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Enhanced antibacterial effect of antibiotics by the essential oil of *Aloysia gratissima* (Gillies & Hook.) Tronc. and its major constituent beta-caryophyllene

Eduardo L. Santos [a], Priscilla R. Freitas [a], Ana Carolina J. Araújo [a], Ray S. Almeida [a], Saulo R. Tintino [a], Cicera Laura R. Paulo [a], Ana Cristina A. Silva [b], Luiz E. Silva [c], Wanderlei do Amaral [c], Cícero Deschamps [d], José Pinto Siqueira Junior [e], José Maria Barbosa Filho [f], Gabriela Ribeiro de Sousa [f], Jaime Ribeiro-Filho [g], Henrique D. M. Coutinho [a,*]

[a] *Department of Biological Chemistry, Regional University of Cariri - URCA, Crato, Brazil*
[b] *Maurício de Nassau College -UNINASSAU, Petrolina, PE, Brazil*
[c] *Post graduate Program in Sustainable Territorial Development, Federal University of Paraná – UFPR, Matinhos, Brazil*
[d] *Post graduate Program in Agronomy, Federal University of Paraná - UFPR, Curitiba, Brazil*
[e] *Department of Molecular Biology, Federal University of Paraiba-UFPB, João Pessoa 58051-900, PB, Brazil*
[f] *Departmen of Pharmacy, Federal University of Paraiba-UFPB, João Pessoa 58051-900, PB, Brazil*
[g] *Gonçalo Moniz Institute, Oswaldo Cruz Foundation (IGM-FIOCRUZ/BA), Salvador, BA, Brazil*

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ABSTRACT

Background: *Aloysia gratissima* (Verbenaceae), popularly known as bee-brush or whitebrush, has been widely used in Brazilian folk medicine as analgesic, expectorant and antimicrobial. Phytochemical studies have identified β-caryophyllene as one of the major components of the essential oil of this plant. This bioactive sesquiterpene has been shown to have anti-inflammatory and antibacterial activities.

Purpose: The present study aimed to characterize the chemical profile and evaluate the antibacterial and antibiotic-enhancing activities of the essential oil obtained from *Aloysia gratíssima* (EOAG) and β-caryophyllene.

Methods: The phytochemical analysis was performed by gas chromatography coupled to mass spectrometry (GC-MS). The antibacterial activity against *Pseudomonas aeruginosa* 24, *Staphylococcus aureus* 10, and *Escherichia coli* 06 was assessed by determining the minimum inhibitory concentration (MIC) using the broth microdilution method. The potentiation of the antibiotic activity by the EOAG and the β-caryophyllene was performed using the MIC evaluation of the antibiotic alone or in association with the essential oil and its main compound. To evaluate the minimum reduction of the drug concentration necessary, the Dose Reduction Index (DRI) was calculated.

Results: A reduction in the MIC of the antibiotics against strains treated simultaneously with the essential oil or β-caryophyllene was observed. The GC-MS analysis of the EOAG identified 30 compounds, including β-caryophyllene as the major component. Both OEAG and β-caryophyllene presented antibacterial activity against *S. aureus*, in addition to potentiating the action of norfloxacin against *S. aureus, P. aeruginosa*, and *E. coli*.

Conclusions: These substances also reversed the antibiotic resistance to gentamicin and erythromycin against of *S. aureus* and *P. aeruginosa*, respectively.

Abbreviations: analysis of variance, ANOVA; Colony forming unit, CFU; essential oil from *Aloysia gratíssima*, EOAG; flame ionization detector, FID; gas chromatography coupled to mass spectrometry, GC-MS; Minimal Inhibitory Concentration, MIC; multi-drug resistant, MDR.

* Corresponding author at: Rua Coronel Antônio Luíz, 1161 - Pimenta, Crato, CE, 63105-010, Brazil.
E-mail addresses: saulo.tintino@urca.br (S.R. Tintino), 011800513@prof.uninassau.edu.br (A.C.A. Silva), luizeverson@ufpr.br (L.E. Silva), wdoamaral@ufpr.br (W. do Amaral), cicero@ufpr.br (C. Deschamps), siqueira@dbm.ufpb.br (J.P.S. Junior), jbarbosa@ltf.ufpb.br (J.M.B. Filho), jaime.ribeiro@fiocruz.br (J. Ribeiro-Filho), hdmcoutinho@urca.br (H.D.M. Coutinho).

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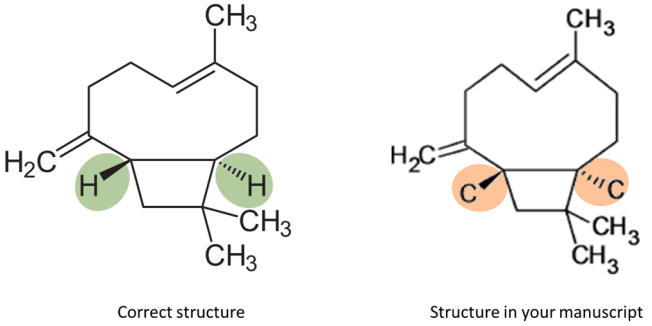

Fig. 1. Chemical structure of β-cariophyllene.

Introduction

Aloysia gratissima (Gillies & Hook.) Tronc. (Verbenaceae) is a plant popularly known as "erva santa" (holy herb). This species is widely used in Brazilian traditional medicine for the treatment of respiratory, digestive (Souza and Wiest, 2007) and central nervous system diseases (Silva et al., 2006; Zeni and Bosio, 2011). Studies have demonstrated that the essential oil of *A. gratissima* has unique pharmacological properties, including antimicrobial (Santos et al., 2015), leishmanicidal (Garcia et al., 2018), anticancer (Yang et al., 2016), sedative (Goleniowski et al., 2006) and larvicidal (Silva et al., 2014). In addition, due to the high content of compounds such as β-caryophyllene, spatulenol, β-pinene, the EOAG has been widely used in the composition of perfumes, foods, and beverages (Yadegarinia et al., 2006).

Previous research has identified β-caryophyllene in the essential oils of various plant species such as cloves, cinnamon, ginger, basil (Kumphune et al., 2011). This sesquiterpene was found to present anti-inflammatory, antioxidant, and antibacterial activities (Dahham et al., 2015; Dorman and Deans, 2000; Rodriguez et al., 2012). Accordingly, studies have demonstrated that many essential oils containing β-caryophyllene presented antibacterial effects against both Gram-positive and Gram-negative strains (Neta et al., 2017; Maia et al., 2010).

Bacterial resistance has become a global public health problem (Grillo et al., 2013). Studies have reported the emergence of multi-drug resistant (MDR) strains of clinically important bacteria such as *S. aureus, Klebsiella pneumoniae, P. aeruginosa* and *Escherichia coli* (Alarco, 2014; Korb et al., 2013; Pierozan et al., 2009), which has significantly hampered the treatment of infections with conventional antibiotics (Lima et al., 2015). *Staphylococcus aureus* is a Gram-positive bacterium with significant impact on public health (Foster et al., 2014). Evidence has indicated that *S. aureus* can encode proteins associated with the development of resistance to fluoroquinolones, penicillins, and cephalosporins. On the other hand, *E. coli* (Trabulsi and Alterthum, 2015) and *Pseudomonas aeruginosa* (Sterhling et al., 2010) are Gram-negative bacteria with remarkable pathogenicity and demonstrated resistance to antibiotics.

Antibiotic resistance occurs mainly through mutations in the bacterial chromosome or through the acquisition of plasmids or transposons containing resistance genes (Andersen, 2015). These phenomena can lead to the development of resistance mechanisms such as the acquisition of antibiotic-inactivating enzymes; decreased membrane permeability to an antibiotic; acquired changes in the target sites of an antibiotic and expression of efflux pumps, leading to antibiotic extrusion (Trabulsi and Alterthum, 2015).

Previous research has demonstrated that the essential oil, as well as the constituents previously identified in *A. gratissima*, have promising antimicrobial properties (Trovati et al., 2009) and therefore have the potential to be used in the development of new drugs useful in combating infections by resistant bacterial strains (Brito and Cordeiro, 2012; Rice, 2008).

By this fact, the aim of this work was to characterize the chemical profile and evaluate the antibacterial and the potentiating-antibiotic activity of, erythromycin and gentamycin alone and in association with the essential oil of *Aloysia gratissima* (EOAG) and its major compound β-caryophyllene (Fig. 1).

Table 1.
Origin and antibiotic resistance profile of the strains.

Bacterial strain	Origin	Resistance Profile
S. aureus 10	Rectum swab	Amc, Amox, Amp, Asb, Azy, Ca Cef, Cf, Cip, Cla, Clin, Eri, Lev, Mox, Oxa, Pen
E. coli 06	Urine	Asb, Ca, Cep, Cfo, Cmp, Cro
P. aeruginosa 24	Nasal discharge	Ami, Cip, Com, Ctz, Imi, Lev, Mer, Ptz

Legend: Amc - Amoxicillin + Clavulanic Acid, Ami - Amikacin, Amox - Amoxicillin, Amp - Ampicillin, Asb - Ampicillin + Sulbactam, Azi - Azithromycin, Ca - Cefadroxil; Cep - Cephalexin, Cfo - Cefoxitin, Cip - Ciprofloxacin, Cla - Clarithromycin, Clin - Clindamycin, Cmp –Cefepime, Cro- Ceftriaxone, Ctz - Ceftazidime, Ery - Erythromycin, Imi - Imipenem, Mer - Levofloxin, Oxa - Oxacillin, Pen - Penicillin and Ptz – Piperacillin.

Materials and methods

Botanical material

The essential oil was extracted from terminal branches of plants collected in Atlantic Forest Areas in the states of Paraná and Santa Catarina, Southern Brazil, at September/2016, at the geographical coordenates: S 25° 19.862′ W 49° 48.338'. A voucher specimen was prepared and registered at the herbaria of the following institutions: Faculdades Integradas Espirita (registry number 8.822) and Royal Botanic Gardens (Kew, England). Terminal branches and inflorescences were randomly collected from at least 10 individual plants and dried with an electric dryer at 40 °C for 24 h.

Essential oil extraction and phytochemical analysis

The essential oil of *A. gratissima* was extracted by hydrodistillation in a Clevenger type apparatus. Briefly, each 50 g of dried leaves were crushed and subjected to three cycles of extraction with 1 l of distilled water at boiling temperature for 2 h (Wasicky, 1963). After extraction, the essential oil was collected and stored under refrigeration (-4 °C) for preservation. The yield of the essential oil on a dry basis was expressed as a percentage of the total weight of the dry leaves used in the extraction.

The chemical composition of the essential oil was determined by gas chromatography coupled to mass spectrometry (CG-MS). Briefly, the essential oil was diluted in dichloromethane (1: 100) and injected with a 1: 20 flow rate in an Agilent 6890 chromatograph (Palo Alto, CA, USA) coupled to an Agilent 5973N selective mass detector, operated at 250 °C. The separation of the constituents was performed in a HP-5MS capillary column (5% -phenyl-95% -dimethylpolysiloxane, 30 m × 0.25 mm × 0.25 μm). For quantification, the diluted samples were injected into an Agilent 7890A chromatograph equipped with a flame ionization detector (FID), operated at 280 °C. The percentage composition was assessed using the electronic integration of the FID signal by dividing the area of each component by the total area.

The identification of the chemical constituents was obtained by comparing their mass spectra with the standards reported in the literature (Nist, 2016) and also by comparing their linear retention indexes, calculated from the injection of a homologous series of hydrocarbons (C7 - C26) with the literature data (Adams, 2007).

Bacterial cultures

The multidrug-resistant strains *Pseudomonas aeruginosa* 24, *Staphylococcus aureus* 10 and *E. coli* 06 were used in the antibacterial tests. The origin and resistance profile of these strains is shown in Table 1, as reported in the study of Bezerra et al. (2017).

All strains were initially kept on blood agar (Difco Laboratories Ltda., São Paulo, Brazil) and maintained in Heart Infusion Agar (HIA, Difco Laboratories) at 4 °C. Samples were transferred from the solid medium to test tubes containing sterile saline, and turbidity was assessed using a value of 0.5 on the McFarland scale, corresponding to 10^5 CFU.

Drugs

Norfloxacin, gentamicin and erythromycin were used for evaluating the effects of their in vitro combinations with the natural products on antibiotic resistance. These antibiotics, as well as the sesquiterpene β-caryophyllene were purchased from Sigma-Aldrich (St. Louis, MO, USA). All drugs were dissolved in DMSO and diluted in water to 1024 μg/ml and serially diluted in test tubes.

Determination of minimum inhibitory concentration (MIC)

The MIC was determined using the broth microdilution method (CLSI, 2009). Bacterial cultures kept on agar under refrigeration at 4 °C were cultured in brain and heart infusion (BHI) broth and incubated at 37 °C for 24 h. Then, each inoculum was prepared using 900 μl of 10% BHI medium and 100 μl of the bacterial solution prepared as described above. Next, 100 μl of inoculum in medium was placed in wells on a 96-well plate followed by addition of 100 μl of the test substance and sequentially diluted, ranging the concentration on the wells between 1024 – 8 μg/mL, and incubation at 37 °C for 24 h. Growth control (medium + inoculum) were included in the last wells of the plate (NCCLS, 2003). After incubation, 20 μl of a 0,01% sodium resazurin solution in saline (p/v) was added to each well, followed by an additional 1 h incubation period at room temperature. A change in the color of the solution (from blue to red), due to the reduction of resazurin, was used as an indicator of bacterial growth. The MIC was defined as the lowest concentration capable of a complete bacterial growth inhibition. All experiments were carried out in triplicate for all bacterial strains.

Analysis of antibiotic resistance modulation by the EOAG and β-caryophyllene

The antibiotic-enhancing activity of the EOAG and β-caryophyllene was assessed by calculating the MICs of the fluoroquinolone antibiotics norfloxacin and ciprofloxacin (Sigma-Aldrich) against resistant strains of *P. aeruginosa, E. coli*, and *S. aureus* in the presence or absence of the essential oil or the isolated compound at concentrations equivalent to their MIC/8 (Coutinho et al., 2008; Tintino et al., 2018).

Each bacterial inoculum was prepared as previously described, and the EOAG and β-caryophyllene were added at concentrations equivalent to their MIC/8. Next, 100 μl of inoculum in medium was placed in wells on a 96-well plate followed by addition of 100 μl of the test substance at concentrations ranging from 1024 to 0.5 μg/ml, and incubation at 37 °C for 24 h. Positive controls (medium + inoculum) were included in the last wells of the plate. The MIC of each antibiotic was determined as described above. All experiments were carried out in triplicate for all bacterial strains.

Analysis of the combination effect

To evaluate how many times the drug dosis can be reduced when associated with the natural products in comparision with the drugs alone, the Dose reduction Index was calculated (Chou and Chou, 1988). This index is importante, mainly inn possible clinical situations due the possibility to reduces the dosis, mantaining the therapeutic efficiency of the drug, determining the potentiation of the drug (Eid et al., 2012). The DRI can be calculated using the follow formulae, replacing IC_{50} by MIC:

DRI = IC_{50} cytotoxic drug alone/ IC_{50} cytotoxic drug in combination

Table 2
GC-MS profile of the EOAG.

Retention Index	Compound	%
937	Alpha-pinene	6.2
979	Beta-pinene	7.2
991	Myrcene	0.5
1030	Limonene	0.9
1098	Linalool	0.4
1139	Nopinone+trans-pinocarveol	1.3
1145	Trans-verbenol	1.5
1163	Pinocarvone	1.2
1194	Myrthenol	1.5
1336	Delta-elemene	0.5
1417	(E)-beta- caryophyllene	17.3
1433	Trans-alpha-bergamotene	1.1
1441	(Z)-beta-bergamotene	3.3
1449	Alfa-humulene	1.3
1456	(E)-beta-farnesene + alpha-aromadendrene	0.8
1477	Gama-muurolene	5.3
1492	Bicyclogemacrene	7.3
1505	Beta-bisabolene	0.5
1509	Beta-curcumene + cubebol	0.8
1519	Delta-cadinene	0.6
1541	Cis-sesquisabinene hydrate	1.1
1565	(E)-nerolidol	9.7
1575	Spathulenol	3.8
1578	Caryophyllene oxide	5.9
1586	Globulol	0.7
1603	Ledol + rosifoliol	1.3
1636	Epi-alpha-cadinol	2.2
1649	Alpha-cadinol	1.2
1666	Epi-beta-bisabolol	1.1
2175	Sandaracopimaral	5.3
TOTAL		91.8%

Statistical analysis

Data are expressed as arithmetic means ± standard deviation and were analyzed by analysis of variance (ANOVA), followed by Bonferroni's post-test using GraphPad Prism software version 7.0. Statistical significance was considered when $p < 0.05$.

Results and discussion

Chemical composition of the essential oil of Aloysia gratissima

The extraction of the EOAG by hydrodistillation presented a yield of 0,33%, considering the dry weight of the botanical material. The phytochemical analysis of the EOAG identified 91.8% of the total constituents, revealing the presence of 30 different compounds, including β-caryophyllene (17.3%) nerolidol (9.7%) and bicyclogermacrene (7.3%), β-pinene (7.2%), α-pinene (6.2%) and caryophyllene oxide (5.9%) as major constituents (Table 2).

The present study characterized the chemical profile and in vitro antibacterial activity of A. gratissima. The GC-MS analysis identified the presence of 30 different constituents in the essential oil of this species, including β-caryophyllene as a major constituent. Accordingly, a study by Trovati et al. (2009) identified the presence of 14 constituents in the essential oil of the same species, including β-caryophyllene as one of the main components (Santos et al., 2015).

In addition, Dambolena et al. (2010) identified β-elemene (35.7%) as the main compound, followed by the β-caryophyllene (28%). On the other hand, Bersan et al. (2014) reported that E-pinocamphone (16.07%) was found as the predominant compound in the essential oil of *A. gratissima*, while β-caryophyllene was not identified among the

Table 3
Minimum Inhibitory Concentrations (MICs) of the EOAG and β-caryophyllene.

Bacterial Strain	EOAG MIC (μg/mL)	β-caryophyllene MIC (μg/mL)
Escherichia coli 6	≥ 1024	≥ 1024
Staphylococcus aureus 10	32	32
Pseudomonas aeruginosa 24	≥ 1024	≥ 1024

constituents. These differences are justified by evidence demonstrating that the chemical composition of a given species may vary according to the method of extraction and collection, as well as the influence of seasonal factors (Figueiredo et al., 1997; Santos et al., 2013).

Antibacterial activities of the EOAG and β-caryophyllene each alone

The antibacterial activity analysis demonstrated that both EOAG and its major compound β-caryophyllene presented MIC values equivalent to 32 μg/ml against *S. aureus* (Table 3). However, the EOAG and the sesquiterpene presented MIC values above 1024 μg/ml against *E. coli* and *P. aeruginosa*. These findings indicate that both natural samples showed antibacterial activities against Gram-positive but not against Gram-negative bacteria.

This finding is in accordance to the work of Pérez-Zamora et al. (2018), who demonstrated that an essential oil obtained from *A. gratissima*, containing β-caryophyllene as a major compound, exhibited antibacterial activity against *S. aureus*.

Accordingly, essential oils of species of the same genus, such as *A. polystachya* and *A. sellowii* showed potent antibacterial actions against *S. aureus*, which may be related to the presence of β-caryophyllene. According to Dahham et al. (2015), this compound is active against both Gram-negative and Gram-positive bacteria. In addition, Maia et al. (2010) demonstrated that the essential oils of *Vernonia remotiflora* and *V. braziliana*, which had approximately 40% of β-caryophyllene in their composition, had antibacterial action against both Gram-negative and Gram-positive bacteria, including S. aureus.

Antibiotic-enhancing effects of the EOAG and β-caryophyllene

Following the antibacterial activity analysis, this study investigated the potential of the essential oil and its major compound to potentiate the activity of conventional antibiotics against resistant strains of *S. aureus, E. coli* and *P. aeruginosa* (Fig. 2). The DRI was calculated and the results are described in the Table 4.

The in vitro treatment with the EOAG potentiated ($p < 0.0001$) the activity of norfloxacin against all bacterial strains analyzed in this study. Of note, the essential oil caused a 6-fold reduction in the MIC of the antibiotic against resistant strains of *S. aureus*. The same combination of substances resulted in remarkable modification of the antibiotic action, inhibiting bacterial growth in *E. coli* cultures, in which the MIC was reduced from 32 μg/ml to 1.58 μg/mL, with a reduction of 20 times. Against P. aeruginosa cultures, the combination between EOAG and antibiotics (norfloxacin) caused a 8-fold (Table 4) reduction in the MIC, indicating potentiation of antibiotic activity. On the other hand, the essential oil did not cause significant changes in the activity of gentamicin and erythromycin against the bacterial strains investigated in the study. These findings suggest that the EOAG selectively modulates the resistance to different classes of antibiotics. Even with this interesting result, this combination must be evaluated carefully due the fact of studies with essential oils must consider the existence of some other compounds in the composition that could affect the antibiotic effect.

The present study demonstrated that both the EOAG and β-caryophyllene increased the antibacterial activity of norfloxacin against multidrug resistant bacteria. This finding is corroborated by a previous study showing that the essential oil of *Lippia menosides*, which has a chemical composition similar to *A. gratissima*, potentiated the action of ampicillin and cephalothin against *S. aureus* (Guimarães et al., 2014). In the present work these substances have potentiated the effect of antibiotics against Gram-negative bacteria, studies with *E. coli* demonstrated variable results from the combination with conventional antibiotics

Table 4
MIC values (μg/mL) of the antibiotic alone and associated with the β-caryophyllene and EOAG. The Dose Reduction Index (DRI) of the antibiotics after associationa (DRI β-c e DRI EOAG). Results represented the media ± standard deviation.

E. coli	MIC Ant	MIC Aβ-c	MIC AEOAG	DRI β-c	DRI EOAG
Gentamycin	512 ± 1	512 ± 1	512 ± 1	1	1
Erythromycin	1024 ± 1	1024 ± 1	1024 ± 1	1	1
Norfloxacin	32 ± 1	1.25 ± 1,49	1.58 ± 1	25.6	20.25
S.aureus					
Gentamycin	256 ± 1	10159 ± 1.49	322.79 ± 1.49	2.51	0.79
Erythromycin	8 ± 1	20.15 ± 2.22	50.79 ± 2.22	0.39	0.15
Norfloxacin	64 ± 1	50.79 ± 1.49	10.07 ± 1.49	1.26	6.35
P. aeruginosa					
Gentamycin	80.63 ± 1.49	161.26 ± 1.49	203.28 ± 1.49	0.5	0.39
Erythromycin	128 ± 1	101.59 ± 1.49	128 ± 1	1.25	1
Norfloxacin	12.69 ± 1.49	4 ± 1	1.58 ± 2.22	3.17	8.03

Ant – Antibiotic alone
Aβ-c – Antibiotic associated with β-caryophyllene
AEOAG – Antibiotic associated with EOAG

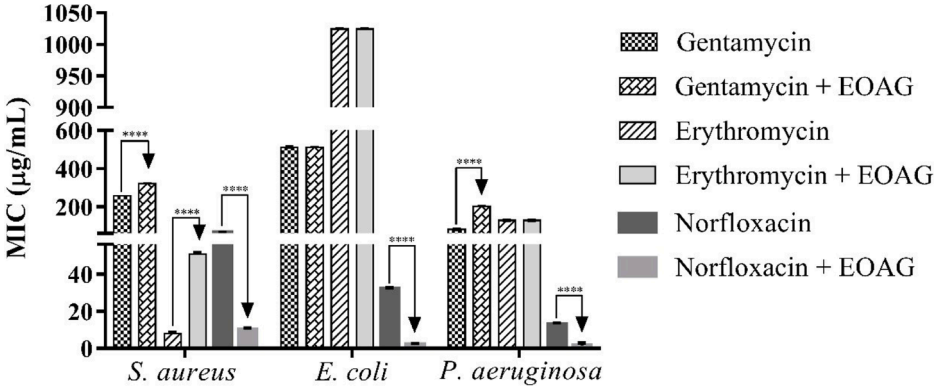

Fig. 2. Modulation of antibiotic resistance by the EOAG in association with norfloxacin, gentamicin or erythromycin against MDR of E. coli, S. aureus and P. aeruginosa. **** $p < 0.0001$ indicates significant differences between groups.

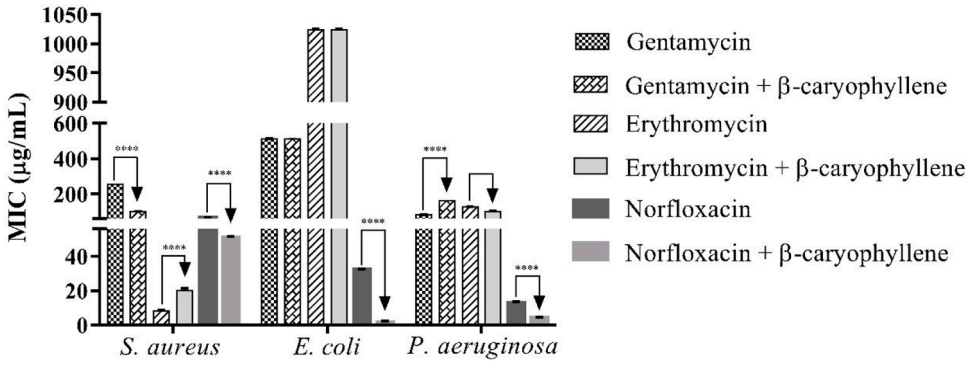

Fig. 3. Modulation of antibiotic resistance by β-caryophyllene in association with norfloxacin, gentamicin or erythromycin against MDR of E. coli, S. aureus and P. aeruginosa. **** $p < 0.0001$ indicates significant differences between groups.

(Oliveira et al., 2006). Accordingly, Goren et al. (2011) demonstrated that β-caryophyllene potentiated the action of sulfamethoxazole in a disk diffusion model.

To analyze whether the antibacterial action of the EOAG would be associated with the activity of its major compound β-caryophyllene, this study investigated the effects of the combination of this compound with conventional antibiotics under the same experimental conditions previously described (Fig. 3). Like the EOAG, the pure compound potentiated the antibiotic activity of norfloxacin against all bacterial strains. However, β-caryophyllene was found to increase the antibacterial activity of erythromycin and gentamicin against P. aeruginosa and S. aureus, respectively. Considering the comparable antibiotic-enhancing activity between the essential oil and the pure compound, it is suggested that the modulating effects of the EOAG on antibiotic resistance result, at least partially, from the action of its major constituent β-caryophyllene.

On the other hand, a study by Aguiar et al., (2015) demonstrated that the EO of *Lantana caatingensis* (Verbenaceae), which has β-caryophyllene as the major component, differentially modulated the activity of aminoglycoside antibiotics against *S. aureus* and *E. coli*. In this context, the association with gentamicina have no potentiating effect in the reduction of the antibiotic MIC against both strains. However, the association caused a 75% reduction in the MIC of amikacin against *S. aureus*. In addition, the association with neomycin caused a 50% reduction in the MIC of this antibiotic against *E. coli,* providing evidence of the existence of a link between the presence of β-caryophyllene as a major constituent, and the antibiotic-enhancing properties of this species.

Dahham et al. (2015), studying the action of β-caryophyllene against Gram-positive and Gram-negative bacteria, demonstrated that this compound has potent activity against strains of *S. aureus*, with MIC values ranging between 3 and 4 µM. The authors also suggested that β-caryophyllene antibacterial activity may be due to interference with antioxidant mechanisms.

However, evidence suggests that lipophilic terpenes can alter the permeability of the bacterial cell membrane, causing damage resulting from the loss of K^+ ions and electrolyte imbalance (Pinto, 2014), leading to the loss of fundamental cellular components such as proteins and lipids (Oliveira et al., 2006; Mahizan et al., 2019).

Conclusion

EOAG as well as its major constituent β-caryophyllene showed antibacterial activity on their own and enhanced the antibiotic activity when tested in combinations. The EOAg and the β-caryophyllene demonstrated clinically relevant antibacterial activity against *S. aureus*, as na antibiotic-potentiating activity against all assayed bacterial strains.

In conclusion, both EOAG and β-caryophyllene have a notable potential for the development of new therapies against bacterial resistance, mainly due the possibility to reduce the MIC of the antibiotics when acting in association with the EOAG and specially with the β-caryophyllene. However, further research is needed to characterize the mechanisms of action of these substances in the model used in the present study.

Author contributions

Conceptualization: E.L.S; experiments were conducted by: A.C.J.A and P.R.F.; data analysis: H.D.M.C.; formal analysis, C.L.R.P.; writing original draft preparation, W.d.A. and C.D.; writing of manuscript: A.C.A.S and J.R.F.; critical review: S.R.T and J.R.F. All authors read and agreed with the final version of the manuscript.

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Declaration of Competing Interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.phyplu.2021.100100.

References

Adams, R.P., 2007. Identification of Essential Oil Components by Gas Chromatography/ Mass Spectroscopy, 4rd ed. Allured Publishing Corporation: Carol Stream, Dupage.

Aguiar, N.U., Lima, G.S., Rocha, S.M., Citó, L.G.M.A., Sousa, P.J.A., Silva, M.R., Silva, A. S.I., Costa, M.G.J., 2015. Chemical composition and modulation of antibiotic activity ofessential oil of *Lantana caatingensis* M. (Verbenaceae). Ind. Crops Prod. 74, 165–170.

Alarco, J.J., 2014. Resistencia bacteriana: una pandemia silente. Rev. Med. Panacea 4, 1–2.

Andersen, J.L., He, G-X., Kakarla, P., Ranjana, K.C., Kumar, S., Lakra, W.S., Mukherjee, M.M., Ranaweera, I., Shrestha, U., Tran, Varela M.F, 2015. Multidrug efflux pumps from Enterobacteriaceae, *Vibrio cholerae* and *Staphylococcus aureus* bacterial food pathogens. Int. J. Environ. Res. Public Health 12, 1487–1547.

Bersan, S.M.F., Galvão, C.C.L., Goes, F.F.V., Sartoratto, A., Figueira, M.G, Rehder, G.L.V., Alencar, M.S., Duarte, T.M.R., Rosalen, L.P., Duarte, T.C.M., 2014. Action of essential oils from Brazilian native and exotic medicinal species on oral biofilms. Comp. Altern. Med. 14, 1–12.

Bezerra, C.F., Camilo, J.C., Silva, N.K.M., Freitas, S.T., Ribeiro-Filho, J., Coutinho, H.D. M, 2017. Vanillin selectively modulates the action of antibiotics against resistant bacteria. Microbial Pathogen 113, 265–26.
Brito, M.A., Cordeiro, B.C., 2012. Necessidade de novos antibióticos. J. Bras. Pat. Med. Lab. 48, 247–249.
Chou, J.H., Chou, T.C., 1988. Computerized simulation of dose reduction index (DRI) in synergistic drug combinations. Pharmacologist 30, A231.
CLSI, 2009. Clinical and Laboratory Standards Institute. Methods for Dilution Antimicrobial Susceptibility Test for Bacteria That Grow Aerobically. Approved Standard- Eighth Edition. Wayne, CLSI document M07-A8.
Coutinho, H.D.M., Costa, M.G.J., Lima, O.E., Falcão-Silva, S.V., Siqueira-Júnior, P.J., 2008. Enhancement of the antibiotic activity against a multiresistant *Escherichia coli* by Mentha arvensis L. and chlorpromazine. Chemotherapy 54, 328–330.
Dahham, S.S., Tabana, N.Y., Iqbal, A.M., Ahamed, K.B.M., Ezzat, O.M., Majid, A.S.A., Majid, A.S.M.A., 2015. The anticancer, antioxidant and antimicrobial properties of the sesquiterpene β-caryophyllene from the essential oil of *Aquilaria crassna*. Molecules 20, 11808–11829.
Dambolena, J.S., Zunino, P.M., Lucini, I.E., Zyglado, A.J., Banchio, E., Biurrun, F., Ahumada, O.E.A., 2010. Aromatic plants of northwest Argentina. Constituents of the essential oils of aerial parts of seven Verbenaceae: *Lantana* and *Aloysia*. J. Essential Oil Res. 22, 289–293.
Dorman, H., Deans, S., 2000. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J. Appl. Microbiol. 88, 308–316.
Z. Eid, S.Y., El-Readi, M., Wink, M., 2012. Synergism of three-drug combinations of sanguinarine and other plant secondary metabolites with digitonin and doxorubicin in multi-drug resistant cancer cells Phytomedicine 19, 1288–1297.
Figueiredo, A.C., Barroso, J.G., Pedro, L.G., Scheffer, J.J.C., 1997. Physiological aspects of essential oil production. In: Essential Oils: Basic and Applied Research. Proceedings of the 27th International Symposium on Essential Oils Vienna, Áustria September 8-11 Edits. Austria. Ch. Franz, Á. Máthé and G. Buchbauer. Carol Stream: Allured Pub. Corp Vienna, pp. 95–107.
Foster, T.J., Geoghegan, J.A., Ganesh, V.K., Höök, M., 2014. Adhesion, invasion and evasion: the many functions of the surface proteins of *Staphylococcus aureus*. Nat. Rev. Microbiol. 12, 49–62.
Garcia, M.C.F., Soares, D.C., Santana, R.C., Saraiva, E.M., Siani, A.C., Ramos, M.F.S., Danelli, M.G.M., Souto-Padron, T.C., Pinto-da-Silva, L.H., 2018. The *in vitro* antileishmanial activity of essential oil from *Aloysia gratissima* and guaiol, its major sesquiterpene against Leishmania amazonensis. Parasitology 145, 1219–1227.
Goleniowski, M.E., Bongiovanni, G.A., Palacio, L., Nuñez, C.O., Cantero, J.J., 2006. Medicinal plants from the "Sierra de Comechingones. Argentina. J. Ethnopharmacol. 107, 324–341.
Goren, A.C., Piozzi, F., Akcicek, E., Kılıc, T., Çarıkçı, S., Mozioglu, E., Setzer, N.W., 2011. Essential oil composition of twenty-two Stachys species (mountain tea) and their biological activities. Phytochem. Lett. 4, 448–453.
Grillo, V.T.R.S., Gonçalves, T.G., Júnior, J.C., Paniágua, N.C., Teles, C.B.G., 2013. Incidência bacteriana e perfil de resistência a antimicrobianos em pacientes pediátricos de um hospital público de Rondônia. Brasil. Rev. Ci. Farma. 34, 117–123.
Guimarães, L.G.L., Cardoso, G.M., Souza, M.R., Zacaroni, B.A., Santos, R.G., 2014. Óleo essencial de Lippia sidoides nativas de Minas Gerais: Composição, estruturas secretoras e atividade antibacteriana. Rev. Ci. Agron. 45, 267–275.
Korb, A., Nazareno, E.R., endonça, F.A., Dalsenter, P.R., 2013. Perfil de resistência da bactéria Escherichia coli em infecções do trato urinário em pacientes ambulatoriais. Rev. Biol. Ci. Terra 13, 72–79.
Kumphune, S., Prompunt, E., Phaebuaw, K., Sriudwong, P., Pankla, R., Thongyoo, P., 2011. Anti-inflammatory effects of the ethyl acetate extract of Aquilaria crassna inhibits LPS-induced tumour necrosis factor-alpha production by attenuating P38 MAPK activation. Int. J. Green Pharm. 5, 43–48.
Lima, M.F.P., Borges, M.A., Parente, R.S., Júnior, R.C.V., Oliveira, M.E., 2015. Staphylococcus aureus e as infecções hospitalares – Revisão de Literatura. Rev. Uningá Rev. 21, 32–39.
Mahizan, N.A., Yang, S-K., Moo, C-L., Song, A-LA., Chong, C-M., Chong, C-W., Abushelaibi, A., Lim, ES-H., Lai, K-S., 2019. Terpene derivatives as a potential agent against antimicrobial resistance (AMR) pathogens. Molecules 24, 1–21.
Maia, A.I.V., Torres, M.C.M., Pessoa, O.D.L., De Menezes, J.E.S.A., Costa, S.M.O., Nogueira, V.L.R., 2010. Volatile leaf oils of Vernonia Brasiliana and Vernonia remotiflora: chemical composition and biological activity. Quim. Nova 3, 584–586.
Neta, M.C.S., Vittorazzi, C., Guimarães, A.C., Martins, J.D.L., Fronza, M., Endringer, D.C., Scherer, R., 2017. Effects of β-caryophyllene and *Murraya paniculata* essential oil in the murine hepatoma cells and in the bacteria and fungi 24-h time–kill curve studies. Pharma. Biol. 1, 190–197.
Oliveira, R.A.G., Lima, O.E., Vieira, L.W., Freire, L.R.K., Trajano, N.V., Lima, O.I., Souza, L.E., Toledo, S.M., Silva-Filho, N.R., 2006. Estudo da interferência de óleos essenciais sobre a atividade de alguns antibióticos usados na clínica. Rev. Bras. Farmacog. 16, 77–82.
Pérez-zamora, C.M., Torres, C.A., Nuñez, M.B., 2018. Antimicrobial activity and chemical composition of essential oils from verbenaceae species growing in South America. Molecules 23, 1–21. https://doi.org/10.3390/molecules23030544.
Pierozan, M., Pauletti, G.F., Rota, L., Santos, A.C.A., Lerin, L.A., Cansian, R.L., Oliveira, J. V., 2009. Chemical characterization and antimicrobial activity of essential oils of salvia L. species. Ci. Tecnol. Alim. 29, 764–770.
Pinto, N.R.P., 2014. Estudo da atividade antibacteriana da Carvona e seus derivados Dissertação (mestrado) Univeridade da Beira Interior. Química Industrial. Covilhã 2–112.
Rice, L.B., 2008. Federal funding for the study of antimicrobial resistance in nosocomial pathogens: no ESKAPE. J. Infect. Dis. 197, 1079–1081.
Rodriguez, E.J., Ramis-Ramos, G., Heyden, Y.V., Simó-Alfonso, E.F., Lerma-García, M.J., Saucedo-Hernández, Y., Monteagudo, U., Morales, Y., Holgado, B., Herrero-Martínez, J.M., 2012. Chemical composition, antioxidant properties and antimicrobial activity of the essential oil of Murraya paniculata leaves from the mountains of Central Cuba. Nat. Prod. Comm. 7, 1527–1530.
Santos, T.G., Laemmle, J., Rebelo, R.A., Dalmarco, E.M., Cruz, A.B., Schmit, A.P., Cruz, R.C.B., Zeni, A.L.B., 2015. Chemical composition and antimicrobial activity of *Aloysia gratíssima* (Verbenaceae) leaf essential oil. J. Essent. Oil Res. 27, 125–130.
Santos, F.M., Pinto, J.E.B.P., Bertolucci, S.K.V., Alvarenga, A.A., Alves, M.N.I., Duarte, M. C.T.I., Sartoratto, A., 2013. Chemical composition and antimicrobial activity of the essential oil from the leaves and flowers of Aloysia gratissima. Rev. Bras. Pl. Med. 15, 583–588.
Silva, C.C., Vandresen, F., Oliveira, C.M.A., Kato, L., Tanaka, C.M.A., Ferreira, H.E., 2006. Chemical composition of *Aloysia gratissima* (Gill. et Hook) Tronc. (Verbenaceae). Biochem. Syst. Ecol. 34, 593–595.
Silva, D.T., Silva, L.L., Amaral, L.P., Pinheiro, C.G., Pires, M.M., Schindler, B., Garlet, Q. I., Benovit, S.C., Baldisserotto, B., Longhi, S.J., Kotzian, C.B., Heinzmann, B.M., 2014. Larvicidal activity of Brazilian plant essential oils against coenagrionidae larvae. J. Econ. Entomo. 7, 1714–1720.
Souza, A.A., Wiest, J.M., 2007. Antibacterial activity of *Aloysia gratissima* (Gill et Hook) Tronc. (garupá, herbsaint), used in the traditional medicine in Rio Grande do Sul State - Brazil. Rev. Bras. Pl. Med. 9, 23–29.
Sterhling, G.E., Leite, D.S., Silveira, W.D., 2010. Molecular typing and biological characteristics of *Pseudomonas aeruginosa* isolated from cystic fibrosis patients in Brazil. J. Infect. Dis. 14, 462–467.
Tintino, S.R., 2018. Avaliação da inibição de bombas de efluxos em linhagens de Staphylococcus aureus por substâncias sintéticas de origem natural. Tese (Doutorado) – Universidade Federal de Pernambuco. Centro de Biociências. Programa de Pós-Graduação em Biotecnologia. Recife, pp. 1–114.
Trabulsi, L.R., Alterthum, F., 2015. Microbiologia, 6rd ed. Atheneu. São Paulo. São Paulo.
Trovati, G., Chierice, O.G., Sanches, A.E., Galhiane, S.M., 2009. Essential oil composition of *Aloysia gratissima* from Brazil. J. Essent. Oil Res. 21, 325–326.
Wasicky, R., 1963. Uma modificação do aparelho de clevenger para extração de óleos essenciais. Rev. Farma. Bioq. USP. 1, 77–81.
Yadegarinia, D., Gachkar, L., Rezaei, M.B., Taghizadeh, M., Astaneh, A.S., Rasooli, I., 2006. Biochemical activities of Iranian *Mentha piperita* L. and *Myrtus communis* L. essential oils. Phytochemistry 67, 1249–1255.
Yang, Q., Wu, J., Luo, Y., Huang, N., Zhen, N., Zhou, Y., Sun, F., Li, Z., Pan, Q., Li, Y., 2016. Guaiol regulates RAD51 stability via autophagy to induce cell apoptosis in non-small cell lung cancer. Oncotarget 7, 62585–62597.
Zeni, A.L.B., Bosio, F., 2011. O uso de plantas medicinais em uma comunidade rural de Mata Atlântica – Nova Rússia. SC. Neotrop. Biol. Cons. 6, 55–63.