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Research Article

Antibacterial and Potentiating Activity of the Essential Oil of *Citrus bergamia* Risso (Rutaceae)

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Abstract

Antimicrobial resistance represents a growing threat to global public health, exacerbated by the improper use of antibiotics. This situation drives the search for alternative therapies, such as essential oils from medicinal plants of the Rutaceae family, including *Citrus bergamia* (bergamot), which is recognized for its potential antibacterial properties. This study evaluated the antibacterial activity of *Citrus bergamia* essential oil (CBEO) against standard and multidrug-resistant (MDR) bacterial strains, and investigated its effects in combination with clinically used antibiotics. The CBEO was commercially obtained, and its inhibitory capacity was determined using the Minimum Inhibitory Concentration (MIC), ranging from 0.5 to 512 μg/mL. Interactions with antibiotics were tested at subinhibitory concentrations, and data were analyzed by one-way ANOVA followed by Tukey's post hoc test. The results revealed that CBEO did not exhibit significant antibacterial activity, and no relevant potentiating effect was observed when combined with antibiotics. Furthermore, an antagonistic effect was observed in the combination with ampicillin against *S. aureus*, as evidenced by an increase in the antibiotic's MIC.

Keywords: *Pharmacology, In Vitro Research, Plant-Derived Essence, Microbiology.*

Introduction

Antimicrobial resistance (AMR) is one of the greatest challenges to global public health, food security, and the economy. This process occurs when microorganisms stop responding to previously effective drugs, making infection treatment more difficult [1,2]. The lack of rigorous action to address this growing problem compromises the effectiveness of existing healthcare systems and may lead to approximately 10 million deaths worldwide by 2050 [3].

Several factors contribute to the development of resistance, notably the inappropriate use of antibiotics, including self-medication, use in cases of non-specific bacterial infections, or administration for periods that deviate from medical recommendations either shorter or longer durations [4].

In this context, spontaneous genetic mutations in bacteria enhance their adaptability and may confer resistance to external agents, making them more resilient to environmental factors [5,6]. The situation is further exacerbated by the fact that most current antibiotics are derived from compounds discovered before the 1980s, underscoring the urgent need for new therapeutic options [7].

Given this scenario, medicinal plants stand out as important sources of antimicrobial agents and are widely recognized for their ability to contribute to the discovery and development of new antibacterial drugs [8,9]. In this context, essential oils have been increasingly investigated as alternative strategies to combat bacterial infections [10].

The Rutaceae family includes 156 genera and over 1,800 species, widely distributed across tropical and subtropical regions. Its species are recognized for their economic value, providing food, medicines, spices, and essential oils [11]. Among the species of the *Citrus* genus is *Citrus bergamia* Risso, commonly known as bergamot. Although still understudied, the essential oil of this plant is known for its antimicrobial activity, primarily attributed to compounds such as limonene, linalool, and linalyl acetate. Studies have confirmed its effectiveness [12,13].

In light of the rise in bacterial resistance and the search for new therapeutic alternatives, this study aimed to evaluate the antibacterial potential of the essential oil of *Citrus bergamia* Risso (CBEO) against standard and multidrug-resistant (MDR) bacterial strains, as well as to investigate the effects of CBEO in combination with commercial antibiotics (gentamicin, ampicillin, and norfloxacin) on bacterial growth inhibition.

Materials and Methods

Essential Oil Source

The essential oil of *Citrus bergamia* (CBEO), in a 10 mL volume, was purchased from the company Via Aroma through its online platform (https://www.viaaromaloja.com.br/). Via Aroma is recognized for providing aromatherapy products, including essential oils with certifications of purity and origin, such as the IBD Natural Ingredients seal and the indication of being a vegan product, as stated on the product page.

Bacterial Strains, Culture Media, and Antibiotics

The antibacterial activity was investigated using both standard bacterial strains (ATCC) and multidrug-resistant (MDR) strains. The ATCC strains used were *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923, while the MDR strains included *E. coli* 06 and *S. aureus* 10. Bacterial cultures were grown in Brain Heart Infusion broth (BHI; Merck KGaA, Darmstadt, Germany), following the manufacturer's instructions. Bacteria were suspended in 3 mL of sterile saline solution (0.9% NaCl), and turbidity was adjusted to 0.5 on the McFarland scale (equivalent to 1.5×10^8 CFU/mL). The commercial antibiotics used as controls were gentamicin, ampicillin, and norfloxacin.

Minimum Inhibitory Concentration (MIC)

To evaluate the ability of CBEO to inhibit bacterial growth, 100 µL of the bacterial inoculum solution was added to 900 µL of BHI medium in 96-well microtiter plates. Subsequently, different concentrations of CBEO, ranging from 0.5 to 512 µg/mL, were added. Plates were incubated in a bacteriological incubator at 37 °C for 24 hours. After the incubation period, liquid resazurin was used as a redox indicator, with the reaction result indicating bacterial growth. Color changes were analyzed after one hour, with a violet color indicating the absence of growth and a light pink color indicating bacterial presence. All assays were conducted in triplicate (n = 3).

Enhancing Activity

The modulatory effect of CBEO in combination with predefined antibiotics was evaluated using sub-inhibitory concentrations of CBEO (MIC/8) in association with the commercial antibiotics gentamicin, ampicillin, and norfloxacin. Assays were performed by broth microdilution in wells containing antibiotic concentrations ranging from 0.5 to 512 µg/mL, with 100 µL per well. Microplates were incubated in a bacteriological incubator at 37 °C for 24 hours, and all experiments were performed in triplicate [14].

Statistical Analysis

Standard deviations were calculated and data were submitted to one-way analysis of variance (ANOVA). To verify statistically significant differences between groups, Tukey's multiple comparisons test was applied, with a 95% confidence level. Statistical analyses were performed using GraphPad Prism software, version 6 (GraphPad Software Inc., San Diego, CA, USA).

Results

The essential oil of *Citrus bergamia* (EOCB) did not exhibit significant antibacterial activity against either ATCC standard strains or multidrug-resistant (MDR) strains. The MIC values were above 512 μg/mL, indicating a lack of relevant antibacterial activity. Furthermore, EOCB did not enhance the efficacy of gentamicin, ampicillin, or norfloxacin. This was confirmed for both *S. aureus* 10 and *E. coli* 06 strains.

However, the combination of EOCB with ampicillin exhibited an antagonistic effect against *S. aureus*, increasing the MIC from 20 to 32 μg/mL, as shown in Figure 1.

In the analysis of *E. coli* presented in Figure 2, the MIC of norfloxacin was reduced in the presence of EOCB; however, the difference was not statistically significant. The MICs of gentamicin and ampicillin remained unchanged even with the addition of EOCB. Therefore, it can be concluded that EOCB did not potentiate the action of these antibiotics against the *E. coli* strain.

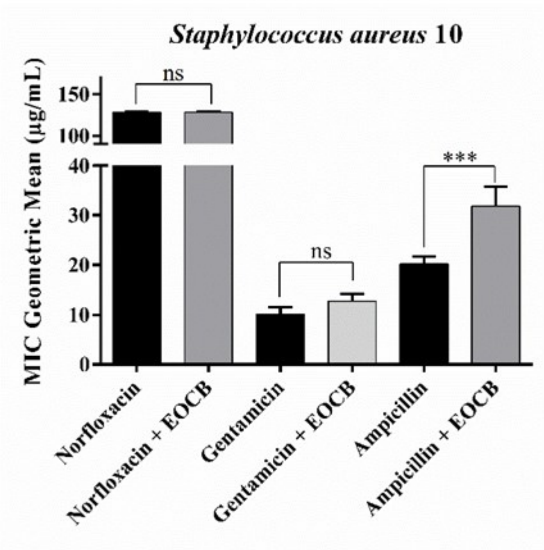

Figure 1. *Geometric mean of the Minimum Inhibitory Concentration (MIC) of Staphylococcus aureus 10 for norfloxacin, gentamicin, and ampicillin, alone and in combination with Citrus bergamia essential oil (EOCB). Results are expressed in μg/mL. Error bars represent standard deviation. (ns) indicates not significant.*

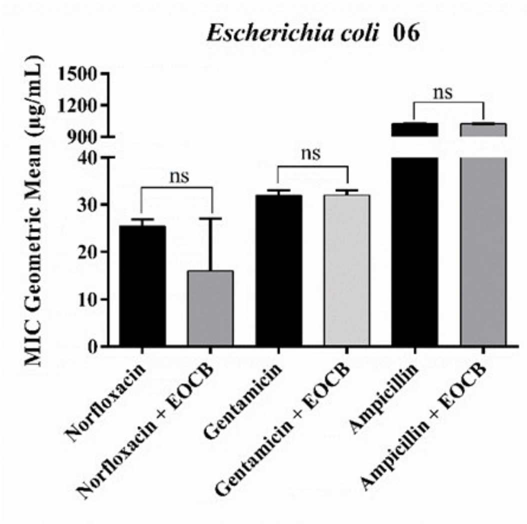

Figure 2. *Geometric Mean of the Minimum Inhibitory Concentration (MIC) of Escherichia coli 06 for norfloxacin, gentamicin, and ampicillin, alone and in combination with Citrus bergamia essential oil (EOCB). Results are expressed in μg/mL. Error bars represent standard deviation. (ns) indicates no statistical significance.*

Discussions

EOCB has been extensively studied due to the presence of compounds such as limonene, linalool, and linalyl acetate, which are known for their antimicrobial activity [13]. Indeed, EOCB has demonstrated relevant antimicrobial properties, with MICs ranging from 0.5% to 2%, showing greater efficacy against *S. aureus*. Although its activity against Gram-negative bacteria is limited by the outer membrane barrier, activity against *E. coli* has also been observed, with MICs ranging from 2% to 4% [15]. However, in contrast to these findings, the results obtained in the present study did not demonstrate significant antibacterial activity for EOCB.

The antagonistic effect observed in the combination of EOCB with ampicillin against *S. aureus* 10 is particularly relevant. Discrepancies in the literature may be attributed to factors such as variations in oil concentrations, the bacterial strains tested, and the methodologies employed in the assays [16]. Therefore, such interactions may result in synergistic, additive, neutral, or even antagonistic effects depending on experimental conditions [17].

EOCB destabilizes the bacterial cell membrane by altering the fluidity and permeability of the lipid bilayer, compromising its integrity and causing leakage of intracellular contents [18]. This resistance is associated with the presence of a viscous extracellular matrix known as slime, which acts as a physical barrier that impairs the diffusion and penetration of hydrophobic substances [19,20].

According to the literature, the combination of medicinal plants and antibiotics presents therapeutic potential, but requires further investigation to avoid possible antagonistic interactions and to maximize treatment efficacy [21]. Thus, it is essential to thoroughly investigate the potential of EOCB, considering its interactions with different classes of antibiotics, in order to enable more effective combined therapies to combat antimicrobial resistance [22,23].

Conclusion

Although *Citrus bergamia* Risso essential oil (CBE) has not demonstrated significant antibacterial activity on its own, its potential as a modulator in combination with antibiotics may contribute to strategies against bacterial resistance. Future research should further analyze its bioactive compounds, mechanisms of action, and safety, exploring different concentrations and combinations. Thus, these investigations may pave the way for more effective and sustainable combination therapies to combat bacterial infections.

Conflict of Interest

The authors declare no conflict of interest.

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