results

3.1. Chemical profile of Ocimum gratissimum L. essential oil

The yield of the essential oil of *Ocimum gratisismum* (EOOg) was 0.39%. Phytochemical characterization, performed by mass chromatography, identified the presence of 10 compounds, as can be seen in Fig. 1.

When analyzing the constituents individually, it is observed that eugenol (63.25%), cineole (10.83%), beta-silinene (8.43%) and *trans*-caryophyllene (6.85%) are the major components (Table 1).

3.2. Minimum inhibitory concentration (MIC)

The values obtained for the MICs of each substance were considered clinically significant when less than 1,000 μg/mL (Houghton et al., 2007). The Minimum Inhibitory Concentration (MIC) obtained in the tests with eugenol (EUG) demonstrated its action against the strains: *Klebsiella pneumoniae* ATCC 4352, *Pseudomonas aeruginosa* ATCC 9027, *Pseudomonas aeruginosa* 24, *Staphylococcus aureus* ATCC 25923 and *Streptococcus mutans* ATCC 0446 (Table 2a).

Following the analysis of antibacterial activity, the EOOg showed results similar to those obtained by the EUG, with clinically significant responses evidenced against the same strains tested (Table 2b).

3.3. Evaluation of the associated activity of eugenol and essential oil of Ocimum gratissimum L

An evaluation of the associated activity of EUG and EOOg with antibiotics was also carried out. In addition, the effect of the combination of chlorpromazine (CPMZ), associated with the antibiotics of interest, and sulbactam (SULB) in combination with ampicillin was also tested. The results obtained in this analysis were distributed in sections, organized according to pairs of antibiotics, which act on the external and internal portion of the bacterial cell.

Fig. 2a and b represent the results of the combination of the compounds mentioned above, against the *Escherichia coli* 06 strain. A possible synergistic effect was evidenced in the association between CPMZ + norfloxacin, and EUG + norfloxacin. The same could be seen with amikacin, when associated with EUG and EOOg. On the other hand, CPMZ when associated with amikacin, caused an increase in MIC, resulting in a possible antagonistic phenomenon (Fig. 2a).

It was observed that the combination of sulbactam and ampicillin caused a reduction in MIC, from 1,024 μg/mL to 128 μg/mL, possibly potentiating the activity of ampicillin against the strain tested (Fig. 2b).

Fig. 2c and d shows a combined activity of the antibiotics norfloxacin, amikacin, penicillin and ampicillin, for the bacteria

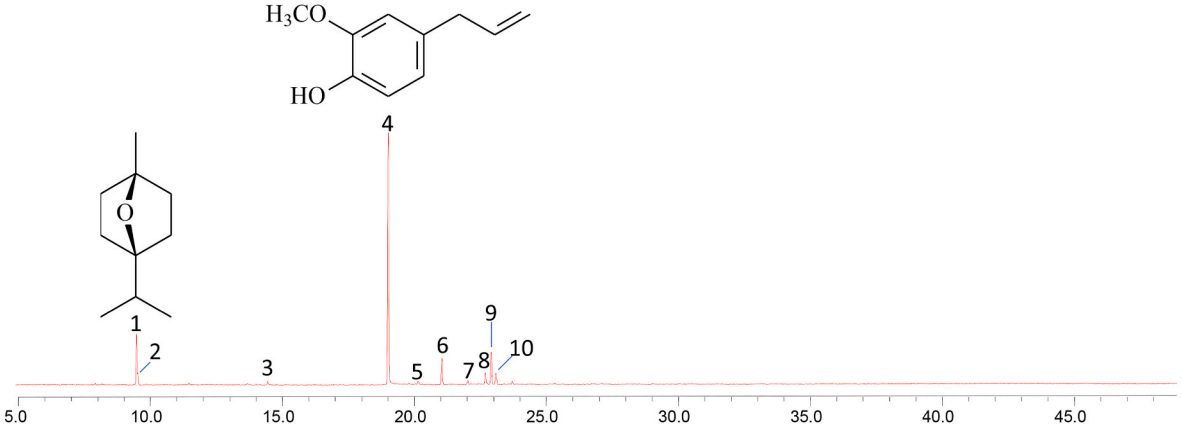

Fig. 1. Chromatogram CG-MS of the essential oil of *Ocimum gratissimum* L. (1) 1,8-cineole-eucalytol; (2) *Cis*-ocimene; (3) 1-alpha-terpineol; (4) Eugenol; (5) Beta-element; (6) *Trans*-caryophyllene; (7) Alpha-humulene; (8) Germacrene-D; (9) Beta-selinene; (10) Alpha-selinene.

Table 1
Relative composition of *Ocimum gratissimum* L. essential oil.

Nº	RT	Similarity (%)	Constituent	Kovats Index	Molecular Formula	Absolute Area	Relative Area
1	9.525	95	1,8-cineole eucalytol	991	$C_{10}H_{18}O$	648767	10,83%
2	9.574	93	*Cis*-ocimene	1050	$C_{10}H_{16}$	112333	1,88%
3	14.428	93	l-alpha-terpineol	1189	$C_{10}H_{18}O$	50805	0,85%
4	18.955	97	Eugenol	1356	$C_{10}H_{12}O_2$	3787946	63,25%
5	20.077	91	Beta-elemene	1375	$C_{15}H_{24}$	50833	0,85%
6	20.959	96	*Trans*-caryophyllene	1419	$C_{15}H_{24}$	410342	6,85%
7	21.921	94	Alpha-humulene	1438	$C_{15}H_{24}$	60036	1,00%
8	22.585	96	Germacrene-D	1480	$C_{15}H_{2}4$	182146	3,04%
9	22.810	95	Beta-selinene	1485	$C_{15}H_{24}$	504974	8,43%
10	22.985	94	Alpha-selinene	1494	$C_{15}H_{24}$	180493	3,01%

*RT – Retention Time.

Table 2
Minimum inhibitory concentration (MIC) of eugenol (a) and essential oil of *Ocimum gratissimum* L. (b) against bacterial strains.

	STRAINS	MIC (μg/mL)
(a) EUGENOL	*Escherichia coli* ATCC 25922	1024
	Escherichia coli 06	1024
	Klebsiella pneumoniae ATCC 4352	512
	Pseudomonas aeruginosa ATCC 9027	406.37
	Pseudomonas aeruginosa 24	322.54
	Staphylococcus aureus ATCC 25923	203.19
	Staphylococcus aureus 10	1024
	Streptococcus mutans ATCC *0446*	322.54
(b) ESSENTIAL OIL	*Escherichia coli* ATCC 25922	1024
	Escherichia coli 06	1024
	Klebsiella pneumoniae ATCC 4352	645.08
	Pseudomonas aeruginosa ATCC 9027	812.75
	Pseudomonas aeruginosa 24	512
	Staphylococcus aureus ATCC 25923	80.63
	Staphylococcus aureus 10	1024
	Streptococcus mutans ATCC 0446	812.75

Pseudomonas auruginosa 24. Chlorpromazine continued to intensify the action of norfloxacin, as well as with *Escherichia coli* 06. This possible synergistic effect was also evidenced in the association CPMZ + amikacin and EUG + amikacin. However, the EUG + norfloxacin combination was possibly antagonistic, with a corresponding increase in the inhibitory concentration of the antibiotic against the strain tested. This information can be seen in Fig. 2c.

Sulbactam continued to intensify the action of ampicillin, by reducing the MIC from 512 μg/mL to 4 μg/mL, on the other hand, there was a possible antagonistic process in the association of this antibiotic with the EUG, given the increase in MIC from 512 μg/mL to 812.7 μg/mL (Fig. 2d).

Fig. 2e shows a possible antagonism in the combination of norfloxacin + CPMZ and norfloxacin + EUG against *Staphylococcus aureus*10. This antagonism was repeated for amikacin + CPMZ and amikacin + EUG. However, there was an intensification of amikacin activity, when combined with essential oil, similarly to what happened in the *Escherichia coli* 06 strain.

Interestingly, EUG and EOOg caused a possible synergistic effect (reduction of MIC from 1,024 μg/mL to 161.3 μg/mL and 128 μg/mL, respectively) when combined with penicillin against *Staphylococcus aureus* 10 (Fig. 2f) which it did not happen with the strains *Escherichia coli* 06 and *Pseudomonas aeruginosa* 24. In view of the Gram-positivity of *Staphylococcus aureus*, it is assumed that this effect for penicillin is not prominent in Gram-negatives. The combination of sulbactam + ampicillin and EOOg + ampicillin also potentiated the control effect, the latter contrary to what was seen in the other strains.

3.4. Toxicological activity

The *Drosophila melanogaster* model was chosen to assess the toxicity of EUG and EOOg. For both, concentrations of 1, 5 and 10 μL/mL were used. EOOg showed a EC50 equal to 2,677 μL/mL after 6 h of exposure. The concentration of 1 μL/mL did not differ significantly when compared to the control. The concentration of 5 μL/mL showed greater toxicity after 48 h of contact. After 6 h of exposure to a concentration of 10 μL/mL of EOOg, it started to increase significantly, demonstrating the toxicity at this concentration. (Fig. 3a).

There was variation in the mortality pattern according to the EUG concentration used, which presented EC50 equivalent to 2,681 μL/mL, after 6 h of exposure. Concentrations of 1 and 5 μL/mL showed a significant degree of mortality after 48 and 24 h of exposure, respectively. On the other hand, the concentration of 10 μL/mL differed from the control after 6 h, being more toxic in relation to the others (Fig. 3b). Thus, EOOg was less offensive when compared to EUG.

The concentration of 1 μL/mL of EOOg was not significant in the impairment of *D. melanogaster* geotaxy. Fact that differs from that visualized for the concentration of 5 μL/mL, which caused an interference in the flight ability from 12 h of the beginning of the tests. The concentration of 10 μL/mL of EOOg caused the negative geotaxis of the fruit flies to be compromised right after the first 3 h of testing, with a substantial degree of impairment (Fig. 4a).

The eugenol also affected the negative geotaxis of *D. melanogaster*. Fig. 4b shows how much each concentration varied compared to the control. In this case, it is verified that the exposure to 1 μL/mL of the compound causes impairment of the mobility of the flies after 48 h of exposure, while the concentration of 5 μL/mL causes the same effect after 12 h of contact. The previous exposure to 10 μL/mL of EUG was even more toxic, in view of the negative geotaxis impairment even in the first counting time.

4. Discussion

Antibiotic resistance has become a serious public health problem in recent years. Some publications try to alert to the need to create research priorities, aiming to find new antibacterial molecules, because of the restricted therapeutics and the lack of new chemical structures, with efficient mechanisms of action (Carneiro et al., 2014; Duval; Grare, Demoré, 2019). Research such as the one carried out by Saraiva et al. (2021), who compared the intrinsic and antibacterial effect of *Laurus nobilis* essential oil and its major compound 1,8-cineole is important to delimit the activity of essential oils and isolated compounds, thus demonstrating which of these are more effective.

The main bacteria associated with the development of resistance mechanisms are Gram-negative, including *Escherichia coli*, *Klebsiella pneumoni*, *Pseudomonas aeruginosa* (Duval; Grare, Demoré, 2019), given the scenario presented above and in addition to the fact that since the production of new antibiotics is practically discontinued nowadays, studies like ours are essential, as they collaborate with the scientific dissemination of antibacterial substances, comparing them and presenting which one is the most expressive in terms of biological activity and tolerability in model organisms.

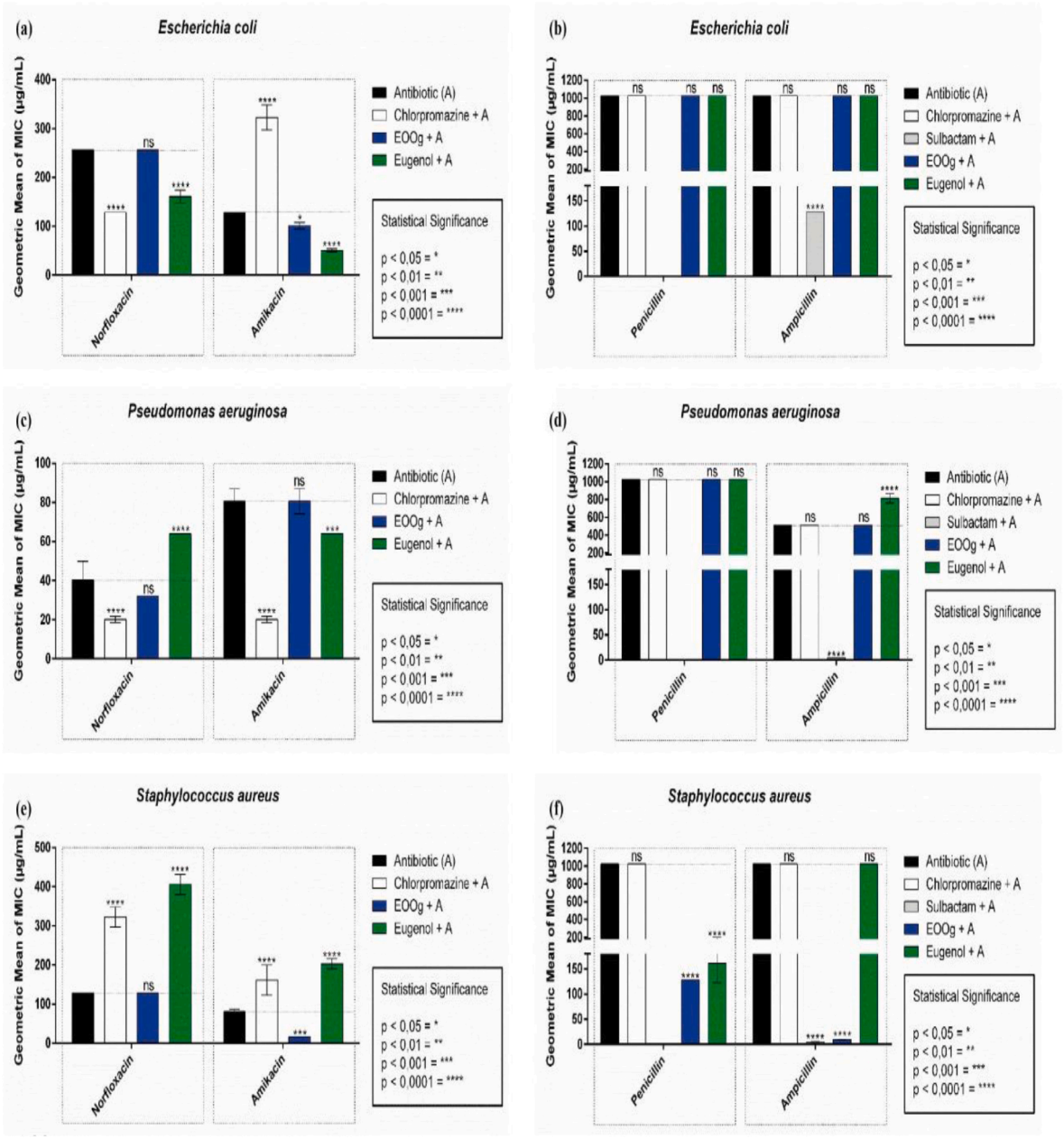

Fig. 2. Evaluation of antibiotics modifying activity (Amikacin, Ampicillin, Norfloxacin and Penicillin) by EUG and EOOg against bacterial strains (*Escherichia coli* 06, *Pseudomonas aeruginosa* 24 and *Staphylococcus aureus* 10).

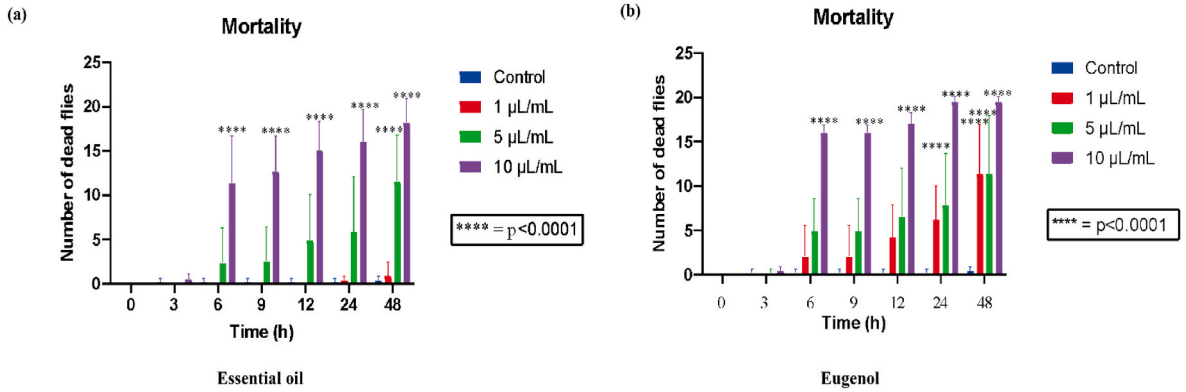

Fig. 3. Effect of Essential oil of Ocimum gratissimum L. (a) and eugenol (b) on the mortality test of *Drosophila melanogaster* at different concentrations and time.

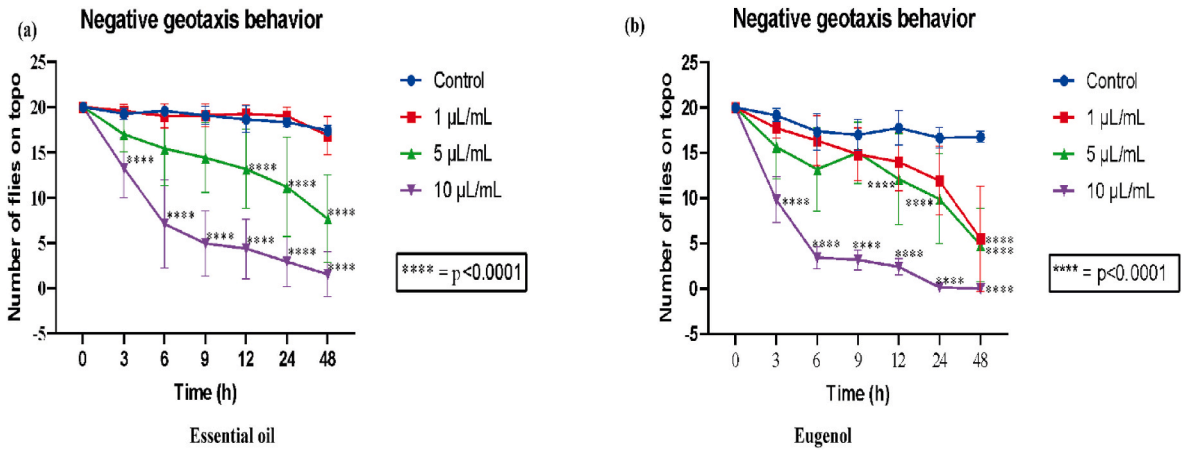

Fig. 4. Effect of the Essential oil of *Ocimum gratissimum* L. (a) and eugenol (b) on the negative geotaxis behavior of *Drosophila melanogaster* in different concentrations and time.

Pessoa et al. (2015) chemically characterized the essential oil of *O. gratissimum* L., distinguishing the following compounds: eugenol (67.30–74.99%), β -selinene (8.18–8.27%), β-ylangene (4 0.97 to 5.09%), viridiflorene (3.38–3.47%), 1,8-cineole (3.07–3.11%) and y-himachalene (2.00–2.62%). The relative composition of EOOg presented in this study was similar to that presented by Pessoa et al. (2015), given what was presented for eugenol (63.25%) and β-selinene (8.43%).

Catherine et al. (2012) mentioned the antibacterial effect of eugenol against *Escherichia coli* and *Staphylococcus aureus* bacteria. Its activity through the microtiter methodology was considerable, demonstrated through the following minimum inhibitory concentrations: *Bacillus cereus* (0.125 µg/mL), *Escherichia coli* (0.125 µg/mL), *Helicobacter pylori* (0.0312 µg/mL), *Staphylococcus aureus* (0.25 µg/mL) and *Streptococcus pyogenes* (0.5 µg/mL). A slightly higher but also significant MIC was obtained for *Pseudomonas aeruginosa* (8 µg/mL) (Jeyakumar & Lawrence, 2021).

Aguiar et al. (2015) examined the antibacterial action of EOOg against the standard and resistant strains of *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. In this one, only the MIC for the standard strain of *Staphylococcus aureus* was significant, which corroborates this study. In our study, EOOg was equally effective against standard strains of *Klebsiella pneumoniae*, standard and resistant strains of *Pseudomonas aeruginosa*, standard strains of *Staphylococcus aureus* and *Streptococcus mutans*. Joshi (2013) also demonstrated that the essential oil of *Ocimum gratissimum* L. has considerable activity against *Escherichia coli* and *Klebsiella pneumoniae*.

In the present study, we demonstrated that the antibacterial activity of the essential oil of *Ocimum gratissimum* L. and the isolated eugenol are equivalent, that is, both compounds showed a response against the same strains, namely: *Klebsiella pneumoniae* ATCC 4352, *Pseudomonas aeruginosa* ATCC 9027, *Pseudomonas aeruginosa* 24, *Staphylococcus aureus* ATCC 25923 and *Streptococcus mutans* ATCC 0446. The best activity was demonstrated for the bacterium *Pseudomonas aeruginosa*, since the substances showed activity against both standard and resistant strains. This result is quite significant since the aforementioned bacterium (Gram-negative) is a relevant opportunistic pathogen, directly associated with nosocomial infections (Tuon, Gortz, & Rocha, 2012).

EUG causes a disturbance in the lipid fraction of the bacterial membrane, causing a change in permeability, which facilitates the interaction of the compound with intracellular portions (Jeyakumar & Lawrence, 2021). The mechanism of action of EOOg is related to its majority composition, with the prevalence of eugenol, as described by Joshi (2013).

The evaluation of the combined activity was performed for the antibiotics amikacin, ampicillin, norfloxacin and penicillin, analyzing the effect of the interaction of EUG and EOOg with these drugs. Studies carried out with *Ocimum basilicum* L. showed that the association of its essential oil, rich in eugenol, with Neomycin and with Amikacin generates an intensifying effect, represented by the reduction of the MIC of these antibiotics against *Staphylococcus aureus* (NUNES et al., 2014). The same was verified in this study for the EOOg and even for the EUG.

Aguiar et al. (2015) studied the direct modulatory effect of the essential oil of *O. gratissimum* L. in association with the aminoglycosides amikacin and gentamicin. The significant results obtained in tests involving the bacteria *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were antagonistic. In this study, we present the possibility of synergism for amikacin (against *Escherichia coli* and *Staphylococcus aureus*), norfloxacin (against *Pseudomonas aeruginosa*), ampicillin and penicillin (against *Staphylococcus aureus*).

Synergistic processes occur through the inhibition of the sequence of a common biochemical pathway, as well as enzymes that protect microorganisms, due to the combination of active agents in the cell wall or their association with components that act in other pathways, which facilitates penetration into the cell (Santiesteban-López, Palou, & López-Malo, 2007).

The factors that cause antagonism in drug combinations are still poorly understood. However, it is known that the association of bacteriostatic and bactericidal agents, the combined use of compounds that compete for the same target in a microorganism and the molecular interactions between different active components, are the main reasons for the development of antagonistic processes (Goñi et al., 2009).

Eugenol acts by creating deformations in the bacterial membrane, impairing its integrity and morphology, which causes an overflow of the intracellular content and inactivation of the microorganism (Debao et al., 2019). In addition, there is also a predisposition of the EUG to bacterial genomic DNA, responsible for the aggregation of DNA molecules. Due to its lipophilic character, typical of monoterpenes, there is interaction with polysaccharides, fatty acids and lipids of the bacterial cell membrane, thus causing a disturbance in the permeable barrier (Limaverde et al., 2017).

A similar mechanism can be seen in the use of essential oils, capable of compromising the integrity of the bacterial membrane and the conformation of its cell wall, facilitating the influx of drugs (Matias et al., 2011). The hydrophobic compounds in these oils, such as eugenol, can adhere both to the plasma membrane and to phospholipids and membrane proteins, thus promoting the assimilation of antibiotics (Luz et al., 2014).

The conformation of the cellular envelope of microorganisms induces variations in the effectiveness of chemical components against bacteria. The thick layers of peptidoglycans in Gram-positive bacteria ensure greater sensitivity to bioactive components, which can interact with the bacterial envelope in different ways and degrees (Requena,

Vargas, & Chiralt, 2019). This phenomenon justifies the action of EOOg by potentiating the action of amikacin and penicillin against *Staphylococcus aureus* 10 and EUG by intensifying the action of penicillin against the same strain in this study.

Chlorpromazine, in non-antibacterial concentrations, has been used as an agent capable of inhibiting efflux pumps, Coutinho et al. (2010) discussed the MIC-lowering effect of chlorpromazine, noting its combined effect with amikacin, kanamycin and tobramycin, in addition to describing an antagonistic effect for gentamicin and neomycin, with data referring to tests with *Escherichia coli*. The combination of SULB with ampicillin considerably broadens the spectrum of action of the latter, against *Klebsiella pneumoniae* and *Staphylococcus aureus*, for example. Specific tests should be performed with both compounds, against strains that express active efflux proteins and the beta-lactamase enzyme, since the results presented in our study are not sufficient to infer the inhibition of possible resistance processes.

According to Zhang et al. (2016), oxygenated monoterpenes are agents of considerable toxicity against *Drosophila melanogaster*, which can be represented by the LC_{50} variation of 0.015 and 0.02 μl/L. This data agrees with that presented for the EUG in this study, taking into account the EC_{50} value equivalent to 2,681 μL/mL. This is associated with the specific characteristics of EUG, such as the presence and position of its hydroxyl group, as well as the characteristic positioning of its double bonds, which can influence differently the degree of mortality of fruit flies, as seen for α-pinene and β-pinene (Xie et al., 2014; Zhang et al., 2016).

The toxicological response of essential oils is related to the additive and synergistic effects of different compounds (Essoung et al., 2020). Tests carried out with *Ocimum tenuiflorum* showed its fumigant capacity against *S. oryzae*, by inhibiting the enzyme acetylcholinesterase (AChE). This action is represented by the LC_{50} value of 479 μL/L (Bhavya, Chandu, & Devi, 2018).

A similar effect was observed for EOOg in this study, whose mortality was similar to that of EUG, which is justified, as the literature describes EUG as one of the main compounds of *Ocimum gratissimum* L. In particular, the compounds present in essential oils confer characteristics volatiles, which facilitate their entry into the intracellular environment, the influx of terpenes, for example, can cause the formation of free radicals, leading to death (Hua et al., 2018).

The toxicological effect on the negative geotaxis of *Drosophila melanogaster* was prominent for EUG and EOOg. As a survival strategy in adverse situations, fruit flies increase the activity levels of the enzyme acetylcholinesterase (AChE), hydrolyzing acetylcholine and preventing it from accumulating under unfavorable environmental conditions or stress (Hu et al., 2019). Thus, the EUG and EOOg acted by inhibiting the action of AChE, allowing the stress of the flies to impair of their locomotor system.

The results of toxicological activity demonstrate that *O. gratissimum* L. essential oil is slightly more tolerated in the model organism *D. melanogaster* than eugenol, as seen in negative geotaxis and mortality assays. Previous studies did not mention the toxicity of EOOg using the chosen model, so the results obtained are also unprecedented for the essential oil. Toxicity tests are important, as the practice of using natural products and their inputs can be unsafe when overused (Duarte et al., 2018).

Considering the costs of hospitalization and treatment of patients affected by infections caused by resistant bacteria, demonstrating that EOOg is less toxic compared to EUG is a way to contribute to greater therapeutic assertiveness, considering the possibility of using EOOg in association with medications of the market, since the agility in choosing the drug to be used is a determining factor in cases of bacterial resistance (Limaverde et al., 2017).

5. Conclusion

Eugenol is the most expressive component of the essential oil of *Ocimum gratissimum* L. The antibacterial activity of the essential oil of *O. gratissimum* L. and eugenol was similar and promising against both standard and resistant strains of *Pseudomonas aeruginosa*, and the combined activity with antibiotics was also significant and similar for both substances. The evaluation of toxicological activity revealed that the essential oil of *O. gratissimum* L. is better tolerated by the model organism *Drosophila melanogaster*, which, comparatively, may collaborate with greater effectiveness in the possibility of administration combined with antibiotics. More studies are recommended, using other toxicity models.

Informed consent

Informed consent was obtained from all individual participants included in the study.

Ethical statement

This article does not contain any studies with animals performed by any of the authors.

Authorship contribution declaration

Júlio César Silva: Methodology, writing and validation. Raimundo Luiz Silva Pereira: Methodology. Thiago Sampaio de Freitas: Software. Janaína Esmeraldo Rocha: Validation. Nair Silva Macedo: Methodology. Carla de Fatima Alves Nonato: Methodology. Marina Leite Linhares: Formal analysis. Daniely Sampaio Arruda Tavares: Formal analysis. Francisco Assis Bezerra da Cunha: Resources, Supervision. Henrique Douglas Melo Coutinho: Conceptualization, Supervision. Sidney Gonçalo de Lima: Resources, Supervision. Francisco Nascimento Pereira Júnior: Writing-review and editing. Francisco Paulo Araujo Maia: Conceptualization, Supervision. Fabiola Fernandes Galvão Rodrigues: Conceptualization, Supervision. George Joaquim Garcia Santos: Conceptualization, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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