



**ANALYSIS OF ANTIMICROBIAL ACTIVITY OF EXTRACTS FROM LEAVES OF
Anacardium occidentale L. (CASHEW) FRONT OF CLINICAL ISOLATED FROM
GRAM-NEGATIVE BACTERIA RESISTANT TO ANTIBIOTICS**

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ABSTRACT

Objective: The aim of this study was to analyze the chemical composition of the hydroalcoholic extract of *Anacardium occidentale* L. (cashew) and the antimicrobial activity of this antibiotic against resistant gram-negative bacteria (GNB).

Theoretical Framework: The spread of resistant microorganisms to available chemotherapeutic drugs is a major public health problem and points to the need for research into new antimicrobials. Plants, due to their secondary metabolism, produce compounds of biological importance, being an important source of bioprospection.

Method: The collected leaves were dried at 37 °C, ground and soaked in 70% ethanol-water solution at a ratio drug / solvent of 1: 5 for seven days. After this time the mash was filtered and dried at 40 °C in a water bath until completely dry and obtain of the dry extract. The phytochemical analysis was based on the qualitative determination of saponins, alkaloids, flavonoids, steroids / triterpenoids and tannins. The study of the susceptibility of the BGN extract was determined by the technique of REMA (Resazurin Microtiter Assay), using the minimum inhibitory concentration (MIC). The maintenance of the blue or pink color in the wells of the plate was interpreted, respectively, as the presence and absence of microbial growth.

Results and Discussion: Phytochemicals in trials observed the presence of saponins, flavonoids and tannins and analysis of antimicrobial activity results demonstrated weak antimicrobial activity against most of the microorganisms evaluated, except for *Pseudomonas* sp. (7370) that showed MIC of 250µg/mL.

Research Implications: Quantitative methods are necessary to better assess and quantify the secondary metabolites of the *Anacardium occidentale* L. in the region.

Keywords: *Anacardium occidentale*, Secondary Metabolites, REMA (Resazurin Microtiter Assay), Phytochemical analysis, Antimicrobial activity.

ANÁLISE DA ATIVIDADE ANTIMICROBIANA DO EXTRATO DAS FOLHAS DE *Anacardium occidentale* L. (CAJUEIRO) FRENTE A ISOLADOS CLÍNICOS DE *Escherichia coli*, *Pseudomonas* sp., *Stenotrophomonas maltophilia* e *Enterobacter* sp. RESISTENTES A ALGUNS ANTIBIÓTICOS FREQUENTEMENTE USADOS NO AMBIENTE HOSPITALAR

RESUMO

Objetivo: O objetivo desse trabalho foi analisar a composição química do extrato hidroalcoólico de *Anacardium occidentale* L.(cajueiro) e a atividade antimicrobiana deste frente a bactérias gram-negativas (BGN) resistentes a antibióticos.

Referencial Teórico: A disseminação de micro-organismos resistentes aos medicamentos quimioterápicos disponíveis representa um importante problema de saúde pública e aponta para a necessidade da pesquisa de novos antimicrobianos. As plantas, devido ao seu metabolismo secundário, produzem compostos de importância biológica, sendo uma importante fonte de bioprospecção.

Método: As folhas coletadas foram secas em estufa a 37 °C, moídas e maceradas em solução hidroalcoólica a 70% em uma relação droga/solvente de 1:5 por sete dias. Após este período, o macerado foi filtrado e seco a 40 °C em banho-maria até a secagem completa e obtenção do extrato seco. A análise fitoquímica baseou-se na determinação qualitativa de saponinas, alcaloides, flavonoides, esteroides/Triterpenoides e taninos totais. O estudo da



susceptibilidade das BGN ao extrato foi determinada pela técnica de REMA (Resazurin Microtiter Assay), utilizando a concentração inibitória mínima (CIM). A manutenção da cor azul ou rósea nos poços da placa foi interpretada, respectivamente, como ausência e presença de crescimento microbiano.

Resultados e Discussão: Nos ensaios fitoquímicos realizados, observou-se a presença de saponinas, flavonoides e taninos totais e a análise da atividade antimicrobiana os resultados demonstraram potencial antimicrobiano fraco frente à maioria dos micro-organismos avaliados, exceto pela *Pseudomonas* sp. (7370) que apresentou CIM de 250 µg/mL.

Implicações da Pesquisa: Métodos quantitativos são necessários para se avaliar melhor e quantificar os metabólitos secundários presentes no *Anacardium occidentale* L. da região.

Palavras-chave: *Anacardium Occidentale*, Metabólitos Secundários, REMA (Resazurin Microtiter Assay), Análise Fitoquímica, Atividade Antimicrobiana.

ANÁLISIS DE LA ACTIVIDAD ANTIMICROBIANA DEL EXTRACTO DE HOJA DE *Anacardium occidentale* L. (ANACARDO) FRENTE A AISLADOS CLÍNICOS DE *Escherichia coli*, *Pseudomonas* sp., *Stenotrophomonas maltophilia* y *Enterobacter* sp. RESISTENTE A ALGUNOS ANTIBIÓTICOS DE USO FRECUENTE EN EL AMBIENTE HOSPITALARIO

RESUMEN

Objetivo: El objetivo de este trabajo fue analizar la composición química del extracto hidroalcohólico de *Anacardium occidentale* L. (anacardo) y su actividad antimicrobiana frente a bacterias gramnegativas (BGN) resistentes a antibióticos.

Marco Teórico: La propagación de microorganismos resistentes a los fármacos quimioterapéuticos disponibles representa un importante problema de salud pública y apunta a la necesidad de investigar nuevos antimicrobianos. Las plantas, debido a su metabolismo secundario, producen compuestos de importancia biológica, siendo una fuente importante de bioprospección.

Método: hojas recolectadas se secaron en estufa a 37 °C, se molieron y maceraron en una solución hidroalcohólica al 70% en una relación fármaco/disolvente de 1:5 durante siete días. Transcurrido este período, el macerado se filtró y se secó a 40 °C en baño maría hasta su completo secado y obtención del extracto seco. El análisis fitoquímico se basó en la determinación cualitativa de saponinas, alcaloides, flavonoides, esteroides/triterpenoides y taninos totales. El estudio de la susceptibilidad del GNB al extracto se determinó mediante la técnica REMA (Resazurin Microtiter Assay), utilizando la concentración mínima inhibitoria (CMI). El mantenimiento de color azul o rosa en los pocillos de la placa se interpretó, respectivamente, como ausencia y presencia de crecimiento microbiano.

Resultados y Discusión: En las pruebas fitoquímicas realizadas se observó la presencia de saponinas, flavonoides y taninos totales y en el análisis de actividad antimicrobiana, los resultados demostraron un potencial antimicrobiano débil frente a la mayoría de los microorganismos evaluados, excepto *Pseudomonas* sp. (7370) que presentó una CIM de 250 µg/mL.

Implicaciones de la investigación: Los métodos cuantitativos son necesarios para evaluar y cuantificar mejor los metabolitos secundarios presentes en *Anacardium occidentale* L. en la región.

Palabras clave: *Anacardium Occidentale*, Metabolitos Secundarios, REMA (Resazurin Microtiter Assay), Análisis Fitoquímico, Actividad Antimicrobiana.

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1 INTRODUCTION

Medicinal plants are used by people from various civilizations for various purposes. It is known that during the metabolism of the plant, they synthesize various chemical compounds. Many of these compounds, called secondary metabolites, may have pharmacological potential, such as tannins, flavonoids, alkaloids, steroids, terpenoids, among other classes. Several scientists have been evaluating the biological activity of plants guided by their popular use (LIMA, 2006).

There are three major groups of secondary metabolites: phenolic compounds, terpenes and alkaloids. Phenolic compounds are derived from shikimic acid and mevalonic acid; flavonoids, tannins and lignins are part of these compounds. Terpenes are produced from mevalonic acid or pyruvate and 3-phosphoglycerate; essential oils, saponins, carotenoids and most plant hormones are terpenes. Alkaloids come from aromatic amino acids (tryptophan, tyrosine), which are derived from shikimic acid and aliphatic amino acids (ornithine): nicotine, caffeine and vincristine are some examples of alkaloids. Of the chemical compounds isolated from plants, those with the greatest pharmacological potential are those that have a phenolic group (SANTOS, 2011).

According to the World Health Organization, medicinal plants are valuable sources for the extraction of various medicines. Furthermore, approximately 80% of the world's population uses them to seek relief from some illness (WHO, 2000). The widespread emergence of antimicrobial-resistant bacterial strains is a serious global public health problem. Despite the great advances in the development of new broad-spectrum antimicrobials, the indiscriminate use of this type of medicine has selected resistant bacterial populations, thus limiting the therapeutic options for infectious processes (FERREIRA et al., 2010).

Infectious diseases are of utmost importance due to the high morbidity and mortality among humans, especially in developing countries. Bacteria have a remarkable ability to transmit and acquire resistance to drugs used as therapeutic agents, and there are frequent reports of isolation of bacteria that were known to be sensitive to drugs used routinely, but that became resistant to all, or almost all, drugs available on the market (SILVA, 2010).

Most pathogens involved in hospital infections are transmitted to the patient by the patient's own microbiota (endogenous route) or through external transmission vehicles such as air, hands, secretions and contaminated materials used in medical procedures (exogenous route) (GARCIA et al., 2013). Certain enteropathogenic bacteria are frequently isolated in infections occurring in hospital wards and pediatric wards. *Escherichia coli* and *Enterobacter* sp. are



frequently isolated in cases of Urinary Tract Infection (UTI), mainly in women, due to the proximity of the vaginal canal to the anus. These bacteria can also be found in infections of the respiratory tract, bloodstream (sepsis), and surgical sites, although less frequently (ARAÚJO; QUEIROZ, 2012).

Stenotrophomonas maltophilia is characterized by being a non-fermenting glucose Gram-negative bacillus (NFGGN), aerobic, with ubiquitous distribution and low virulence, but in recent decades it has been standing out as an emerging nosocomial pathogen, mainly in immunocompromised patients, with high morbidity and mortality, being, in many centers, the third most important NFGGN in hospital infections, second only to *Pseudomonas aeruginosa* and *Acinetobacter baumannii* (RODRIGUES, et al., 2011).

Pseudomonas aeruginosa is an opportunistic pathogen that has been highlighted for its prevalence in cases of hospital infections. This bacterium can infect any region of the body in a susceptible patient, being more frequent in reports of respiratory tract infections. Years of evolution have given this microorganism natural and acquired resistance mechanisms that sometimes frustrate modern therapeutic conduct. One of the great challenges in containing this pathogen in the hospital environment is its resistance to chemical disinfectants and antiseptics such as quaternary ammonium compounds, phenol and hexachlorophene (PIRES et al., 2009).

Bacterial resistance to antibiotics is effective through three basic mechanisms: decreased absorption of antibiotics, alteration of the target site of the antimicrobial, or acquisition of the ability to destroy or modify the chemotherapeutic agent. Microorganisms also have the genetic ability to acquire and/or transmit resistance through mutations and mechanisms of conjugation, transduction and transformation, involving genes present in transposons and plasmids. The development of resistance is a natural biological phenomenon, which followed the introduction of antimicrobials into clinical practice, becoming alarming due to the excessive and irrational use of these agents (WOODFORD; ELLINGTON, 2007; DZIDIC et al., 2008). This growing number of pathogenic microorganisms resistant to currently available chemotherapeutic agents has driven the research into new antimicrobial products from plants (SOUZA et al., 2012).

Brazil is the country with the greatest biodiversity of plants in the world, with an estimated number of more than 20% of the total number of species on the planet. This rich biodiversity is accompanied by a long-standing acceptance of the use of medicinal plants and associated traditional knowledge. Almost half of the medicines used in therapy come from natural products, especially medicinal plants, which are an important source for obtaining medicines (SILVA, 2010).



The cashew tree is a species of the Anacardiaceae family, rich in tannins, which give it important antibiotic (SANTOS, 2011; GONÇALVES; GOBBO, 2012), anti-inflammatory (SANTOS et al., 2009, AREKEMASE et al., 2011) and hemostatic (SCHIRATO et al., 2006) activities. The decoction of the bark and the infusion of the leaves are used as: tonic, antidiabetic and anti-inflammatory, in addition to having the ability to prevent the formation of oral bacterial plaque (OLIVEIRA, 2010). *Anacardium occidentale* L. (cashew tree) stands out as a medicinal plant due to its wide variety of applications, being used in ethnopharmacology, mainly in the Brazilian Northeast, especially as an antibacterial and anti-inflammatory. It is used in traditional medicine to treat toothache, bronchitis, arthritis, intestinal colic, jaundice, diabetes, asthma and is even used as an aphrodisiac (FILHO et al., 2010).

The cashew tree is among the 71 plant species in RENISUS (National List of Medicinal Plants of Interest to the Unified Health System), made up of plants with the potential to advance in the stages of the production chain and generate pharmaceutical products (RENISUS, 2009).

Popular use is not enough to scientifically validate medicinal plants as effective and safe medicines, and preclinical and clinical trials are necessary, requiring official authorization for their medicinal use. This validation is obtained through experimental evidence proving that the risk to which those who use them are exposed is outweighed by the benefits that may arise (SOUZA JÚNIOR et al., 2009).

Medicinal plants have played a prominent role in modern medicine, providing resources for the production of medicines that would be difficult to obtain through chemical synthesis. In addition, they provide substances that can be slightly modified, making them more effective and less toxic. (SOUZA et al., 2012).

In view of the above, carrying out tests with plants used in folk medicine aims both to evaluate their therapeutic potential and toxicological character, as well as to discover new drug prototypes, especially with antimicrobial properties, necessary in view of the limitations caused by bacterial resistance. The present work characterized some secondary metabolites present in the extract of the leaves of *Anacardium occidentale* L, as well as analyzed its antimicrobial activity against clinical isolates of gram-negative bacteria: *Escherichia coli*, *Pseudomonas* sp., *Stenotrophomonas maltophilia* and *Enterobacter* sp., resistant to the antibiotics: Amoxicillin + Clavulanic acid (AMC), Levofloxacin (LVX), Nitrofurantoin (NIT), Norfloxacin (NOR), Ofloxacin (OFX), Sulfazotrim (SUT), Ticarcillin + Clavulanate (TIC), Ampicillin (AMP), Ampicillin / Sulbactam (ASB), Cefaclor (CFC), Cephalothin (CFL), Cefepime (CPM), Ceftazidime (CAZ), Ceftriaxone (CRO), Clindamycin (CLI), Ciprofloxacin (CIP).



2 METHODOLOGY

2.1 OBTAINING THE EXTRACT

To carry out the study, *Anacardium occidentale* L. leaves were collected in the city of Brasília de Minas and dried in an oven at 37 °C, and were subsequently ground and macerated in a 70% hydroalcoholic solution, in a 1:5 ratio (plant material/solvent), for seven days (Reis et al., 2012). After this period, the macerate was filtered and dried at 40 °C in a water bath until completely dry and the dry extract was obtained. The final mass of 282.7 grams of dry crude extract showed a yield of 9.5% in relation to the initial weight.

2.2 PHYTOCHEMICAL TESTS

The phytochemical analysis was performed in triplicate and was based on the methods described by Silva et al. (2010), for the qualitative determination of saponins, alkaloids, flavonoids, steroids/triterpenoids and tannins. To perform the phytochemical tests, the crude extract was diluted in a hydroalcoholic solution (96:4 v/v) in a 1:1 ratio (extract/hydroalcoholic solution).

In the saponin research, 5 mL of distilled water was added to 2 mL of the extract and the tube was shaken continuously for 3 minutes and the formation of persistent foam was observed. The result was read after 15 minutes of rest.

For the determination of alkaloids, 2 mL of the extract were taken in a test tube, alkalized with 15 drops of 1% sodium hydroxide and added with 2 mL of water and 2 mL of chloroform. The aqueous fraction was discarded and the chloroform fraction was added with 2 mL of water. The chloroform fraction was discarded and the tests were performed with the acidic aqueous fraction to which 3 drops of Drangendorff reagent had been added.

Flavonoids were determined by the Shinoda test, 2 mL of the extract was added to a tube, approximately 0.5 cm of magnesium ribbon with 2 mL of concentrated HCl. The end of the reaction was given by the end of effervescence.

analysis was performed by the Lieberman-Burchard reaction, taking 2 mL of the extract and mixing it with 2 mL of chloroform. The aqueous solution was then filtered through a cotton funnel covered with a few decigrams of anhydrous Na₂SO₄. In a test tube, 1 mL of acetic anhydride was added, shaking gently, and 3 drops of concentrated H₂SO₄ were gently added, shaking gently and observing the color development.



The test to determine the presence of tannins was carried out in a test tube containing 2 mL of the extract by adding 3 drops of the alcoholic FeCl₃ solution, shaking vigorously and observing the color change.

2.3 ANTIMICROBIAL ACTIVITY

The study of the susceptibility of clinical isolates of *Escherichia coli*, *Pseudomonas* sp., *Stenotrophomonas maltophilia* and *Enterobacter* sp., all gram-negative rods (GNB), to *Anacardium occidentale* L. extract was determined by the REMA (Resazurin Microtiter Assay) technique, using the minimum inhibitory concentration (MIC) (PALOMINO et al., 2002; MIYATA et al., 2013). The microorganisms evaluated were isolated and identified by the Microbiology Laboratory of the Ipatinga Municipal Hospital, and their antimicrobial sensitivity profile was established by antibiogram. In parallel, standard strains were also used: *Escherichia coli* (ATCC 25921) and *Pseudomonas aeruginosa* (ATCC 27853).

The samples used were frozen in brain-heart infusion broth (BHI) containing 10% glycerin, in a freezer at the Microbiology Laboratory of Faculdades Santo Agostinho. In this study, the Brain-Heart Infusion Broth (HIMEDIA) culture medium was used to determine the MIC of the extract. Initially, the stock culture was thawed and then the bacteria [*Escherichia coli* (ATCC), *Pseudomonas aeruginosa* (ATCC), *Escherichia coli* (7304), *Pseudomonas* sp. (7370), *Stenotrophomonas maltophilia* (7328), *Stenotrophomonas maltophilia* (7334), *Enterobacter* sp. (7281), *Stenotrophomonas maltophilia* (7307)] were cultured into test tubes containing brain heart infusion broth (BHI) and incubated at 37 °C for approximately 24 hours. These strains were then cultured onto plates containing CLED Agar (Prodimol Biotecnologia) and incubated at 37 °C for 24 hours. In all three tests performed, 24-hour cultures were used.

To perform the REMA technique, the extract was diluted in 10% dimethyl sulfoxide (DMSO) to obtain a complete dilution of the extract. Positive and negative controls of the growth of microorganisms in 10% DMSO were performed to ensure the viability of the results. Then, according to the specifications of the technique, the extract was diluted and tested at concentrations between 1000 and 15.6 µg/mL (serial dilution on a plate – 1000, 500, 250, 125, 62.5, 31.2 and 15.6) against the bacterial strains, and the plate was incubated at 37 °C for 24 hours. After this period, a cell viability indicator, resazurin, was used to analyze the presence and absence of live microorganisms. After adding 30 µL of the resazurin solution (0.1%) to all wells, the plates were incubated at 37 °C for 6 hours to read the results. The maintenance of the blue color in the wells was interpreted as the absence of microbial growth and the development



of a pink color as the presence. The MIC is defined as the lowest concentration of the extract capable of preventing the color change from blue to pink. The tests were performed in triplicate of independent experiments, and Gentamicin was used at a concentration of 80 µg/mL, with serial dilution at concentrations between 80 and 1.25 µg/mL as the reference standard antibiotic. To read the results of the activity of the standard antimicrobial, it was expected to observe activity of this in vitro up to the concentration of 10 µg/mL as described in the literature (NCCLS, 2014).

3 RESULTS AND DISCUSSIONS

Table 1 shows the results of the phytochemical tests on the leaves of *A. occidentale*. The presence of saponins, flavonoids and total tannins was evidenced, with no divergence between three independent tests. These findings are consistent with studies found in the literature such as those by Bouzada et al. (2009) and Santos (2011), which confirm the presence of tannins in the cashew leaves. However, they differ from those that evidenced alkaloids and triterpenes in the plant species, which were not found in this study.

Table 1

Results obtained in the characterization tests of some secondary metabolites of the extract of A. occidentale leaves.

Substance	Test*
Saponin	+
Alkaloid	-
Flavonoid	+
Steroid	-
Triterpenoids	-
Total Tannins	+

Positive sign (+) means positive result for characterization of the substance. Negative sign (-) means negative result for characterization of the substance. *Tests performed in triplicate.

Source: The authors (2024)

For Gobbo Neto; Lopes (2007), factors such as circadian rhythm, age or stage of development, seasonality, temperature, water availability, ultraviolet radiation, among others, can influence the quantity and nature of the active constituents in the plant. Seasonal variations can alter the content of practically all classes of secondary metabolites, including saponins, alkaloids and tannins. There is also a correlation between the intensity of solar radiation and the production of phenolic compounds, such as flavonoids and anthocyanins. The way in which plants are able to alter the production of these secondary compounds, due to stimuli from biotic



and abiotic factors imposed by the environment in which they are found, can explain why the compounds found in this study differ from those of the authors cited above, since the plants analyzed were found in different locations under different stress situations. Another explanation for this divergence may be given by the type of tests used, since the qualitative nature of the survey in this work may not be capable of identifying very low concentrations of these compounds in the extract.

Saponins are substances mainly related to the defense system. They are found in tissues that are more vulnerable to attack by microorganisms or predatory insects. The amphiphilic behavior of saponins and the ability to form complexes with steroids, proteins and membrane phospholipids enable varied biological actions, being able to act on cell membranes, altering permeability or even leading to cell lysis (CASTEJON; STRIGHINI, 2011; SCHENKEL *et al* ., 2001).

Tannins and saponins have already been identified as active principles of several plant extracts researched by global pharmacognosy. Several studies on tannin activity have shown important antibacterial action, action on protozoa, in tissue repair, enzymatic and protein regulation, among others (CASTEJON; STRIGHINI, 2011). Antimicrobial activity occurs through three mechanisms: antioxidant and free radical scavenging activity, complexation with metal ions, and the ability to complex with other molecules, especially proteins and polysaccharides (GONÇALVES; GOBBO, 2012).

The flavonoid class can interfere in the course of certain diseases, such as ulcerative lesions in the human gastrointestinal tract, as well as in the survival of microorganisms. Its antioxidant activity promotes the inhibition of enzymes that participate in the production of reactive oxygen species and in the complexation of ions such as iron and copper. This class also presents pharmacological activities such as: antiproliferative, antiulcerative, antimicrobial, among others. (DIAS-SOUZA, 2013)

Better knowledge of the antimicrobial activity of these plants provides an impetus for improving the living conditions of populations in developing countries, both as a therapeutic resource and as a source of economic resources (GONÇALVES *et al* ., 2005).

Gonçalves *et al* . (2005) found that the hydroalcoholic extract of cashew tree had an inhibitory effect on the growth of *Proteus mirabilis* , *Shigella sonnei* , *Staphylococcus aureus* and *Staphylococcus spp* .. Araújo *et al* . (2005) evaluated the *in vitro* antifungal activity of cashew tree extract against yeasts of the genus *Candida* , being effective against *C. tropicalis* and *C. stellatoidea* .



It has already been observed in tests that cashew leaf extract also presents antimicrobial activity against strains of *E. coli* (PEREIRA *et al* , 2006), agreeing with the data of other authors who demonstrated activity of this species against *Aspergillus niger* (KUDI *et al* , 1999), as well as *P. aeruginosa* and *Helicobacter pylori* (ELLOF, 1998). Bouzada *et al.*, (2009), through the disk diffusion technique, showed that cashew leaf extract presented activity against strains of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Salmonella typhimurium* and *Klebsiela pneumoniae* .

Table 2 shows the results of the *in vitro* antimicrobial analysis of *A. occidentale* leaves , showing that the extract showed activity against the standard strain of *P. aeruginosa* ATCC 27853, and against the Gram-negative microorganisms, *Pseudomonas sp.* and *S. maltophilia* , both multidrug-resistant clinical isolates. It is worth noting that the microorganisms cited as resistant refer to those that did not have inhibited growth at the concentrations tested, i.e., in the range between 1000 and 15.6 µg/mL.

Table 2

Minimum Inhibitory Concentration of A. occidentale leaf extract.

Micro-organismos	Extract concentration (µg/ml) *						
	1000	500	250	125	62,5	31,2	15,6
<i>E. coli</i> (ATCC 25921)	+	+	+	+	+	+	+
<i>P. aeruginosa</i> (ATCC 27853)	-	+	+	+	+	+	+
<i>E. coli</i> (7304)	+	+	+	+	+	+	+
<i>Pseudomonas sp.</i> (7370)	-	-	-	+	+	+	+
<i>S. maltophilia</i> (7334)	-	+	+	+	+	+	+
<i>Entorobacter sp.</i> (7281)	+	+	+	+	+	+	+
<i>S. maltophilia</i> (7328)	+	+	+	+	+	+	+
<i>S. maltophilia</i> (7307)	+	+	+	+	+	+	+

Positive sign (+) means microbial growth; Negative sign (-) means absence of microbial growth. *Tests performed in triplicate.

Source: The authors (2024)

According to Holetz *et al* . (2002), plant extracts that present antimicrobial activity in concentrations above 500 µg/mL have weak activity, being difficult to use pharmaceutically in the treatment against bacteria or fungi. Thus, the extract presented low activity against the bacterial strain of *S. maltophilia* , since its MIC was 1000 µg/mL, however, it should be considered that *S. maltophilia* is an important emerging hospital pathogen, resistant to carbapenems, with only sulfamethoxazole/trimethoprim as a therapeutic option (RODRIGUES *et al* ., 2011).

According to Kobayashi *et al* . (2009), resistant microorganisms from hospital infections such as *S. aureus* and *Pseudomonas sp.* are responsible for a major clinical and



economic impact, since infections caused by these pathogens are a clinical challenge, both because they have a high capacity to develop resistance and because of their natural susceptibility to a limited number of antimicrobial agents and high rates of acquired resistance to several available drugs. These characteristics give the analysis of the extract significant importance, since, despite being considered to have low activity, these strains presented MICs of 1000 µg/mL. The antibiotic-resistant strain of *Pseudomonas* sp. (7370) presented MICs of 250 µg/mL, indicating high antimicrobial activity for this bacterium, since it has MICs of less than 500 µg/mL. This fact demonstrates the need to carry out more detailed analyses to determine the possibility of creating a new antimicrobial prototype to be used with this class of bacteria.

Research into plant extracts with antimicrobial action has become an alternative for combating microorganisms, due to the large increase in resistance of pathogenic species to multiple drugs, due to the indiscriminate use of antimicrobials, thus leading to the search for these new therapeutic alternatives (PALMEIRA *et al.*, 2010).

4 CONCLUSION

The phytochemical results showed the presence of saponin, flavonoids and total tannins, which are consistent with studies found in the literature that confirm the presence of these compounds in cashew leaves. Therefore, it is necessary to continue the study through quantitative research to determine the concentration of each of the compounds found, since these secondary metabolites are extremely important in pharmacological studies that seek to develop drugs with anti-inflammatory, antimicrobial and antioxidant characteristics.

The results of the antimicrobial activity analysis demonstrated weak antimicrobial potential against most microorganisms evaluated, except for *Pseudomonas* sp. (7370), which presented a MIC of 250 µg/mL, which encourages studies on new antimicrobials for the control of multiresistant bacterial strains targeting this family of bacteria. New studies should be conducted in an attempt to confirm this activity in other microorganisms of the family, as well as the fractionation of the crude extract aiming to find an antibiotic prototype.

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