

Antimicrobial activity of Mamica de Porca (*Zanthoxylum rhoifolium* Lam) Extract against Gram-positive and Negative bacteria

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Abstract— Mamica de porca (*Zanthoxylum rhoifolium* Lam., Rutaceae) is a native medicinal plant of occurrence in the Brazilian Cerrado Biome, the most diverse and rich savannas worldwide. It's present in the states such as Tocantins, Roraima, Amapá, Pará, Amazonas, Acre, and Rondônia. The leaves are used to treatment of microbial infections and parasitic diseases, conferring alternative for health promotion and recovery. However, little is known about the action against Gram-positive and negative bacteria, expanding the knowledge about the potential of the antibacterial. To analyze the antimicrobial activity of leaf crude extract of *Z. Rhoifolium* L. front the standard strains Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli*. The ethanolic extract was obtained in the Natural Products Laboratory (UAP) of the University UnirG, Gurupi-TO, Brazil, allotted to Muller Hinton Agar plates, in triplicate, inoculated with bacterial strains Standard American Type Culture Collection (ATCC): Gram-Positive *S. aureus* ATCC® 29213 and Gram-Negative *E. coli* ATCC® 25922, with the technique of wells for depositing 50 µL saline (negative control) and extract, with Gentamicin® on disk 10µg for positive control. The plates were incubated in a greenhouse at 35 ± 1 ° C for 24 hours, microbiology laboratory, measuring the diameter of the growth inhibition halos. The crude leaf extract of *Z. Rhoifolium* showed biological activity against Gram-positive bacteria *S. aureus* and Gram-negative *E. coli*, with moderately sensitive response and growth inhibition halos ranging from 14 to 16mm and 10 to 10.3mm, respectively. The antimicrobial potential of the crude leaf

extract *Z. Rhoifolium* was checked against microorganisms of different cellular structures, expanding the possibilities of antimicrobial action, especially Gram-negative bacteria, being incipient studies in this perspective and not commonly identified for other medicinal species.

Keywords— Medicinal Plants, Antibacterial Agents, Natural Products.

I. INTRODUCTION

The medicinal plants traditionally used confer an alternative for health promotion and recovery, recommended the safe and effective use according to the World Health Organization and National Policy of Medicinal Plants and Phytotherapy. Considering the chemical and biological diversity of Brazil, this review highlights the Brazilian natural products that were successfully used to develop new products and the value of secondary metabolites from Brazilian biodiversity with potential application for new products and technologies [1]. The therapeutic properties are attributed to the presence of bioactive constituents, many of which are employed in the development of drug production [2]. Although research in the field of complementary and alternative medicine has revealed the mechanisms of action and efficacy of this type of treatment, many extracts vegetables yet comply with research to be properly used for therapeutic purposes [3]. This is one of the ways to the rational use of this drug alternative antimicrobial therapy and combating bacterial multidrug resistance, a worldwide problem that persists in the area of hospital health.

The phytotherapy characterized by the use of medicinal plants in its different pharmaceutical forms was implanted, being widely used effectively in primary health care, with the purpose of preventive or curative of pathologies [4, 5]. In Brazil, as an integrative therapeutic option, it is extremely useful in primary health care programs, due to its efficacy, low operational cost and great potential for the development of this therapy, since it is the Country with the highest plant diversity in world, possess broad biodiversity and studies linking traditional knowledge technologies to scientifically validate this knowledge [6]. It is estimated that 75-80% of the entire population uses this practice, especially in developing countries where it also requires very often primary health care, greater cultural acceptability, and accessibility to treatment, in addition to the safety treatment, due to better compatibility with the human body and less adverse effects deleterious [7, 8]. It was verified in studies evaluating Methanol extracts of *Colocasia esculenta* (Araceae) an activity against several Gram-positive bacteria, highlighting the *S. aureus*, with MIC of 250 µg/mL; among Gram-negative bacteria, the emphasis was on the MIC of 500 µg/mL against *Pseudomonas aeruginosa* [8]. The Mamica de porca (*Zanthoxylum rhoifolium*) other is a percent plant species the order Sapindales, Family Rutaceae, being native of occurrence in the Cerrado Biome, Brazil, the most diverse and rich savannas worldwide. It's present in the north of the Country, in States such as Tocantins, Roraima, Amapá, Amazonas, Acre, Rondônia [9,10]. The extracts of this vegetal species have been used in the treatment of microbial infections and parasitic diseases. In Recent studies, the antileishmanial effect induced by ethanolic extract of stem bark from *Z. Rhoifolium* and its n-hexane fraction in the infection and infectivity of murine macrophages by promastigotes forms of *Leishmania amazonenses*, where the antileishmanial effect was significant in the reduction of macrophage infection, probably underlying the activation of defense mechanisms in these cells, evidencing the potential application of this species in the treatment of parasitic diseases such as leishmaniasis [11].

There are few studies with *Z. Rhoifolium* and there are unknown investigations that evaluate its biological activity both against Gram-negative and Gram-positive bacteria, which would indicate certain ease or not of the extract acting through microorganisms of different cellular structures. It is justifiable to broaden the scientific search for the biological action of plant species of the genus *Zanthoxylum* spp. due to its wide ethnobotany and biological importance, therefore, a promising source of substances with different biological activities [10]. In view of the above, the study aims to

analyze the antimicrobial activity of the crude leaf plant extract of Mamica de porca when subjected to contact with Gram-positive and Gram-negative microorganisms, respectively *Staphylococcus aureus* and *Escherichia coli*.

II. MATERIALS AND METHODS

The collection of Leaves of *Z. Rhoifolium* was held in the Legal Reserve Area (ARL) of the Vale Verde Settlement, municipality of Gurupi-TO, Brazil, located between the coordinates S 11 52,582 W 048 ° 58,913, identified by comparison accessing *Zanthoxylum rhoifolium* Lam. in GBIF/Checklist dataset <https://doi.org/10.15468/39omei/HUTO-Herb'Optareo> from University of Tocantins – Version 1.45 [11]. The confirmation of the occurrence of the species and conference of the scientific name was also carried out on the site Flora do Brazil 2020 [12].

The foliar extract of *Z. Rhoifolium* it was obtained from the Natural Products Laboratory (UAP) of the University of UnirG, Gurupi-TO, Brazil, from the collection and drying of the leaves in a greenhouse at a temperature of 40 ° C (± 0.5) for 03 days, followed by milling in Willey knife Mill (EDB-5), weighing 100 g of the FO powder and subsequent extraction in 500 ml of ethanolic solution (ethanol dynamic brand) at 95% for 07 days.

The ethanolic filtrate was concentrated in a rotational evaporator model 801 (Fisaton) under reduced pressure (temperature up to 50 ° C) and then weighed. This concentrate was dried in a greenhouse for 24h at 50 °C and weighed again in order to obtain the ratio between the mass (g) of the concentrated extract (m) and after its drying (m), presenting for the *Z. Rhoifolium* the yield (M/m) of 8.5%.

The crude leaf extract was tested at the concentration of 50ml front the bacteria pattern strains Gram-positive *Staphylo-coccus aureus* ATCC® 29213 e Gram-negative *Escherichia coli* ATCC® 25922.

Four 140x15 mm plates containing Muller Hinton Agar (MH) were used at room temperature. For inoculation, we used the good technique of 12mm diameter and Agar perforation with a sterile and, distally 2.4 cm from each other following the recommendations Clinical and Laboratory Standards Institute – CLSI, 2006 [13]. Bacterial eyeglasses with Mac Farland scale 0.5 turbidities (1 a 2 x 10⁸ UFC/mL) were evenly distributed on the plates on the agar surface using sterile Swab [13]. For positive control, we used standard chemotherapeutic, Gentamicin in Disk 10µg, being allocated to the wells in order to allow contact with the surrounding surface of the medium containing the bacteria. The plates were

incubated in a greenhouse at $35 \pm 1^\circ\text{C}$ for 24 hours. The tests were performed in triplicate, thus, every 4 MH plates the same microorganism was tested 4 times before the extract, to the positive control group (Gentamicin $10\mu\text{g}$) and the negative control group (saline). The antibacterial activity was verified from the growth inhibition halo, in millimeters, using the graduated halometer.

The evaluation of the antibiotic response was made from the comparison against the biological chemotherapeutic reference standard (positive control) and the zone or halo of growth inhibition, being measured starting from the circumference of the well following the margin where there is the growth of microorganisms [13,14]. The sensitivity of Gram-negative and Gram-positive bacteria against antimicrobials was verified from the dimension of the halo formed, according to the classification: sensitive, when the diameter of the inhibition zone was greater or not more than 3 mm less than the Positive control; moderately sensitive, with a halo greater than 2 mm, but less than the positive control of more than 3 mm; and resistant, where the diameter was equal to or less than 2 mm [15, 16].

III. RESULTS AND DISCUSSION

In the evaluation of the present study, the extract leaf crude *Z. rhoifolium* presented antimicrobial effect for Gram-negative and positive, with the formation of the largest halos of inhibition of bacterial growth when compared with negative control, being effective to antibacterial action through the dilution technique in wells against Gram-positive bacteria *S. aureus* and Gram-negative *E. coli*, with moderately sensitive response and growth inhibition halos ranging from 14 to 16mm and 10 to 1mm, respectively. The answer was considered moderately sensitive, given that his halo was greater than negative control but less than positive control [17]. This result is relevant through the antimicrobial potential, also, in the face of Gram-negative bacteria, being more common scientific evidence of action only against Gram-positive bacteria. Was verified a higher antimicrobial potential against Gram-positive bacteria to the detriment of Gram-negative, from the dimension of the formed halo, although such results still do not overcome the action evidenced against the positive control (Tables 1 and 2).

Table.1: Antimicrobial activity of *Z. rhoifolium* front of gram-negative bacteria, assessed by the size (mm) of inhibition of bacterial growth. UnirG/Gurupi-TO, 2019.

<i>Escherichia coli</i> (Gram-negative)					
Surrounding Halo (mm)					
Test number (n)	1	2	3	4	Average of the Halo (mm)
<i>Zanthoxylum rhoifolium</i>	12 mm	10 mm	10 mm	10 mm	10,5 mm
Positive control (Gentamicina $10\mu\text{g}$)	24 mm	24 mm	24 mm	24 mm	24 mm
Negative control (Sterile saline)	Absent	Absent	Absent	Absent	Absent

Table.2: Antimicrobial activity of *Z. rhoifolium* front Gram-positive bacteria, assessed by the size (mm) of inhibition of bacterial growth., UnirG/Gurupi-TO, 2018.

<i>Staphylococcus aureus</i> (Gram positive)					
Surrounding Halo (mm)					
Test number (n)	1	2	3	4	Average of the Halo (mm)
<i>Zanthoxylum rhoifolium</i>	16 mm	12 mm	12 mm	14 mm	13,5 mm
Positive control (Gentamicina $10\mu\text{g}$)	24 mm	24 mm	24 mm	24 mm	24 mm
Negative control (Sterile saline)	Absent	Absent	Absent	Absent	Absent

The antimicrobial activity of plant extract front *E. coli* Gram-negative bacteria (Figure 01) and *S. aureus* Gram-positive (Figure 02) was flagged from the formation of the halo.

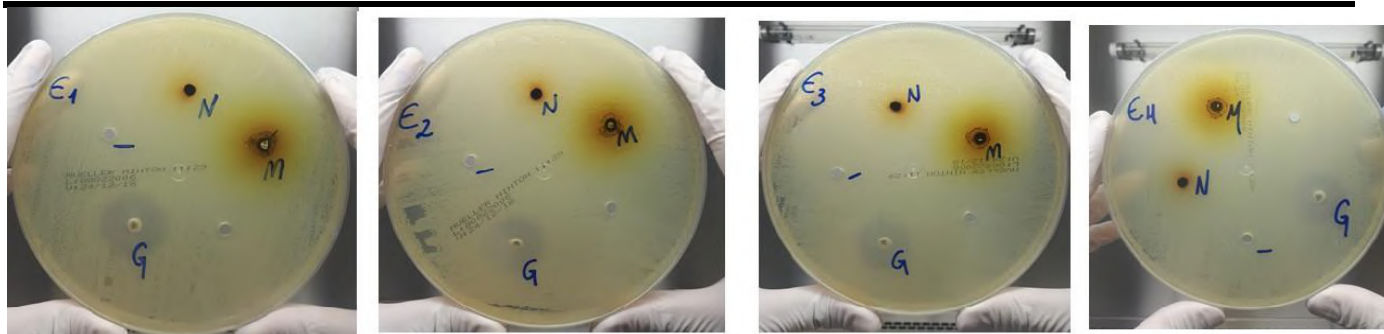


Fig.1: Formation of halo (mm) inhibition of bacterial growth of *E. coli* (E-Gram-negative) by the effect of leaf extract of *Z. rhoifolium*., UnirG/Gurupi-TO, Brazil. 2019. N = negative control; G-positive control; M-mamica of the porca/*Z. rhoifolium*.



Fig.2: Formation of halo (mm) inhibition of bacterial growth of *S. aureus* (S-Gram-positive) by the effect of leaf extract of *Z. rhoifolium*, UnirG/Gurupi-TO, Brazil. 2019. N = negative control; G-positive control; M-mamica of the porca/*Z. rhoifolium*

O antimicrobial potential of volatile oil from leaves of *Z. Rhoifolium* was confirmed in previous studies, being highly virulent against 6 species Gram-negative bacteria, being *Proteus mirabilis*, *Serratia marcescens*, *Klebsiella pneumonia*, *Escherichia coli*, more efficiently in front of *Shigella sonnei* e *Salmonella enteritidis* and negative inhibitory effect in front of *E. coli* [18]. More recent studies have shown the antimicrobial potential within the genus Gram-negative bacteria, the ethanolic extract, ethylacetate, acetone and methanol of *Z. bungeanum* presented activity in front of *Cepa E. coli*. [19]. In relation to the characteristic of the body, from the MIC tests, studies point out that extracts from *M. linifera* showed more effective action only against Gram-posit bacteria, and that this is must, probably. The differentiated cellular structure of Gram-negative bacteria once these have a cellphon wall air composed of peptidoglycan and an external membrane containing lipopoly Saccharides, which confers protection against environmental substances, plant extracts, and antiobióticos [20].

The efficiency of plant extracts has been better evaluated against the Gram-positive bacterias [6, 18, 21]. The extracts polar *Clusia burlemarxii* were evaluated and the only verified against Gram-Positive, among them *S. aureus*, where the ethanolic extract of the leaves inhibited the microbial growth with a CIM (Concentration of the Minimum inhibitory) 62,5 µg/ML,

although you have not observed Gram-Negative activity [22]. The extract ethanolic leaves of *Clusia nemorosa* G. Mey. (Clusiaceae) was shown to be effective against gram-positive multidrug-resistant bacteria *Staphylococcus aureus* and non-effective against gram-negative *Escherichia coli* [23].

It is understood as positive the findings of this study, since the existing medicinal use of this species as well as potential for bacteria, consistent with the national policy of medicinal plants and phytotherapy, regarding the results of research to foundation The safe and effective use of native species in the treatment and complementary practices of Brazilian Single Health System- SUS. Although They do not overcome the antimicrobial action of allopathic drugs, as in the present study, there are alternatives with resolution ability in the presence of bacteria, especially Gram-negative, and positive. Such evidence does not always occur, because it is microorganisms whose cellular structures are different, certainly conferring different barriers to the attack of antimicrobial agents.

The action of plant extracts against bacteria is also intrinsically related to the secondary components present. Keskin et al. (2001) [24] verified that propolis extracts showed antibacterial activity against Gram-positive bacteria (*Streptococcus aureus* and *Streptococcus*) and weak activity against Gram-negative bacteria (*Escherichia coli* and *Pseudomonas*

aeruginosa) and that this effect may be related to the chemical composition of propolis concentrates, with the phase-in flavonoid content [25]. Identified the most representative active principles, such as alkaloids, coumarins, terpenes, flavonoids are present in the secondary metabolites of the genus *Zanthoxylum*, although faced with the difficulty still in the greater understanding of extract specialization, antimicrobial analysis, and pathogens [26]. There is empirical use of the *mamica porca* with medicinal direction. Two of the specimens of this genus (*Rhoifolium*, *Ekmanii*) are used for the treatment of cancer and malaria, pain relief in the teeth and reduction of microbial processes by the inhabitants living on the banks of the Madeira River in the state of Rondônia [17, 27]. In French Guiana is used the bark of *Z. Rhoifolium*, with antimalarial remedies due to their curative and preventive cities, where boil in water to shell, alone or mixed with other ingredients [28]. The therapeutic indications of Brazilian folk medicine prioritize oral administration as teas and infusions against various diseases, where the medicinal properties of this species may be related to its alkaloid composition [17].

There are other factors influencing the biological activity, if not the potential of the plant extract, and also attributed to the inoculation technique used, even though the good technique is one of the most recommended for bioactivity studies with Leaf extracts. Depending on the substance, whether natural or artificially obtained, it is different diffusion capacities and dissociation in agar, with causes associated with: the presence of bacterial enzymes; composition of the medium; the substance in the middle; Inoculum density; incubation period; Temperature and finally stability of the substance in use [29]. O Agar diffusion method has a higher efficiency for water-soluble substances, enabling the diffusion of these through the culture medium. However, the molecular weight and the presence of particulate matter in the sample can also hinder the diffusion in the culture medium [30]. In this sense, it is plausible report that the extract of *Z. rhoifolium*. In the present bioassay showed to be poorly disseminated in the medium in the culture medium when using antibiogram technique in wells, and this fact may have its antimicrobial potential masked and forming inhibition halos smaller than the standard positive control. This suggests the verification of the physicochemical characteristics of the extracts in correspondence to the respective extractors, diluents and even methods used.

Research with medicinal plants and phytotherapy allows detecting vegetables with an active microbial and therefore a number of plants has been investigated for the You can medical, in view of the growing problem of

bacterial resistance front synthetic antimicrobials. However, still, there is a lot of discussion as to the validity and feasibility of the reported information about plant extracts activity, mainly due to the diversity of techniques used in research because of the lack of standardization [31].

IV. CONCLUSION

The antimicrobial potential of the crude leaf extract *Z. Rhoifolium* was checked against microorganisms of different cellular structures, which is unusual for other medicinal plant species. This extends the efficacy against Gram-negative bacteria, being incipient studies in this perspective. There was little diffusion the extract of *Z. rhoifolium* in this bioassay when using the antibiogram technique in wells, and this fact may have compromised the antimicrobial potential. Observed inhibition halos lower than the standard positive control. This suggests the verification of the physicochemical characteristics of the extracts of this species in correspondence to the respective extractors, diluents and even methods of inoculation used.

The existing medicinal use of this species as well as the potential for bacteria, consistent with the national policy of Medicinal plants and phytotherapy in Brazil, in relation to the results of research to increasingly underfloor the safe and effective use of the species Therapies and complementary practices of the Unified Health System. Although It does not overcome the antimicrobial action of allopathic agents, there are alternatives with resolution ability in the face of bacteria, especially the Gram-negative and positive. Such evidence does not always occur because they are microorganisms with different cellular structures and, for this reason, differ in response to the attack of antimicrobial agents.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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