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Research Article

Antibacterial Evaluation *in vitro* of the Essential Oil of *Astronium urundeuva* (M.Allemão) Engl. Against Multidrug-Resistant Strains

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Abstract

Given the current disastrous situation of bacterial resistance resulting in deaths worldwide due to the misuse of antibiotics, it is necessary to explore natural means that can reverse this situation. Thus, the essential oil of *Astronium urundeuva*, which possesses properties directly related to its antibacterial effects, was the focus of this research. The objective was to analyze the antibacterial activity of the essential oil from *A. urundeuva* leaves, as well as its antibiotic-enhancing action. Initially, approval was obtained from the Biodiversity Authorization and Information Systems (SISBio) and the National System for Management of Genetic Heritage and Associated Traditional Knowledge (SisGen). Subsequently, collections were made, and extracts from *A. urundeuva* leaves were obtained. In addition, in vitro tests were conducted to determine the minimum inhibitory concentration (MIC) of the essential oil, along with tests to identify any modifying activity on antibiotic action. Bacterial strains such as *Pseudomonas aeruginosa*, *Escherichia coli,* and *Staphylococcus aureus* were used for these tests. It was found through the minimum inhibitory concentration that the essential oil (EO) alone could not prevent bacterial growth, considering that its result was > 512 µg/mL. However, it was observed that the essential oil, in conjunction with certain antibiotics, had a potentiating effect on its antibacterial action. The most significant enhancers were Norfloxacin, as it achieved higher action against all three tested strains when combined with the EO, and Gentamicin, which, when combined with the EO, strengthened its action against *E. coli* and *S. aureus.* However, Ampicillin, when combined with the EO, showed significance only against *S. aureus*. Therefore, this study revealed that the essential oil of *A. urundeuva* may be promising, as it can enhance the action of antibiotics against bacterial growth.

Keywords: *Myracrodruon Urundeuva; Phytotherapy; Aroeira Do Sertão, Terpenes.*

1. Introduction

Over decades of irresponsible use and excessive prescription of the oldest and conventional antibiotics, we have witnessed the emergence of an alarming crisis of microbial resistance. This distressing scenario results in approximately 700,000 annual deaths worldwide, including 23,000 deaths in Brazil, due to infections resistant to these antibiotics [1]. Although there is a variety of antibiotics available to treat bacterial infections currently, the improper use of these medications, without the proper guidance from qualified healthcare professionals, can lead to serious consequences. Increasingly, there is the emergence of bacterial strains that no longer respond to these treatments, making the approach to infections increasingly challenging [2].

In 2011, the World Health Organization (WHO) claimed that bacterial resistance would pose a threat to humanity; however, since 2014, the WHO stated in a global report (The Global Strategy for Combating Antimicrobial Resistance) that it is no longer just a threat or prediction but a reality that influences everyone. Among antimicrobials, the most commonly used and prescribed drugs are antibiotics, which can be classified as bactericidal, causing bacterial death, or bacteriostatic, inhibiting bacterial growth [3]. These antibiotics are derived from the secondary metabolism of bacteria and fungi and can be classified as natural or synthetic [4].

In the search for new antimicrobial agents, attention should be given to those derived from plants, as Brazil possesses the richest biodiversity on the planet, and many plants have been used for this purpose [5]. Even plant species of the same genus may have significant differences in the production of secondary metabolites, influenced by climate changes and soil types [6]. Thus, since the dawn of humanity, the therapeutic use of plants has been a traditional practice that has spread among different peoples, with a focus on rural communities that hold valuable knowledge about the uses of plants [7,8,9,10].

In recent decades, essential oils have gained considerable attention due to the antimicrobial activities they exhibit [11]. These essential oils are volatile substances resulting from the secondary metabolism of aromatic plants, produced in specialized cells found in roots, stems, seeds, flowers, and fruits. Many natural products, such as compounds like flavonoids, terpenes, phenolics, and coumarins, have been described as metabolites because they facilitate transport through cell membranes, thereby inducing various biological activities, including antibacterial activity [5].

Astronium urundeuva, commonly known as *Myracrodruon urundeuva* Fr. All, is a plant primarily found in arid environments in South America and is widely used in traditional medicine in northeastern Brazil [12]. It belongs to the Anacardiaceae family and naturally grows in the Northeast, Southeast, and Midwest regions. This tree is native to the Cerrado, Caatinga, and Atlantic Forest regions of Brazil and has various common names that vary by region, including genuine aroeira, aroeira, urundeúva, black aroeira, aroeira da serra, and aroeira-do-campo [13].

In this manner, extracts obtained from various parts of the *A. urundeuva* plant, such as leaves and seeds, have revealed antibacterial, neuroprotective, and cytotoxic capabilities against cancer cells [14,15,16]. Therefore, *A. urundeuva* is a plant widely used in herbal therapies and is known to produce essential oil. Consequently, the present project aims to analyze the antibacterial activity of the essential oil obtained from the leaves of *A. urundeuva* (M.Allemão) Engl. (Anacardiaceae), as well as its potential as a drug-strengthening agent.

2. Materials and Methods

2.1 Botanical Material License and Collection

Initially, it was necessary to obtain approval from the Biodiversity Authorization and Information Systems (SISBio) and the National System for Management of Genetic Heritage and Associated Traditional Knowledge (SisGen). Subsequently, plant material from *A. urundeuva* was collected in the municipality of Quixelô – CE.

2.2 Essential Oil Extraction

After the collection process, the leaves of *A. urundeuva* were kept at room temperature with the aim of drying and manually ground to increase the contact area with the extraction solvent. Subsequently, they were placed in a 5-liter glass flask with 2 liters of distilled water and subjected to continuous boiling for 2 hours. After this period, the oil was collected, its yield assessed, placed in an amber bottle, and refrigerated at -4 °C until the chemical description in the *Clevenger* system and the execution of tests [17].

2.3 Antibacterial Activity

2.3.1 Bacterial Strains, Culture Media, and Drugs

In this research, the following strains from the Standard American Type Culture Collection (ATCC) will be used: *Pseudomonas aeruginosa* ATCC 25853, *Escherichia coli* ATCC 25922, and *Staphylococcus aureus* ATCC 25923, along with multidrug-resistant strains: *E. coli* 06, *S. aureus* 10, and *P. aeruginosa* 03, which were maintained at the Laboratory of Microbiology and Molecular Biology of the Regional University of Cariri at -20°C and subcultured on Brain Heart Infusion (BHI) Agar, stored in an incubator at 37°C for 24 hours. After the growth stage, individual colony samples were diluted in test tubes containing 3 ml of sterile saline solution (0.9% NaCl). The suspension was shaken using a vortex mixer, and its turbidity was compared and adjusted to the McFarland 0.5 scale (1.5 x 10^8 colony-forming units/ml).

A total of 10 mg of essential oil from *A. urundeuva* and the antibiotics gentamicin, erythromycin, and norfloxacin (Sigma Aldrich Co., St. Louis, USA) were dissolved in 1 ml of dimethyl sulfoxide (DMSO) and 8,765 µL of sterile distilled water. The concentrations of these solutions were then increased to 1,024 µg/ml [17].

2.3.2 In Vitro Assay

The method described by [17] was employed to determine the minimum inhibitory concentration (MIC) of the natural products. Eppendorf tubes were filled with 100 µL of inoculum and 900 µL of 10% brain heart infusion broth, and aliquots of 100 µL from each solution were dispensed into a 96-well Number Sense ELISA plate. Subsequently, ordered microdilutions (1:1) with 100 µL of essential oil were performed up to the penultimate well, as the last well was used as a control for bacterial growth. The oil concentrations on the plates ranged from 512 to 8 µg/ml. The tests were conducted in triplicate, and the plates were placed in an incubator at 37°C for 24 hours. Afterward, 20 µL of sodium resazurin (0.4 mg/ml) was added to the wells of the plate and allowed to stand for 1 hour at room temperature (25 °C) before reading. The continuation of the blue color of resazurin indicated inhibition of bacterial growth, while a change to pink color indicated bacterial growth occurrence.

2.3.3 Modifying Antibiotic Action Activity

In Eppendorf tubes, 150 µL of the inoculum, a volume of essential oil equivalent to its subinhibitory concentration (MIC/8), and 10% BHI needed to complete its total volume (1.5 ml) were added. The Eppendorf tubes related to the modification controls were completed with 1,350 µL of 10% BHI and 150 µL of inoculum. Thus, the solutions were numerically divided into 96-well microdilution plates (100 µL/well). Serial microdilution (1:1) was performed using 100 µL of antibiotic from the first to the penultimate well, with plate concentrations ranging from 512 to 0.5 µg/ml. The tests were conducted in triplicate, and the plates were incubated in an incubator at a temperature of 37°C for 24 hours. The results will be read in the same way as MIC [18].

2.4 Statistical Analysis

All means and their respective standard errors of the mean were calculated. Subsequently, a one-way analysis of variance (ANOVA Oneway) was conducted using the Tukey test with a confidence level of 95%. All evaluations were performed using GraphPad Prism 6.0 software.

3. Results

3.1 Antibacterial Activity

3.1.1 *In vitro* Assays

Through the minimum inhibitory concentration, it was possible to identify that the essential oil of *A. urundeuva* does not have an inhibitory effect on the bacterial growth of the three ATCC strains analyzed (*P. aeruginosa, E. coli,* and *S. aureus*), as its result was > 512 ug/mL.

3.1.2 Modifying Antibiotic Action Activity

From the conducted tests, it was found that when the essential oil of *A. urundeuva* was combined with antibiotics, it achieved a higher potential against the tested MDR bacteria (Figure 1). Thus, the antibiotic that had the most significant effect in association with OEAU was Norfloxacin, as it potentiated the antibacterial action against the three analyzed strains: *P. aeruginosa, E. coli* (gram-negative), and *S. aureus* (gram-positive). Additionally, Gentamicin also increased the antibacterial effect when combined with essential oil; however, it only showed synergistic action against *E. coli* and *S. aureus*.

Nevertheless, Ampicillin had a lower significance compared to the previous ones, as it increased antibacterial activity against only one of the analyzed strains, *S. aureus*, but had an antagonistic effect on the other bacteria.

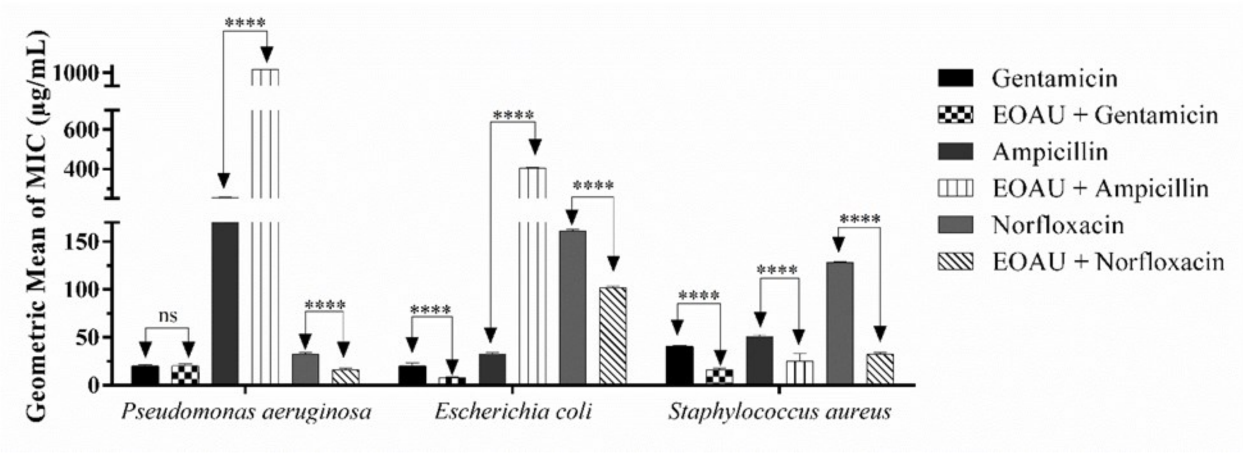

Figure 1 *- Geometric mean of the minimum inhibitory concentration (MIC) in µg/mL of the essential oil of Astronium urundeuva (EOAU) in combination with three antibiotics (Gentamicin, Ampicillin, and Norfloxacin) against multidrug-resistant bacterial strains (P. aeruginosa, E. coli, and S. aureus). ns = p > 0.05, ** = p < 0.01, **** = p < 0.0001.*

4. Discussions

Thus, *Astronium urundeuva* has in its chemical composition various secondary metabolites, such as phenols, flavonoids, terpenes, tannins, and chalcones. These components, whether acting individually or in combination, are directly associated with the therapeutic effects of the plant, as evidenced in previous studies [20, 21, 22, 23, 24]. However, it is important to note that the leaves of *A. urundeuva* exhibit a lower concentration of antimicrobial properties compared to the plant's bark, as indicated by previous studies [25].

According to [26] and [27], phytochemical exploration of the extracts revealed the presence of various classes of secondary metabolites, demonstrating diverse activities, including antimicrobial effects. This activity is directly associated with the widely documented antioxidant and anti-inflammatory properties of "aroeira-do-sertão" [20]. [28] assert that the antimicrobial potential is related to the presence of flavonoids and tannins in hydroalcoholic extracts from the leaves of "aroeira-do-sertão."

Therefore, when a substance is combined with an antibiotic, the antibiotic's activity increases (usually by reducing the MIC), a phenomenon classified as a synergistic effect. However, when there is an opposite effect to the antibiotic effect, i.e., an increase in the MIC, it indicates antagonism [29]. As obtained, Norfloxacin combined with EOAU had a synergistic effect against the tested strains. Gentamicin had a synergistic effect against two of the bacteria and antagonistic against only one of them. Ampicillin had a synergistic action against only one of them and antagonistic against two.

Given this, gentamicin, belonging to the aminoglycoside class, was able to enhance its effects against *E. coli* and *S. aureus*. This is consistent with the research by [21], which asserts that the combination of phytotherapeutic products with aminoglycosides can be a way to reduce the adverse effects of antibiotics in the treatment of infections caused by Gram-positive or Gram-negative bacteria. This combination can produce a synergistic effect, allowing the use of lower doses for successful treatment [21].

Therefore, ampicillin is a beta-lactam antibiotic of the aminopenicillin class, classified as semi-synthetic and is currently the most widely used antimicrobial. Due to its antibacterial spectrum, clinical indications, and pharmacokinetic characteristics, beta-lactams are antibacterial medications capable of penetrating placental and blood-brain barriers. However, the misuse of these antibiotics since their discovery has greatly reduced their effectiveness, leaving society at risk of losing effective antibiotics [30]. Thus, interactions between natural products and drugs can be either favorable or unfavorable, potentiating the effect of a medication or reducing its efficacy [31].

Norfloxacin is a synthetic antibiotic belonging to the fluoroquinolone class, which represents the second generation of quinolones and differs due to the presence of a fluorine atom and a piperazinyl group. As a result, this antibiotic has antibacterial action 1,000 times greater than nalidixic acid, which is a quinolone, acting primarily against gram-negative bacteria and certain gram-positive bacteria [32, 33]. Therefore, herbal products are composed of a variety of bioactive substances and, consequently, have different mechanisms of action that can lead to drug interactions when ingested with medications [31]. Regarding the combination of Norfloxacin with EOAU, it resulted in a synergistic effect against the analyzed MDR bacteria.

5. Conclusion

This research suggests that the essential oil from the leaves of *A. urundeuva* may be favorable since, when combined with antibiotics, it has a potentiating effect on the inhibition of bacterial growth. Therefore, it is deduced that herbal studies against bacterial resistance are essential, as they may bring fewer side effects since they come from natural products like plants, and are also more accessible.

Conflict of Interest

The authors declare no conflict of interest.

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