

Evaluation of the Microbiological Activity of *Myracrodruon urundeuva* Allemão Extract Against Clinically Relevant Pathogens

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Abstract

Antimicrobial resistance is a global concern, affecting public health and generating economic and social impacts, which requires global strategies to contain its spread and reduce associated deaths. Medicinal plants demonstrate efficacy against pathogenic microorganisms, offering alternatives in the fight against microbial resistance. As a highlight, *Casearia javitensis* has antimicrobial and antiparasitic properties of therapeutic relevance to cope with these microorganisms. This research aimed to evaluate the antibacterial activity of ethanolic extract of *C. javitensis* leaves (EECJ), as well as its antibiotic potentiating activity. Leaves of the species were collected, dried, crushed and subjected to extraction using ethanol. For the antimicrobial assays, conventional and multidrug-resistant bacterial (MDR) strains were used. The inhibition capacity was analyzed by means of the Minimum Inhibitory Concentration (MIC), at concentrations from 0.5 to 512 µg/mL. The potentiating activity was evaluated using subinhibitory concentrations of EECJ (MIC/8) in association with the antibiotic's gentamicin, ampicillin and norfloxacin. The data obtained was submitted to statistical analysis. The results indicated that EECJ did not present isolated antibacterial activity (MIC > 512 µg/mL); however, it has been shown to be effective as an antibiotic enhancer, reducing the MIC of gentamicin, ampicillin, and norfloxacin against the MDR strains of *E. coli*, *S. aureus*, and *P. aeruginosa*. These findings suggest that ethanolic extract of *C. javitensis* may be a promising alternative in combination therapies.

Keywords: Bacterial Resistance, Aroeira-do-sertão, Phytochemicals, Flavonoids, and Catechins

1. Introduction

Infections caused by microorganisms resistant to antimicrobials have become one of the major public health challenges worldwide. According to recent estimates, bacterial resistance was responsible for approximately 4.95 million deaths in 2019, making it the third leading cause of death globally [1]. Projections indicate that by 2050, drug-resistant infections could result in around 10 million annual deaths [2]. This scenario highlights the urgency of effective therapeutic alternatives to contain the spread of resistant pathogens and reduce the morbidity and mortality associated with these infections [2,3,4].

Bacterial resistance occurs due to the ability of microorganisms to develop mechanisms that make them insensitive to the actions of antimicrobials, compromising the effectiveness of traditional treatments [2]. Among the main factors contributing to this phenomenon is the indiscriminate and inappropriate use of antibiotics [4,5,6,7]. The increase in the consumption of antibacterials between 2000 and 2015 was 39%, with a very high growth projection in the coming decades, raising the risks of emergence and spread of resistant strains [5,6,8].

Microorganisms such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* stand out for their ability to acquire multiple antimicrobial resistance, significantly complicating therapeutic options [9,10]. This phenomenon has a direct impact on global health, increasing hospital costs, prolonging hospitalization time, and raising mortality rates. Thus, there is an urgent need to investigate new approaches to combat resistant microorganisms and expand the available therapeutic arsenal.

In this context, natural products emerge as a promising strategy in the search for new compounds with antimicrobial activity. Throughout history, plants have been widely used to treat infectious diseases, with several of their secondary metabolites exhibiting antibacterial and antifungal effects [11,12]. Recent studies show that bioactive substances extracted from plants can act synergistically with conventional antimicrobials, enhancing their action or reducing microbial resistance [7].

Within this scope, species such as *Myracrodruon urundeuva* have attracted attention due to their therapeutic potential. This botanical species is commonly known as "aroeira-do-sertão," a plant from the Anacardiaceae family, widely used in folk medicine for its anti-inflammatory, healing, anti-allergic properties, and in the treatment of skin and mucosal infections [11,13,14].

Through their secondary metabolism, higher plants produce metabolites such as terpenoids, alkaloids, phenolic compounds (quinones, tannins, and flavonoids), carotenoids, and saponins, which act as defense mechanisms against microorganisms by altering enzymatic reactions and cell membrane structures [15,16]. Aroeira-do-sertão has stood out for its antimicrobial and antioxidant potential due to the presence of secondary metabolites [17]. Phenolic compounds, such as those found in the plant, are recognized for their antimicrobial [12] and antioxidant [18] properties, making them promising for the food preservative industry.

2. Materials and Methods

2.1 Obtaining a license and collecting the botanical material

The bark of *Myracrodruon urundeuva* was collected in the municipality of Quixelô in the Caatinga area. The species identification was performed in the field by botanist Dr. José Weverton Almeida-Bezerra. From the collected material, the ethanolic extract of the plant was prepared for further analysis.

2.2 Preparation of ethanolic extract (EE)

The bark (120 g) was collected in the morning, washed under running water to remove impurities, and then subjected to dehydration. After drying, the material was ground to reduce its size and increase the surface area for contact with the solvent, ethanol. The fragments were then transferred to autoclaved glass jars and subjected to maceration in 1500 mL of 96% ethanol for 72 hours. After this period, the suspension was filtered, and the solvent was removed by rotary evaporation under reduced pressure [19]. The obtained extracts were stored at room temperature until the tests were conducted.

2.3 Phytochemical Prospecting

In order to identify the main classes of secondary metabolites, phytochemical screening was conducted according to the methodology described by Matos (1997) [20]. The analysis covered phenolic compounds, hydrolyzable tannins, flavonoid tannins, anthocyanins, anthocyanidins, flavones, flavonols, xanthonenes, chalcones, aurones, flavonoids, leucoanthocyanidins, catechins, and flavanones, as detailed in Table 1.

To verify the presence or absence of these compounds, cross tests were applied, classifying the results into different intensities: (-) absence, (+) weak presence, (++) moderate presence, and (+++) intense presence, following adaptations of the methodologies described by Hernández et al. (2018) [21] and Barbosa et al. (2004) [22]. The identification of catechins was based on qualitative colorimetric reactions, as described by Matos (1997) [20]. Their presence was confirmed through reactions in acidic and alkaline media, showing positive results of low intensity (+).

Table 1. Methods for qualitative phytochemical determination.

Classes	Method	Citation
Phenols	FeCl ₃ reaction	BARBOSA <i>et al.</i> , 2004 HERNÁNDEZ <i>et al.</i> , 2019
Hydrolyzable tannins	FeCl ₃ reaction	BARBOSA <i>et al.</i> , 2004
Flababnian tannins	FeCl ₃ reaction	BARBOSA <i>et al.</i> , 2004
Anthocyanins	Acid and alkaline reaction	BARBOSA <i>et al.</i> , 2004
Anthocyanidins	Acid and alkaline reaction	BARBOSA <i>et al.</i> , 2004
Flavones	Alkaline reaction	BARBOSA <i>et al.</i> , 2004
Flavonols	Alkaline reaction	BARBOSA <i>et al.</i> , 2004
Xanthonenes	Alkaline reaction	BARBOSA <i>et al.</i> , 2004
Chalcones	Acid and alkaline reaction	BARBOSA <i>et al.</i> , 2004
Aurones	Acid and alkaline reaction	BARBOSA <i>et al.</i> , 2004
Flavanonols	Alkaline reaction	BARBOSA <i>et al.</i> , 2004
Leucoanthocyanidins	Acid and alkaline reaction	BARBOSA <i>et al.</i> , 2004
Catechins	Acid and alkaline reaction	BARBOSA <i>et al.</i> , 2004
Flavonones	Acid and alkaline reaction	BARBOSA <i>et al.</i> , 2004
Foamy saponins	Foam time	BRAGA <i>et al.</i> , 2019

2.4 Evaluation of antibacterial activity

2.4.1 Strains, culture media, and drugs

The antibacterial activity of the extract was evaluated using both standard and multidrug-resistant strains. *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 9027, and *Staphylococcus aureus* ATCC 25923, along with resistant variants *E. coli* 06, *P. aeruginosa* 24, and *S. aureus* 10, obtained from the Microbiology and Molecular Biology Laboratory (LMBM) of the Regional University of Cariri (URCA), were tested. The bacteria were stored at 4 °C until the time of the tests and cultured on Heart Infusion Agar (HIA) and BHI broth at 10%, incubated at 37 °C for 24 hours to ensure adequate growth. The inoculum was prepared with a bacterial suspension in sterile saline solution (0.9% NaCl), adjusted to the McFarland scale 0.5 (approximately 1.5×10^8 CFU/mL).

The antibiotics gentamicin, norfloxacin, and ampicillin (Sigma Co., St. Louis, USA) were solubilized in sterile distilled water at an initial concentration of 1.024 µg/mL. The compounds tested were weighed (10 mg) and dissolved in 1 mL of dimethyl sulfoxide (DMSO) and sterile distilled water to achieve the same concentration. The antimicrobial activity was evaluated by the colorimetric assay with sodium resazurin (Sigma-Aldrich, St. Louis, MO), used as an indicator of cell viability through redox reactions. The experimental protocol followed the guidelines of CLSI M7-A113, with adaptations as needed.

2.4.2 Minimum Inhibitory Concentration (MIC)

The determination of the Minimum Inhibitory Concentration (MIC) was performed using the broth microdilution method [23]. The bacterial strains were prepared as previously described and added to liquid BHI culture medium (1.350 µL) along with 150 µL of the inoculum, totaling 1.5 mL. Serial dilutions were performed in 96-well plates, with successive dilutions up to the second-to-last well; the last well was reserved as the growth control. An additional control ensured the sterility of the culture medium.

After incubation at 37°C for 24 hours, the MIC was determined by adding 20 µL of sodium resazurin solution, and waiting for one hour to allow the redox reaction. A color change from blue to pink indicated bacterial growth, while the absence of this change confirmed inhibition. The lowest concentration without any color change was recorded as the MIC. The experiments were performed in triplicate to ensure the reproducibility of the results.

2.5 Statistical analysis

All assays were performed in triplicate, and the results were analyzed using GraphPad Prism version 6 (GraphPad Software Inc., San Diego, CA, USA). The data were analyzed using two-way ANOVA with Bonferroni post hoc. Results were considered significant when $p < 0.05$, $p < 0.0001$, and not significant when $p > 0.05$. The means \pm standard deviation were expressed.

3. Result

3.1 Phytochemical Screening

The phytochemical screening, according to Table 2, highlights that the Ethanolic Extract of *Myracrodruon urundeuva* (EEMU) in this study revealed the presence of hydrolyzable tannins, flavanonols, catechins, and flavanones. On the other hand, phenols, flavabene tannins, anthocyanins, anthocyanidins, flavones, flavonols, xanthonones, chalcones, auronones, leucoanthocyanidins, and saponins were not detected.

Table 2. Phytochemical Screening of the Ethanolic Extract of *Myracrodruon urundeuva* (EEMU). Chemical class absence (-); Chemical class presence (+).

Classes	EEMU
Phenols	-
Hydrolyzable tannins	+
Flavabene tannins	-
Anthocyanins	-
Anthocyanidins	-
Flavones	-
Flavonols	-
Xanthonones	-
Chalcones	-
Auronones	-
Flavononols	+
Leucoanthocyanidins	-
Catechins	+
Flavanones	+
Foaming saponins	-

3.3 Antibacterial activity

The determination of the Minimum Inhibitory Concentration (MIC) of EEMU, as shown in Table 3, revealed that all the tested strains exhibited MIC values equal to or greater than 512 $\mu\text{g/mL}$. This result was observed for both standard strains (*Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 25923, and *Escherichia coli* ATCC 25922) and multidrug-resistant strains (*P. aeruginosa* 24, *S. aureus* 10, and *E. coli* 06), indicating a low inhibitory activity of the extract under these experimental conditions.

Table 3. Determination of Minimum Inhibitory Concentration (MIC $\mu\text{g/mL}$).

Bacteria	PA ATCC 9.027	SA ATCC 25.923	EC ATCC 25.922	PA 24	SA 10	EC 06
EEMU	> 512	> 512	> 512	> 512	> 512	> 512

Abbreviations: PA: *Pseudomonas aeruginosa*; SA: *Staphylococcus aureus*; EC: *Escherichia coli*, EEMU: Ethanol Extract of *Myracrodruon urundeuva*.

3.4 Modifier effect on antibiotic action

Figure 1 presents the geometric mean of the Minimum Inhibitory Concentration (MIC) of gentamicin, norfloxacin, and ampicillin, both individually and in combination with EEMU, against *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus*. For *P. aeruginosa*, the combination of the extract with gentamicin showed no significant difference, while the combination with norfloxacin increased the MIC, suggesting an antagonistic effect. In *E. coli*, EEMU significantly reduced the MIC of gentamicin, indicating a possible synergistic effect, but had no significant impact on norfloxacin. For *S. aureus*, EEMU significantly reduced the MIC of gentamicin, norfloxacin, and ampicillin, suggesting a strong potentiation of the antimicrobial activity of these antibiotics. These results indicate that EEMU may act as a modulator of antibacterial action, promoting synergy with certain antibiotics, especially against *S. aureus* and *E. coli*, while potentially antagonizing the effect of norfloxacin against *P. aeruginosa*.

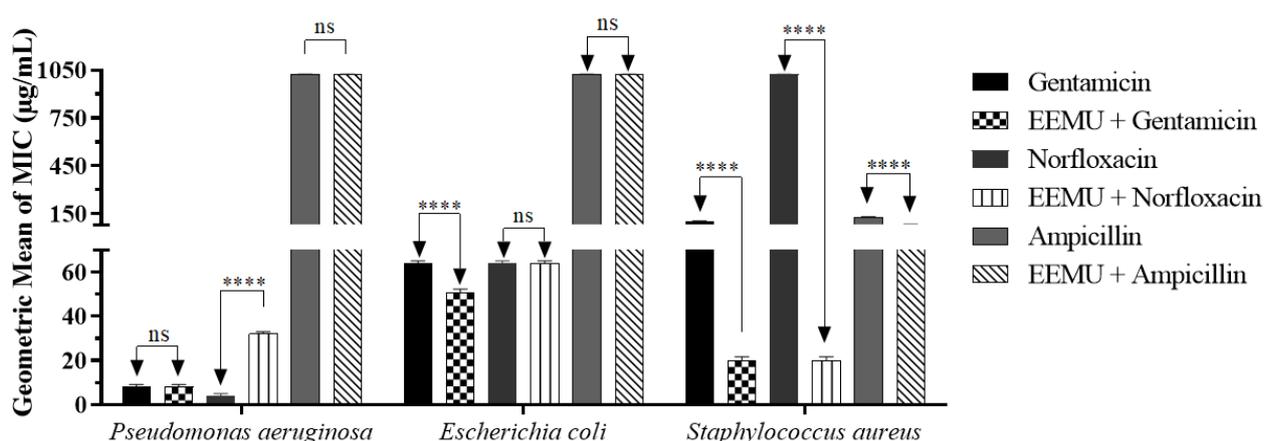


Fig 1. Combined effect of the Ethanol Extract of *Myracrodruon urundeuva* (EEMU) on the antimicrobial activity of gentamicin, norfloxacin, and ampicillin against *Pseudomonas aeruginosa* 24, *Staphylococcus aureus* 10, and *Escherichia coli* 06. The values represent the geometric mean of the Minimum Inhibitory Concentration (MIC) of the antibiotics, both isolated and in combination with EEMU. Bars indicate standard deviation. Statistically significant differences are represented by **** ($p < 0.0001$), while "ns" indicates no statistical significance ($p > 0.05$).

4. Discussion

The growing issue of bacterial resistance has driven the search for new therapeutic strategies, and the combination of natural products with antibiotics has proven to be a promising approach to counteract this scenario. In this context, the ethanolic extract of *Myracrodruon urundeuva* exhibited significant antimicrobial activity, particularly when combined with widely used antibiotics in clinical practice.

The phytochemical composition of EEMU contains compounds such as catechins, flavanones, and flavanols. Catechins, which are already known for their antimicrobial and antioxidant properties, may contribute to the synergistic effect observed with antibiotics [24,25]. The presence of phenolic compounds, vitamin C, and carotenoids further reinforces its therapeutic relevance, as these substances have well-established antimicrobial properties.

Compared to other species of the *Myracrodruon* genus, *M. urundeuva* stands out for the presence of hydrolyzable tannins and flavonoids, both associated with antimicrobial and anti-inflammatory effects. This chemical composition may justify its activity against Gram-positive bacteria, such as *Staphylococcus aureus*, supporting previous studies on its antimicrobial potential [26].

Environmental factors and agricultural practices can influence the chemical profile of EEMU, affecting its therapeutic efficacy [27,28]. Therefore, standardizing cultivation and processing conditions becomes essential to ensure the reproducibility of the observed biological effects.

The phytochemical screening confirmed the presence of hydrolyzable tannins, catechins, flavanones, and flavanols, compounds widely recognized for their antimicrobial and antioxidant properties [29]. The absence of flavonols and flavones may suggest a more selective composition, with an emphasis on specific secondary metabolites, such as catechins, which have a strong antibacterial effect.

The antimicrobial activity tests revealed significant inhibition of the growth of Gram-positive bacteria, possibly due to the interaction of phenolic compounds with the bacterial cell wall [30]. The presence of condensed tannins and phenolic acids may contribute to the destabilization of the microbial cell membrane, leading to the loss of cell integrity and subsequent bacterial death [31,32].

The synergy between EEMU and clinical antibiotics was evidenced by the reduction of the minimum inhibitory concentration (MIC) against multidrug-resistant strains of *S. aureus*. This effect may be related to the inhibition of bacterial efflux pumps by flavonoids and tannins, increasing the retention of antibiotics within the bacterial cell [26]. Therefore, EEMU may act as a modulator of bacterial resistance, highlighting the need for further investigations into its mechanisms of action.

The effectiveness of the extract in potentiating aminoglycosides and lincosamides suggests different interactions between its secondary metabolites and antibiotics. Studies indicate that the phenolic compounds and flavonoids in EEMU may alter bacterial cell membrane permeability, facilitating the action of antibiotics and expanding their antimicrobial spectrum [33]. The synergy observed between *M. urundeuva* and antibiotics such as gentamicin, norfloxacin, and ampicillin reinforces its therapeutic potential in the fight against bacterial resistance.

Although the results are promising, further studies are needed to assess the toxicity of EEMU and its effectiveness in in vivo models. Elucidating the mechanisms of action and standardizing its phytochemical composition are crucial for its application as a natural antimicrobial agent or adjunct in antibiotic therapy. Given the rise of bacterial resistance, the exploration of natural products like *M. urundeuva* represents a viable and innovative alternative for the development of new therapeutic strategies.

5. Conclusion

The results of this study indicate that the ethanolic extract of *Myracrodruon urundeuva* has significant antimicrobial potential, especially when combined with conventional antibiotics, showing the ability to enhance their effectiveness. The phytochemical composition of the extract, rich in phenolic compounds, flavonoids, and catechins, may be responsible for this synergistic effect. However, further studies are needed to investigate the underlying molecular mechanisms and to evaluate the safety and efficacy of the extract in in vivo models, in order to confirm its potential as a viable therapeutic alternative in the fight against bacterial resistance.

Conflict of Interest

The authors declare no conflict of interest.

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