Anacardium occidentale L. (ANACARDIACEAE): ANTIBACTERIAL AND ANTIBIOTIC POTENTIATING ACTIVITY

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ABSTRACT: Alexander Fleming discovered the first antibiotic, which was instrumental in saving millions of lives. However, improper use has contributed to the emergence of resistant microorganisms. Medicinal plants are alternatives to drugs, because they have biologically active compounds that can act against microorganisms and even enhance the effects of antibiotics. *Anacardium occidentale* is a species with medicinal properties traditionally used to treat illnesses and, in this work, its antibacterial and antibiotic potentiating activity was evaluated. *A. occidentale* bark collection was carried out in the municipality of Lavras da Mangabeira, Brazil. After cleaning, dehydration and grinding, the material was extracted using ethanol. Antibacterial activity tests were performed with standard strains and clinical isolates. The minimum inhibitory concentration (MIC) was determined and the modulating capacity of the extract with gentamicin, erythromycin and norfloxacin was evaluated. The tests were performed in Brain Heart Infusion culture medium. The results showed that the natural product has no intrinsic antibacterial activity, but when combined with standard drugs it was able to change its activities, intensifying its action in the treatment of infections. These results include the importance of using plant extracts in the treatment of infections, highlighting the importance of further studies to better understand their action, as well as the search for new sources of medication.
KEYWORDS: Drugs, Antibiotics, Resistance, Extracts, Medicinal plants.

Anacardium occidentale L. (ANACARDIACEAE): ATIVIDADE ANTIBACTERIANA E POTENCIALIZADORA DE ANTIBIÓTICOS

RESUMO: Alexander Fleming descobriu o primeiro antibiótico, que foi fundamental para salvar milhões de vidas. No entanto, o uso inadequado contribuiu para o aparecimento de microrganismos resistentes. Plantas medicinais são alternativas às drogas, pois possuem compostos biologicamente ativos que podem agir contra microrganismos e até mesmo potencializar os efeitos dos antibióticos. *Anacardium occidentale* é uma espécie com propriedades medicinais tradicionalmente utilizadas para tratar enfermidades e, neste trabalho, foi avaliada sua atividade antibacteriana e potencializadora de antibióticos. Coleta de cascas de *A. occidentale* foi realizada no município de Lavras da Mangabeira, Brasil. Após limpeza, desidratação e trituração, o material foi extraído usando etanol. Testes de atividade antibacteriana foram realizados com cepas padrão e isolados clínicos. Determinou-se a concentração mínima inibitória (CIM) e foi avaliada a capacidade moduladora do extrato com gentamicina, eritromicina e norfloxacina. Os testes foram realizados em meio de cultura

Brain Heart Infusion. Os resultados mostraram que o produto natural não possui atividade antibacteriana intrínseca, mas ao ser combinado com fármacos padrões foi capaz de alterar suas atividades, intensificando sua ação no tratamento de infecções. Estes resultados incluem a importância da utilização de extratos vegetais no tratamento de infecções, destacando a importância de mais estudos, para melhor compreensão de sua ação, bem como a busca por novas fontes de medicamentos.
PALAVRAS-CHAVE: Fármacos, Antibióticos, Resistência, Extratos, Plantas medicinais.

1 I INTRODUCTION

In the first half of the 20th century, scientist Alexander Fleming's discovered the first antibiotic, which years later would save the lives of thousands of people throughout the world. This discovery was so important in the world of microbiology that it earned him a Nobel Prize in 1945. In his speech upon receiving his prize, he warned everyone about the possibility of the emergence of microorganisms resistant to such an antibiotic. Unfortunately, his points were neglected, so that antibiotics were used wildly by populations for the treatment of various diseases. Such inappropriate use has resulted in one of the biggest public health problems worldwide, the well-known "bacterial resistance" (SAND, 2020; ABADI et al., 2019; TAN; TATSUMURA, 2015).

This bacterial resistance is quite worrying, as it is estimated that in 2050 there will be around 10 million annual deaths due to infections that are now considered treatable (KRAKER; STEWARDSON; HARBARTH, 2016). The resistance mechanisms of bacteria to chemotherapeutic agents are responsible for their survival and multiplication, which are the enzymatic destruction or inactivation of the drug, changes in the entry of the antibiotic, changes in the target sites of the drug, protection or blocking of the target site and efflux of the antibiotic (ANDERSSON; HUGHES; KUBICEK-SUTHERLAND, 2016; FAIR; TOR, 2014).

Thus, it is evident that the search for new antibiotics is the primary solution. However, the search and discovery of new molecules with a biological effect on pathogenic bacteria has not followed its evolution. As an alternative, there are medicinal plants, which are rich in biologically active compounds, derived from their secondary metabolism (ANAND et al., 2019; MANANDHAR; LUITEL; DAHAL, 2019). Such metabolism originates molecules that have been optimized over thousands of years during the course of evolution, so that they act as defense mechanisms, being able to act against herbivores and even microbes (ANAND et al., 2019). These constituents, in addition to causing death to microorganisms, can affect cellular events in the pathogenic process, so that the ability to develop resistance to products of plant origin becomes more difficult (MAHADY, 2005). In addition to direct biological activity, natural products can act as antibiotic intensifiers against resistant microorganisms (BEZERRA et al., 2020; COUTINHO et al., 2008). This success of medicinal plants is so notorious that the World Health Organization (WHO) lists 21,000 plant species that are used to treat diseases throughout the world (YUAN et al., 2016).

The Anacardiaceae family is a taxon that includes numerous species of great medicinal importance, with emphasis on the treatment of infections, such as *Mangifera indica* L. (mango), *Pistacia vera* L. (pistachio), *Spondias tuberosa* Arruda (umbu), *Schinus terebinthifolia* Raddi (pink pepper), *Astronium urundeuva* Engl. (aroeira) and *Anacardium occidentale* L. (cashew) (OLIVEIRA et al., 2022; SALESSE et al., 2018; SCHULZE-KAYSERS; FEUEREISEN; SCHIEBER, 2015). The latter is a plant species native to tropical regions of the American continent, and which, in addition to medicinal potential, has global socioeconomic relevance, as its fruits are consumed all over the world. However, despite the popularization of its fruit, its medicinal properties are not restricted to this organ. The bark and leaves are used by the population in the form of teas (infusions and/or decoctions) for the treatment of illnesses. Among the uses, stand out as an analgesic, anti-inflammatory, antipyretic, headache, healing, diabetes, diarrhea, flu, against infections and gastritis (OLIVEIRA et al., 2022; COSTA et al., 2020). Such ethnomedicinal properties attributed to *A. occidentale* may be related to its secondary metabolism. Because it is quite heterogeneous, mainly presenting phenolic compounds, such as flavonoids and tannins (SALEHI et al., 2019).

Having exposed this, it is hypothesized that the bark of *A. occidentale* has biologically active constituents against pathogenic bacteria. Thus, this work aimed to evaluate the antibacterial and potentiating effect of antibiotics from extracts of the stem bark of A. occidentale, as well as to determine its chemical constituents.

2 I MATERIALS AND METHODS

2.1 Collection of Botanical Material

In October 2018, *Anacardium occidentale* barks were collected in the municipality of Lavras da Mangabeira – CE, Brazil (-6º45’12” W and -38º58’18”S). The fertile branches were pressed and dehydrated, being later identified by Professor Dr. João Tavares Calixto-Júnior and deposited at the Herbário Caririense Dárdano de Andrade Lima (HCDAL/URCA), under number 13.690. The collection was carried out during the morning period (09:00±00:30).

2.2 Preparation of extracts

After cleaning in running water to remove residues, the peels (180 g) were dehydrated at 45 °C for 24 hours and crushed. Subsequently, the material was submerged in 1.3 L of 96% ethanol in amber glasses for 72 hours. After exhaustive extraction, the extract was obtained by filtering and concentrating the material in a rotary evaporator coupled to a water bath.

2.3 Antibacterial activity

2.3.1 Bacterial strains, culture media and drugs

To perform antibacterial activity tests, standard bacterial strains and clinical bacterial isolates were used. The standard strains were: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 25853 and *Staphylococcus aureus* ATCC 25923. The clinical isolates were: *Escherichia coli* 06, *Pseudomonas aeruginosa* 24 and *Staphylococcus aureus* 10, with resistance to several drugs ((FERNANDES et al., 2022).

For the execution of the antibacterial assays, the Brain Heart Infusion (BHI) culture medium was used, which was prepared according to the manufacturer's recommendations. In addition, the drugs that were used to evaluate the modulating activity of the A. occidentale extract were gentamicin, belonging to the aminoglycoside class, erythromycin, from the macrolide group, and norfloxacin, a component of fluoroquinolones. These were chosen in order to better assess the capacity of the extract to modulate the action of drugs.

2.3.2 Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was determined according to the methodology used by Bezerra et al., (2019). For this, a 1,000 µL solution containing 100 µL of inoculum and 900 µL of liquid culture medium (10% BHI) was prepared. This solution was distributed in 96 wells of previously numbered plates, adding 100 µL to each well. Then, 100 µL of extract was added to the first well and serially microdiluted, with concentrations ranging from 512 µg/mL to 1 µg/mL. Finally, the plates were incubated for 24 hours at 37°C to determine the MIC responsible for fully inhibiting bacterial growth.

After incubation, 20 µL of resazurin solution were added to each well for oxidoreductive reactions to take place, signaling possible microbial growth. After 1 hour, the color of each of the wells was evaluated. Those that changed color from blue to red indicate growth while those that remained blue indicate no growth. The color change in the wells allows the identification of the presence or absence of bacterial growth.

2.3.3 Modulator Effect

To evaluate the potentiating capacity of the extract, the methodology proposed by Coutinho et al., (2008) was adopted, which consisted of carrying out MIC tests with several resistant bacteria. After that, the sub-inhibitory concentrations (MIC/8) of the antibiotics were established, which ranged from 512 µg/mL to 1 µg/mL. To carry out the tests, 1,162 µL of 10% BHI were prepared, comprising 150 µL of each inoculated strain and the natural product in volume corresponding to a sub-inhibitory concentration. The control group was prepared with 1,350 μL of BHI (10%) and 150 μL of bacterial suspension. Then, serial microdilution with the antibiotic was performed, with 100 µL of each drug up to the penultimate well. After incubation (24 hours at 37 °C), the MIC was read, with the addition of 20 µL of resazurin. In this way, it was expected that the results obtained would demonstrate the potentiation capacity of the *A. occidentale* extract.

2.4 Statistical analysis

The results obtained were analyzed using the GraphPad Prism program, version 6, with the aid of the One-way Anova and Tukey's *post hoc* test. The results were considered to be significant when the p value <0.05, which was fulfilled. Therefore, the results obtained were considered relevant and significant.

3 I RESULTS AND DISCUSSION

When evaluating the intrinsic antibacterial effect of the *A. occidentale* extract against standard and multiresistant bacteria, it was possible to observe that the natural product was not able to inhibit bacterial growth at clinically relevant concentrations. So all inhibitory concentrations were >512 *µ*g/mL. Although the bark does not have an antibacterial effect, Doss and Thangavel (2011) demonstrated that the leaves and seeds have antibacterial properties against different bacteria. These include Gram-positive bacteria such as *Micrococcus luteus* and *Staphylococcus aureus*, and Gram-negative human pathogenic bacteria *Salmonella typhi*, *Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa*.

In addition to these, Agedah et al. (2010) also identified the antibacterial effect of extracts derived from the leaves of *A. occidentale* on two clinically important pathogens, *Escherichia coli* and *Staphylococcus aureus*. Thus, *S. aureus* was more sensitive due to its structural organization, since it does not have an external membrane. While *E. coli* has an external membrane, called the lipopolysaccharide layer attached to its cell wall, which may have prevented the entry of a substantial amount of the extract into the interior of the bacteria. Such antibacterial properties observed for the leaves may be linked to the chemical composition of such organs, since they present polyphenols, anacardic acid and other compounds.

Despite the absence of intrinsic antibacterial activity, the extract when combined with standard drugs was able to alter their activities. An intensifying effect of the drug was observed when associated with oxacillin and norfloxacin against multidrug-resistant strains of *S. aureus* (Figure 01). So the natural product in sub-inhibitory concentration was able to reduce the MIC of the drugs. Making these more effective in treating infections.

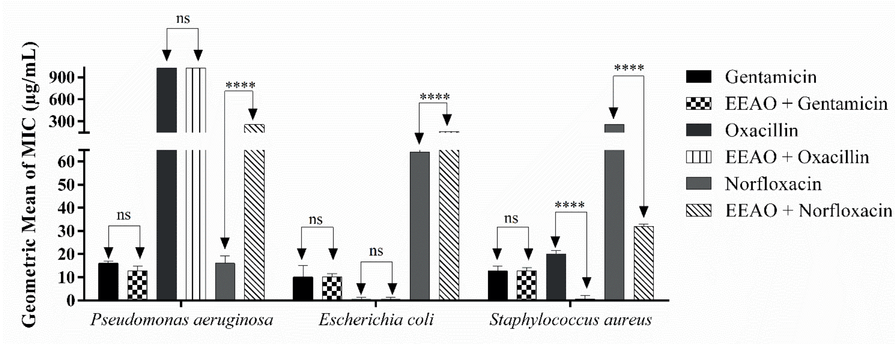

Figure 01: Geometric mean minimum inhibitory concentration (MIC) in µg/ml of ethanolic extract of *Anacardium occidentale* (EEAO) against different multiresistant bacterial strains. ****: p<0.0001.

4 I CONCLUSIONS

Evaluating the *A. occidentale* extract, it was possible to verify that it has no intrinsic antibacterial activity. However, when combined with standard drugs, it was able to change its activities and increase its effectiveness, intensifying its action in the treatment of infections.

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