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EVALUATION IN VITRO OF ANTIMICROBIAL ACTIVITY OF TUCUMÃ OIL (*ASTROCARYUM VULGARE*)

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Abstract: Hospital Infection is a major health problem and affects around 1.5 million people annually around the world. The Amazon region has a wide diversity of native palm trees that have fruits and oilseeds. *Astrocaryum vulgare*, commonly known as Tucumã in Brazil, belongs to the family Arecaceae. This palm has orange, fleshy, single-egg-shaped fruits that are used for therapeutic purposes in diseases of the eyes and skin due to the high content of carotenoids, oil is used in cooking, health treatment and massage. This study evaluated the antimicrobial activity of the Tucumã oil against 18 microorganisms. The antimicrobial activity of Tucumã was measured through the determination of the Minimum Inhibitory Concentration (MIC), as well as the determination of the Minimum Microbicidal Concentration (CMM) aiming to contribute to the discovery of new antimicrobials against pathogenic microorganisms' human health and may contribute to the treatment of nosocomial infections. The results showed that the oil of Tucumã presented antimicrobial activity against five important bacteria, four Gram - positive bacteria (*Enterococcus faecalis*, *Enterococcus faecium*, *Staphylococcus epidermidis* and *Streptococcus agalactiae*) and one Gram - negative (*Acinetobacter baumannii*).

Keywords: *Astrocaryum vulgare*. Antimicrobial Activity. Microorganisms.

1 INTRODUCTION

The resistance of microorganisms to an antimicrobial against which they were originally sensitive is defined as antimicrobial resistance. Despite being a natural evolutionary phenomenon, it has been accelerated by the misuse of medications and insufficient infection control practices. New antibiotics have been challenged with the threat of resistance over the past decades[1]. Antimicrobial resistance is recognized as one of the greatest threats to human health around the world. Only one organism, methicillin-resistant *Staphylococcus aureus* (MRSA), kills more Americans each year than emphysema, HIV / AIDS, Parkinson's disease and combined homicide.[2]

Hospital Infection (IH) is a major health problem affecting about 1.5 million people annually around the world. It is estimated that, in developing countries, every 100 hospitalized patients will be affected by IH, causing ethical, legal and social problems, as well as increased costs related to hospitalization, lengthening hospitalization time, and in more severe cases leading to deaths.[3]

Staphylococcus, especially *S. aureus* and *S. epidermidis*, are among the most important microorganisms associated with nosocomial infections. These infections constitute an important problem in hospitals, and intensive care units (ICUs) have the highest incidence of this type of infection. S. epidermidis is a colonizing species of the skin, often being inoculated during invasive procedures or carried out by the health team, and this situation is exacerbated by the emergence of endemic multiresistant strains in the hospital environment.[4]

Enterococcus are non-spore-forming, facultative anaerobic, Gram-positive, catalase negative bacteria, usually arranged in pairs and in short chains. The two most important species, *Enterococcus faecium* and *Enterococcus faecalis*, are found mainly in the gastrointestinal and genitourinary tract, when present in other parts of the body they are opportunistic pathogens and because it is an opportunistic bacterium, it has a high incidence in hospitalized patients. According to NNIS (National Nosocomial Infection Surveillance), *Enterococcus faecalis* is the third most common pathogen worldwide in the following hospital infections: urinary tract, intra-abdominal, surgical and catheter-associated.[5]

The genus *Streptococcus* includes Gram-positive organisms molded into cocci and organized into chains. They are opportunistic commensals, pathogens, and pathogens for humans and animals. *Streptococcus agalactiae* is an important human pathogen that colonizes the urogenital tract and/or lower gastrointestinal tract of up to 40% of healthy women of reproductive age and is a major cause of sepsis and meningitis in neonates. *S. agalactiae* can also infect elderly and immunocompromised adults. Moreover, like other Gram-positive bacteria, *Streptococcus* can form three-dimensional biofilm-like structures that may increase its ability to colonize and persist in the host.[6]

The genus *Acinetobacter sp*. is characterized as Gram-negative bacilli, catalase positive, oxidase-negative and non-fermentor. Among the various species, *Acinetobacter baumannii* is an opportunistic pathogen commonly associated with outbreaks of nosocomial infections, with a higher incidence in intensive care units.[7]

Most current antibiotics have significant limitations in terms of antimicrobial spectrum, side effects and overuse have led to increased clinical resistance of previously sensitive microorganisms and to the occurrence of difficult-to-treat infections. Documentally, the plants provided a good source of anti-infective agents and the demand for plants with antimicrobial activities gained increasing importance. With the accelerated emergence of resistant bacteria and strains of resistant fungi, there has been an increase in the universal demand for natural antimicrobial therapy. Thus, herbal medicines have been widely used, making them part of primary health care in many countries.[8]

In the last four decades, numerous studies on plant substances with antimicrobial activity have been carried out to find effective therapeutic alternatives against infections caused by antibiotic resistant microorganisms.[9] Brazil, especially the Amazon rainforest, has extensive plant diversity and, therefore, a high potential for the development of new herbal medicines. Numerous vegetables and fruits present technological, economic and nutritional potential, arousing the interest of scientific studies. In this way, oleaginous plant species have been the target of several works due to their antimicrobial efficiency, as well as the absence of side effects.[10]

Astrocaryum vulgare Mart. (Tucumã) is a native palm tree of the Amazon region, belonging to the family Arecaceae. Its fruit has an ellipsoid shape, orange and measuring

3 to 5 cm in length. They present high content of lipids, carotenes, fibers and tocopherol, giving the fruit high energy and nutritive content.[10]

Considering the chemical composition of this fruit, mainly associated to the high lipid content and the presence of carotenoids, as well as the scientific evidence that lipids may present antimicrobial potential, the purpose of this study was to test the tucumã oil, evaluating its antimicrobial activity, against some bacterial and fungal pathogenic strains.

2 MATERIAL AND METHODS

This study was conducted at the Microbiology Laboratory located at the Universidade Franciscana - UFN, Santa Maria - RS, Brazil.

The oil of tucumã commercially acquired from Amazonoil - Industry - Pará - Amazonas - Brazil, was characterized by Baldissera *et al.*[11] by the gas chromatographic method where the composition of the fatty acids was determined. Regarding the fatty acid content, tucumã presented oleic (unsaturated) acid with 368.7 mg of ag / g of sample and palmitic acid (saturated) with 198.23 mg of ag / g of the sample as majorities.

The oil was diluted in Dimethylsulfoxide (DMSO) according to protocols approved by the Clinical and Laboratory Standards Institute (CLSI), where a 16,000 µg / mL stock solution was prepared, 16.41 µL of the oil and 983.59 µL of DMSO (concentration of DMSO, 4%). From this mother solution, a working solution was prepared, with 200 µL of the stock solution and 1,800 µL of Mueller Hinton broth (M.H), for bacterial testing and 1,800 µL of RPMI 1640 for filamentous and yeast fungi. The tested concentration range of tucumã oil was concentrations lower than 800 µg/mL.

To carry out the study we used clinical isolates of inmates of the University Hospital of Santa Maria that were previously identified by specific phenotypic methods for each genus. These microorganisms are part of the strain collection of the Laboratory of Microbiology of the Universidade Franciscana. Yeast fungus - *Candida albicans, Candida glabrata, Cryptococcus neoformans.*; Filamentous fungi - *Aspergillus fumigatus* e *Aspergillus flavus*; Bacteria - *Escherichia coli, Enterococcus faecalis, Enterococcus faecium, Klebsiella pneumoniae, Micrococcus luteus, Pseudomonas aeroginosa, Staphylococcus aureus,* *Staphylococcus epidermidis, Streptococcus agalactiae, Streptococcus mutans, Serratia liquefaciens, Salmonella* enteritidi*, Acinetobacter baumanni*. Before using on tests, the strains were seeded on Mueller Hinton Agar plates (M.H), Sabouraud Agar Dextrose and Potato Agar for viability and purity verification.

Sensitivity tests for determination of Minimal Inhibitory Concentration (MIC) of Tucuman oil were performed according to protocols approved by the Clinical and Laboratory Standards Institute (CLSI). The protocols followed for this experiment were: M27-A3 for tests with yeast fungi,[12] M38-A2 for filamentous fungal tests[10] and M07-A8 for aerobic bacteria tests.[13]

For the antimicrobial evaluation of the oil of Tucumã against the tested microorganisms, the MIC was determined, which refers to the lower concentration capable of inhibiting microbial growth. In this sense, bacterial samples were cultured on MH agar medium and after colonies were inoculated into 5 mL of sterile Saline, the absorbance was monitored until a transmittance of 0.5 on the Mac Farland scale (1×10^6 a 5×10^6 cells per mL), then a 1:10 dilution in MH broth was performed resulting in a concentration of 10^4 cells per mL. Samples of yeast and filamentous fungi were cultured on Sabouraud Agar Dextrose and Potato Agar Dextrose Agar, respectively. Colonies of yeast fungi were inoculated into 5 mL of sterile saline, the absorbance was monitored until a transmittance of 0.5 on the Mac Farland scale was obtained, then the inoculum was diluted 1:50 in sterile saline and 1: 20 in RPMI 1640 medium, resulting in a concentration of $1.5 \pm 1.0 \times 10^3$ cells per mL. Filamentous fungi were harvested in tubes containing Potato Agar Dextrose, where after the fungal growth was added to all 2 mL of sterile saline and 2 mL of Tween 20 leaving it to stand for 5 min after the supernatant was removed and passed to another tube and this solution was diluted 1:50 in RPMI 1640 medium resulting in a concentration of about 0.4 x 10 4 to 5 x 10 4 cells per mL.

After preparation of the inoculums, MIC was performed in 96-well polystyrene plates. The tests were performed in triplicate where 100 µL of M.H broth and 100 µL of RPMI1640 were added to wells 1 to 12 for bacterial and fungal tests, respectively. Rows 11 and 12 represented the negative controls (without the presence of the inoculum) and positive (without the presence of the tested solution), respectively. Controls were also made using DMSO (4% concentration) to ensure that the concentration used was not interfering with the test results. After adding the culture medium, 100 µL of the working solution containing the oil was added to the first well and serial dilutions were made to the well equivalent to 800, 400, 200, 100, 50, 25, 12, 5, 6, 3,12 and 1,56 µg / mL, neglecting in well 12 where the negative control was done. After inoculation of the microorganisms, each well of the microdilution plate was then added 100 µL of the standardized inoculum for the tests with filamentous and yeast fungi. For the tests with the bacteria was added 10 µL of the standardized inoculum. After pipetting the plates were incubated at 37 ° C for 24 hours for bacteria and at 37 °C ± 1 °C for 48 hours for the fungi. The plates were read by the addition of 20 µl of 1% solution of the 2,3,5-triphenyl tetrazolium chloride dye (Vetec®).

The determination of MICs consisted in registering the lowest concentration of essential oil capable of causing total inhibition of bacterial growth.

3 RESULTS

Toucan oil was tested for antimicrobial activity against 18 clinically important microorganisms using the broth dilution method. The MIC was verified from the lowest concentration at which Tucuman oil inhibited bacterial and fungal growth, the results obtained are described in table 1. The present results demonstrated the inhibition of the antimicrobial activity of the microorganisms *Acinetobacter baumannii, Enterococcus faecalis, Enterococcus faecium, Staphylococcus epidermidis* and *Streptococcus agalactiae* at a concentration of 800 µg/mL.

We can also verify, from the results in Table 1, that the microorganisms *Escherichia coli, Klebsiella pneumoniae, Micrococcus luteus, Pseudomonas aeroginosa, Staphylococcus aureus, Serratia liquefaciens, Salmonella sp., Proteus mirabilis, Candida albicans, Candida glabrata, Cryptococcus neoformans, Aspergillus fumigatus* and *Aspergillus flavus* were resistant to the action of the Tucuman oil, because there was no change in their growth.

Table 1 – Results of Minimum Inhibitory Concentration (µg/mL) of tucumã oil against isolated microorganisms. (-) means no bacterial growth; (+) means bacterial growth

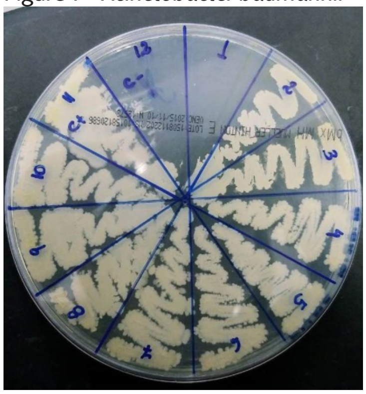
Concentrations	800 µg/mL	400 µg/mL	200 µg/mL	100 µg/mL	50 µg/mL	25 µg/mL	12,5 µg/mL	6,25 µg/mL	3,12 µg/mL	1,56 µg/mL
Microorganisms										
P. aeruginosa	+	+	+	+	+	+	+	+	+	+
E. coli	+	+	+	+	+	+	+	+	+	+
S. aureus	+	+	+	+	+	+	+	+	+	+
E. faecalis	-	+	+	+	+	+	+	+	+	+
K. pneumoniae	+	+	+	+	+	+	+	+	+	+
S. liquefacens	+	+	+	+	+	+	+	+	+	+
E. faecium	-	+	+	+	+	+	+	+	+	+
S. agalactiae	-	+	+	+	+	+	+	+	+	+
S.enteritidis	+	+	+	+	+	+	+	+	+	+
S. epidermidis	-	+	+	+	+	+	+	+	+	+
A. baumannii	-	+	+	+	+	+	+	+	+	+
M. luteus	+	+	+	+	+	+	+	+	+	+
P. mirabillis	+	+	+	+	+	+	+	+	+	+
C. albicans	+	+	+	+	+	+	+	+	+	+
C. glabrata	+	+	+	+	+	+	+	+	+	+
C. neoformans	+	+	+	+	+	+	+	+	+	+
A. fumigatus	+	+	+	+	+	+	+	+	+	+
A. flavus	+	+	+	+	+	+	+	+	+	+

Source: the authors.

Note: - = there was no bacterial growth, + = there was bacterial growth.

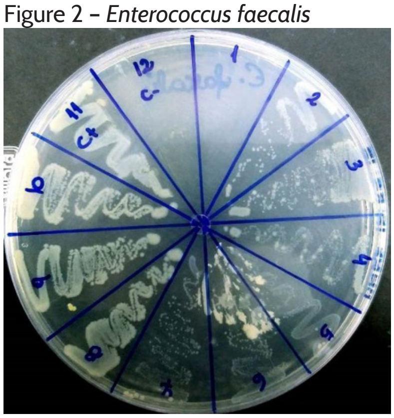
After the MIC results, a minimum Microbicidal Concentration (CMM) was performed, where 10 µL were pipetted from the wells which there was no detectable growth and seeded in petri dishes containing Mueller Hinton Agar (bacteria) and Sabouraud Agar Dextrose (fungi) and incubated at 37° C for 24 / 48h respectively. CMM was considered where there was no fungal or bacterial growth. A well of 800 µg / mL for the microorganisms *Acinetobacter baumannii, Enterococcus faecalis, Enterococcus faecium, Staphylococcus epidermidis* and *Streptococcus agalactiae* was evident.

Figure 1 – *Acinetobacter baumannii*



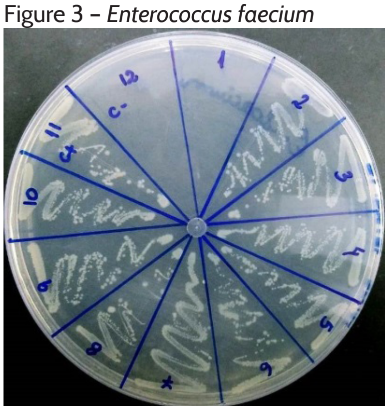
Note: 1= 800 µg/mL; 2=400 µg/mL; 3=200 µg/mL; 4=100 µg/mL; 5=50 µg/mL; 6=25 µg/mL; 7= 12,5 µg/mL; 8=6,25 µg/mL; 9=3,12 µg/mL; 10= 1,56 µg/mL; 11=c+; 12=c-

Figure 2 – *Enterococcus faecalis*



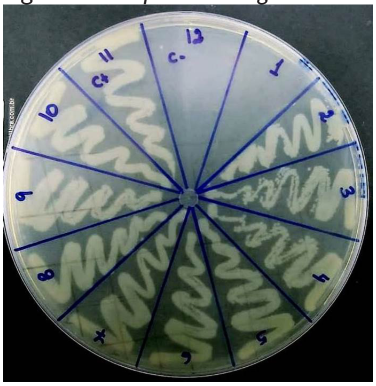
Note: 1= 800 µg/mL; 2=400 µg/mL; 3=200 µg/mL; 4=100 µg/mL; 5=50 µg/mL; 6=25 µg/mL; 7= 12,5 µg/mL; 8=6,25 µg/mL; 9=3,12 µg/mL; 10= 1,56 µg/mL; 11=c+; 12=c-

Figure 3 – *Enterococcus faecium*



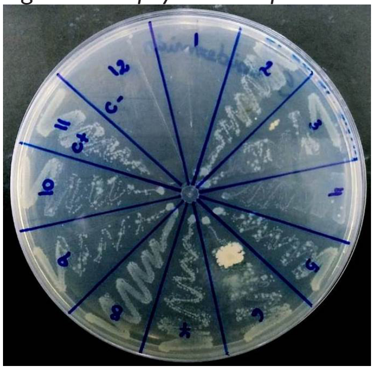
Note: 1= 800 µg/mL; 2=400 µg/mL; 3=200 µg/mL; 4=100 µg/mL; 5=50 µg/mL; 6=25 µg/mL; 7= 12,5 µg/mL; 8=6,25 µg/mL; 9=3,12 µg/mL; 10= 1,56 µg/mL; 11=c+; 12=c-

Figure 4 – *Streptococcus agalactiae*



Note: 1= 800 µg/mL; 2=400 µg/mL; 3=200 µg/mL; 4=100 µg/mL; 5=50 µg/mL; 6=25 µg/mL; 7= 12,5 µg/mL; 8=6,25 µg/mL; 9=3,12 µg/mL; 10= 1,56 µg/mL; 11=c+; 12=c-

Figure 5 – *Staphylococcus epidermidis*



Note: 1= 800 µg/mL; 2=400 µg/mL; 3=200 µg/mL; 4=100 µg/mL; 5=50 µg/mL; 6=25 µg/mL; 7= 12,5 µg/mL; 8=6,25 µg/mL; 9=3,12 µg/mL; 10= 1,56 µg/mL; 11=c+; 12=c-

The results described above suggest that the oil of Tucumã shows antimicrobial activity against four Gram - positive bacteria (*Enterococcus faecalis, Enterococcus faecium, Staphylococcus epidermidis* and *Streptococcus agalactiae*) and a Gram - negative *(Acinetobacter baumannii*).

4 DISCUSSION

Several efforts towards drug discovery and the safe use of antimicrobial agents are the basis for overcoming the global problem of microbial resistance. Resources for the discovery of new drugs are natural products of medicinal plants.[14] In this work, the antimicrobial activity of tucumã oil was evaluated against several species of microorganisms pathogenic to human health.

The antimicrobial activity of some essential oils and their main constituents against pathogenic microorganisms has been well recognized. The oil of *Astrocaryum vulgare* is rich lipids, carotenes, fibers and tocopherol, giving the fruit high energetic and nutritious content. Probably the antimicrobial effect of tucuman oil may be associated with its chemical composition which includes various types of polyphenol and fatty acid molecules. According to Daglia,[15] polyphenols are secondary metabolites produced by higher plants, which play several essential roles in the physiology of plants possessing healthy potential properties in the human body, mainly as antioxidants, anti-inflammatory, anti-allergic and antimicrobial.

Fatty acids act as anionic surfactants, have antibacterial properties at low pH, and can further alter the structure and function of bacterial cell walls affecting the bacteria's ability to tolerate acids.[16] Among these fatty acids, oleic (monounsaturated) and linoleic (polyunsaturated) acids were identified by Osawa *et al.*[17] as being the main bactericidal agents of the cocoa bean in *S. mutans* cells. These authors attribute this property to their lipophilic characteristics and membranotropic effects, causing damage to the cell membrane integrity of the microorganism.

The pulp and bark extracts of tucumã, *Astrocaryum aculeatum*, were tested for antimicrobial activity against 37 microorganisms, the results showed that the pulp and bark extract from tucumã (*Candida albicans*), causing the death of four microorganisms: *E. faecalis, B. cereus, C. albicans and L. monocytogenes.*[18] In this study, the antimicrobial activity of six compounds (separately and in combination) at similar concentrations found in tucuman extracts was also evaluated. The results showed that the antimicrobial activity is influenced by the chemical compounds that were studied, but this activity is dependent on the specific organism and generally related to the combination of two chemical compounds. These results corroborate the idea that the antimicrobial effect found in the fruit of the tucumã, and thus in the oil of the tucumã, is not homogeneous and possibly acts in different mechanisms to induce increasing inhibition and death in each specific microorganism.

The results of this work also corroborate with studies by Jeong, Lee, Kang, Lee, Shin, and Kim,[19] that investigated the effect of some specific polyphenols (a flavone and 11 hydroxyflavones) on the viability of *Enterococcus faecalis* and suggested that these compounds could be effective antimicrobials, which explains the antimicrobial activity of tucuman oil against *E. faecalis* in this study.

5 CONCLUSION

Hospital infections are currently considered a public health problem, affecting more than 15% of patients hospitalized, worsening with the emergence of bacterial resistance. The solutions for this are generally based on the development of new and more effective antimicrobial agents.[20] Brazil, and especially the Amazon, has a very rich biological diversity, with numerous medicinal and pharmacological properties that should be explored.[14]

In conclusion, in this study, tucumã oil presented significant antimicrobial activity against five important bacteria that are pathogenic to human health. Taking into account that these microorganisms present great ease in the production of new multiresistant strains, worsening the cases of difficult to treat hospital infections, the effects of this study could be considered relevant.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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