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Original Article

Potentiation of the Antibiotic Activity by the Essential Oils of *Eugenia brasiliensis* Lam. and *Piper mosenii* C. DC.

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Abstract: The present study aimed to investigate the antibiotic-enhancing activity of the essential oils obtained from the leaves of *Eugenia brasiliensis* Lam (EOEb) and *Piper mosenii* C. DC. (EOPm) against multiresistant strains of *Escherichia coli* 06 and *Staphylococcus aureus* 10. The essential oils were obtained by hydrodistillation, and the minimum inhibitory concentrations (MIC) were determined through the broth microdilution method. The EOEb enhanced the activities of norfloxacin and erythromycin against *Staphylococcus aureus* and *Escherichia coli*, respectively. At the same conditions, the EOPm was found to potentiate the effects of gentamicin and erythromycin against *E. coli*. Of note, this is the first study to date that has reported the ability of these extracts to enhance the antibacterial activity of conventional antibiotics.

Keywords: *Eugenia brasiliensis*; *Piper mosenii*; Bacterial resistance; Enhanced antibiotic activity

Introduction

The discovery of antibiotics has revolutionized the treatment of bacterial infections, causing a significant impact on public health. While antibiotic therapy significantly reduced both morbidity and mortality rates worldwide [1], the irrational use of antibiotics has contributed to the rising of resistant bacteria, as a result of genetic adaptation related to selective pressure [2].

Antibiotic resistance is the phenomenon by which some bacteria develop the ability to overcome the action of specific or multiple antibacterial drugs, which may involve the following mechanisms: limiting uptake of a drug, modification of a drug target, enzymatic inactivation of a drug, and active efflux of a drug [3]. While most cases of bacterial resistance occur in the hospital environment, resistant strains have been increasingly isolated from patients with community infections. Thus, as bacterial resistance against most available antibiotics has been currently reported [4,5], the development of new drugs to combat bacterial resistance is crucial for the management of diseases caused by resistant bacteria strains.

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Considering the remarkable pathogenicity and demonstrated resistance, the World Health Organization (WHO) recommended that *Escherichia coli*, as well as *Staphylococcus aureus* [6], should be prioritized in targeted research for the development of new antibiotics. *E. coli* is a Gram-negative enterobacterium with a wide variety of virulence mechanisms associated with complex clinical manifestations [7]. In this context, in addition to presenting a wide range of resistance genes, *E. coli* has been recognized as the causative agent of many severe infectious diseases [8]. Accordingly, *S. aureus* is one of the most virulent species of the genus. Despite being present in the microbial flora of healthy individuals, this Gram-positive bacterium is capable of causing a spectrum of manifestations, from mild disease to severe infections [9], such as pyoderma, impetigo, furuncle, erysipelas, cellulite, folliculitis [10].

In this context, several studies have shown that essential oils represent a group of substances with immense pharmacological potential. Besides having antioxidant, antifungal, and antiparasitic properties, essential oils were found to present antibacterial activities against both Gram-positive and Gram-negative strains. Therefore, these complex substances, as well as their isolated constituents, represent promising sources of molecules for the development of new drugs to combat bacterial resistance [11,12].

Eugenia brasiliensis Lam. is popularly known as "grumixama." This species, native to Brazil, is now widely cultivated due to the consumption of its tasty fruits [13,14]. In addition, the infusion of their leaves is popularly used in the treatment of rheumatism and arthritis. Furthermore, there is evidence that *E. brasiliensis* has antidiarrheal and diuretic actions [15].

Piper mosenii C. DC. belongs to a genus of immense therapeutic importance. Accordingly, the pharmacological properties of *Piper crassinervium* H.B. & K. (jaborandi) and *Piper nigrum (black pepper)* have been widely reported in the literature. Moreover, β-caryophyllene, α-copaene, and β-pinene, which are found in many species of this genus, are compound with proven pharmacological activity [16,17]. However, despite the chemical and taxonomic importance of the genus, the medicinal properties of *P. mosenii* remain to be better investigated.

The search for substances capable of combating bacterial resistance to conventional antibiotics is fundamental to reduce both the mortality rates and complications resulting from these infections. In this context, accumulating evidence has demonstrated that the Brazilian biodiversity is a source of extracts and compounds with promising antibacterial effects [18].

Thus considering the evidence that some species of *Piper* and *Eugenia* could revert bacterial resistance *in vitro*, the present study aimed to characterize the chemical profile and evaluate the antibiotic-enhancing activity of the essential oils of *Eugenia brasiliensis* Lam (EOEb) and *Piper mosenii* C. DC. (EOPm) against multiresistant strains of *Escherichia coli* and *Staphylococcus aureus*.

Materials and methods

Plant material and essential oil extraction

The species *Eugenia brasiliensis* Lam was collected in the municipality of Atalanta, Santa Catarina, Brazil, while *Piper mosenii* C. DC. was collected in the Bom Jesus Biological Reserve, located in the municipality of Guaraqueçaba, Paraná, Brazil. The collection and registration of the specimens were performed as described by Silva *et al.* [19].

Antibiotics, culture media and microorganisms

The liquid antibiotics norfloxacin, gentamicin, and erythromycin were obtained from LaborClin (Parana, Brazil). Heart Infusion Agar (HIA) and Brain Heart Infusion (BHI) culture media were purchased from HIMEDIA (Pennsylvania, USA).

The following standard and resistant strains were used: *Escherichia coli* 06 and *Staphylococcus aureus* 10. These microorganisms were obtained from the Laboratory of Microbiology and Molecular Biology (LMBM) of the Regional University of Cariri (URCA).

Sample preparation

A total of 10 mg of each essential oil was diluted

in 0.5 mL DMSO and then diluted in water. Both essential oils and antibiotics were prepared at initial concentration of 1,024 µg/mL and serially diluted in test tubes.

Determination of the minimum inhibitory concentration by direct contact

Each bacterial strain was cultured in HIA medium at 37°C for 24 h. After this period, samples were transferred from the solid medium to test tubes containing sterile saline and turbidity was compared with the 0.5 value of the McFarland scale. Next, a 100 µL solution containing 10% BHI:inoculum (10:1) was transferred to each well of a 96-well plate with 100 µL of each essential oil at concentrations ranging from 512 to 8 µg/mL, followed by incubation at 37ºC for 24 h. Wells containing only the inoculum in BHI were used as a growth control. After incubation, sodium resazurin was added to each well, followed by an additional 1 h incubation period at room temperature. A change in the color of the solution was used as an indicator of bacterial growth. The MIC was defined as the lowest concentration capable of inhibiting bacterial growth. All experiments were carried out in triplicate for both bacterial strains.

Analysis of antibiotic resistance modulation

In order to evaluate the ability of the essential oils to enhance the activity of antibiotics against resistant bacteria, the MIC values of norfloxacin, gentamicin, and erythromycin against resistant strains of *P. aeruginosa*, *E. coli*, and *S. aureus* were determined in the presence or absence of these natural products at concentrations equivalent to their MIC÷8 [21]. Bacterial cultures, controls and readings were performed as described above.

Statistical analysis

Data are expressed as arithmetic means ± standard deviations and were analyzed by analysis of variance (ANOVA), followed by Bonferroni's post-test using GraphPad Prism software version 5.0. Statistical significance was considered when $p < 0.05$.

Results and discussion

A study conducted by Silva *et al.* [19], who evaluated the chemical composition of *Eugenia brasiliensis* and *Piper mosenii* C. DC., found α-muurolol (12.01 %) as the major component of the OEEb, while α-pinene (14.59 %) and bicyclogermacrene (12.25 %) were identified as the major compounds in the OEPm. Of note, major components were defined as those representing more than 10 % of the total composition of the essential.

A previous study by Siebert *et al.* [22] demonstrated that the essential oil of the leaves of *E. brasiliensis* is constituted predominantly of sesquiterpenes, such as spathulenol and T-cadinol and the monoterpene α-pinene. According to Coutinho *et al.* [21], extracts obtained from this species are rich in flavonoids such as quercetin, rutin, galangin, and myricetin, in addition to other phenolic compounds such as catechin, gallocatechin, and gallic acid. In a study by Fidyt and collaborators [16], α-pinene was identified as one of the main components of essential oils of *Piper* species, corroborating the results of the present research. On the other hand, a study by Krinski *et al.* [23] identified β-pinene, limonene, (E)-caryophyllene, and caryophyllene oxide in the composition of the essential oil of *Piper mosenii*, indicating that variations in the constitution of this species may occur. In fact, consistent evidence has demonstrated that quantitative and qualitative changes in the chemical composition of essential oils might arise under the influence of seasonal, environmental, and technical factors [24].

Our analysis of the effect of a subinhibitory concentration of *Eugenia brasiliensis* essential oil in combination with antibiotics of different pharmacological classes against *S. aureus* 10 is shown in Fig. 1. The combination with the oil significantly reduced the MIC of norfloxacin, but increased the MIC of gentamicin, indicating the occurrence of synergism and antagonism, respectively. However, the MIC of erythromycin was not significantly changed, indicating that the EOEb selectively enhanced the antibacterial activity of antibiotics against this bacterial strain.

On the other hand, in tests using *E. coli*, the MIC values of norfloxacin and gentamicin were not affected, while the MIC of erythromycin was

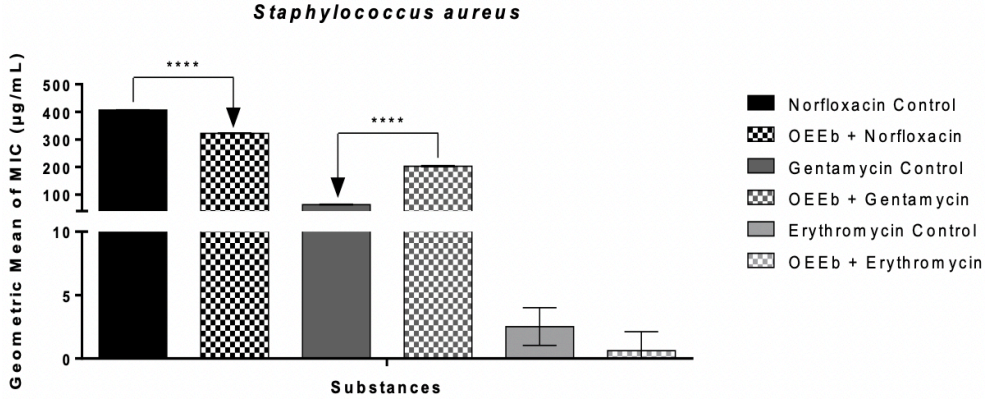

Figure 1. Minimal inhibitory concentration (µg/mL) of different antibiotics in the presence or absence of the OEEb against *S. aureus* 10

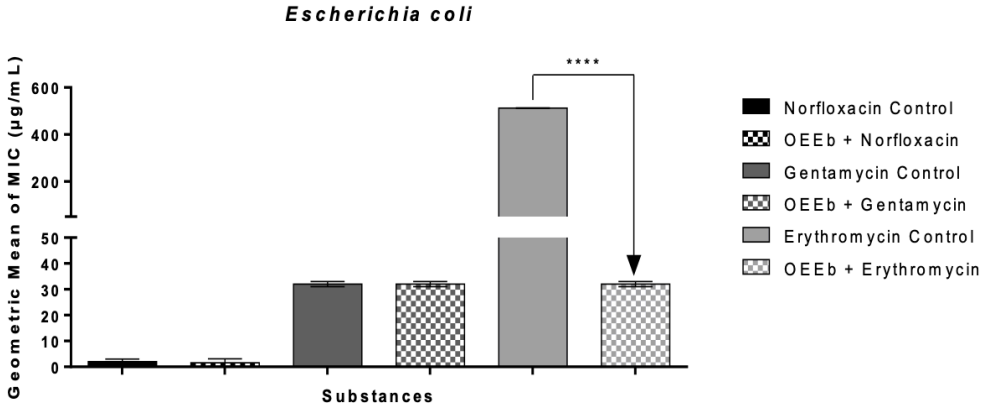

Figure 2. Minimal inhibitory concentration (µg/mL) of different antibiotics in the presence or absence of the OEEb against *E. coli* 06

significantly reduced by the combination with the essential oil demonstrating the EOEb enhanced the activity of this antibiotic against *E. coli* (Fig. 2).

Our data demonstrated that the essential oil of *E. brasiliensis* exhibited the most promising antibiotic-enhancing effects when associated with norfloxacin against *S. aureus* and when associated with erythromycin against *E. coli*. Studies indicate that the association of plant-derived essential oils with antibacterial drugs can vary significantly Gram-positive and Gram-negative strains, which may justify the differences observed in our study [25]. The present research is a pioneer study demonstrating that the essential oil obtained from the leaves of *E. brasiliensis* enhanced the antibacterial activity of antibiotics. Accordingly, Simonett and collaborators [26], found that species of the genus *Eugenia* present bioactive substances with antibacterial activity against both Gram-positive and Gram-negative bacteria, corroborating the data obtained in the current research.

Following the analysis of the essential oil of *E. brasiliensis*, we investigated the ability of the essential oil obtained from *Piper mosenii* to enhance the activity of antibiotics against the same strains. While the MIC values of erythromycin and gentamicin against *S. aureus* were not altered by the association with the oil, the MIC of norfloxacin was significantly increased (Fig. 3), suggesting that the association of the EOPm with these antibiotics against *S. aureus* is not clinically useful. On the other hand, in the tests with *E. coli 06*, the combination with the oil reduced the MIC values of gentamicin and erythromycin. In contrast, the MIC of norfloxacin was not affected (Fig. 4).

Our analysis revealed that the species *Piper mosenii* presented the most promising effects when tested against the *Escherichia* coli strain, reducing

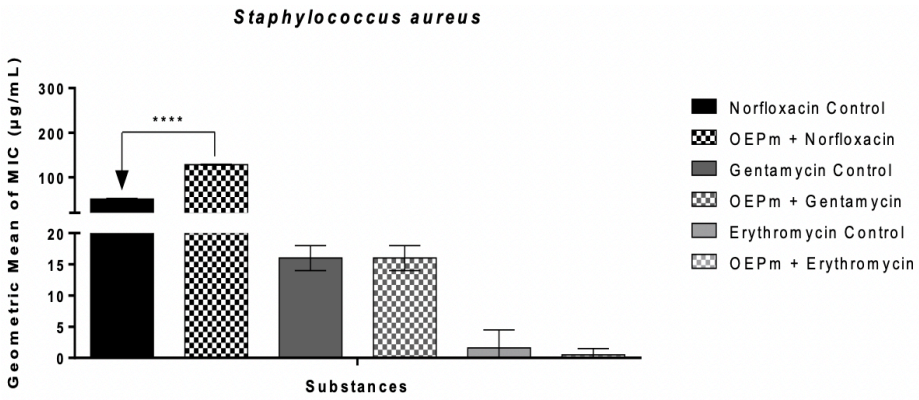

Figure 3. Minimal inhibitory concentration (µg/mL) of different antibiotics in the presence or absence of the OEPm against *S. aureus* 10

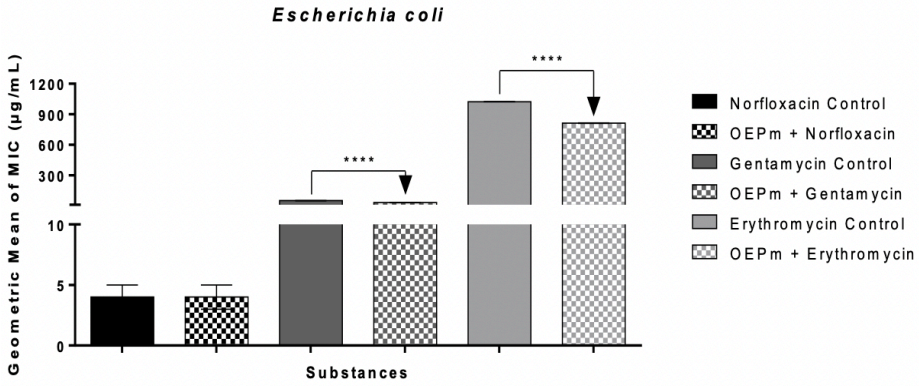

Figure 4. Minimal inhibitory concentration (µg/mL) of different antibiotics in the presence or absence of the OEPm against *E. coli* 06

the MIC of two antibiotics commonly used in antibiotic therapy. There is evidence that the Gram-negative bacterial cell wall hinders the penetration of essential oils [27]. Nevertheless, research using medicinal plants has proven the efficacy of several natural products in combating bacterial resistance in multiresistant *E. coli* strains [28,29]. Here, we hypothesize that the activity of the EOPm may be related to the presence of α-pinene. Accordingly, a study by Ramdani and collaborators [30] demonstrated that the essential oil of *Juniperus phoenicea*, which has α-pinene as the major component, exerted a significant activity against *E. coli*. Leite *et al.* [31] also highlighted the antibacterial potential of this plant constituent, emphasizing the importance of its application in antibacterial therapy.

Importantly, this is to date, the first study reporting the antibacterial ant antibiotic-enhancing effects of *Piper mosenii*. According to Coutinho *et al.* [32], the main mechanisms associated with the modulation of bacterial resistance mediated by natural products involve alteration of the bacterial membrane permeability and inhibition of efflux pumps. These mechanisms could justify the synergistic interactions demonstrated in this study. On the other hand, antagonistic interactions are likely to result from mutual chelation between essential oils and antibiotics [33].

Conclusion

The present study demonstrated that both EOEb and EOPm enhanced the activity of antibiotics against multidrug resistant strains of *E. coli* and *S. aureus*. However, the effects of these substances varied significantly according to the type of strain and associated antibiotic, which could result in clinically ineffective combinations. Therefore, further research is required to determine the conditions under which these extracts and their isolated constituents may be useful in the combat of bacterial resistance.

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