

Antibacterial Potential of the Hydroalcoholic Extract of *Mauritia Flexuosa* in Bacteria

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The antibacterial potential of the hydroalcoholic extract of *Mauritia flexuosa* leaves was evaluated against gram-positive bacteria *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* (ATCC 25524) and *Bacillus subtilis* ATCC 6633; Gram negative *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Salmonella typhi* ATCC 11011. Control group (erythromycin) was used, the biological material used was 500g of leaves; placed in an amber bottle with 500mL of 96° alcohol at rest for 15 days under constant agitation; concentrations of 20, 50 and 80mg/ml were prepared. Phytochemical screening was carried out to identify its secondary metabolites, 18 plates were prepared with Muller Hinton agar culture medium, adding 1ml of standardized inoculum at a 0.5 McFarland scale, the diffusion method was used, making 4 holes of 5mm each, 3 of them with concentrations of the extract and one with a control group (erythromycin), leaving it to stand for 24 to 48 hours at 37°C in the incubator, the inhibition halo was measured in mm using a digital vernier. The results show that the extract of *Mauritia flexuosa* presented high antimicrobial activity against gram positive bacteria, at a concentration of 50 and 80 mg/ml, observing a greater diameter of the inhibition halo than that caused by the control; and in gram negative bacteria (*Escherichia coli* and *Salmonella typhi*) there was greater inhibition at a concentration of 80 mg/ml.

1. Introduction

Plants possess phytochemical compounds used as a natural alternative to pharmaceuticals for the treatment of diseases caused by fungi, bacteria and other organisms (Chilquillo et al., 2018). Plant-derived compounds are used as substitutes for pharmaceuticals. It is known that one in three people use medicinal plants for healing purposes in Europe (Gallegos, 2016; Rodriguez et al., 2015), Studies have identified that 25% of the US population uses plant-derived medicines (Corrales and Reyes, 2015; Heisler et al, 2015).

In Peru, the San Martin region is considered a tropical region for its diversity, climate and gastronomy. In this geographical area grows *Mauritia flexuosa* known as "aguaje" which is a palm tree widely distributed in South America and belongs to the *Arecaceae* family. Some studies have emphasized the pharmacological potential of parts of the plant such as the fruit for the production of sweets, ice cream, juices, jams, baby food, oils, among others. The seeds were transformed into activated carbon to minimize gas adsorption, and the leaves into infusions (Lins et al, 2015; Sandoval et al, 2021). It is known to contain secondary metabolites belonging to chemical groups, such as alkaloids and cyanogenic glycosides and non-nitrogenous compounds, including tannins, flavonoids, terpenes and anthocyanins and play an important role in antimicrobial defense (Aquino et al, 2015; Pereira et al, 2016; Marchi et al, 2019).

Bacteria have become resistant to many drugs. Gram-positive bacteria, such as *Staphylococcus epidermidis*, *Staphylococcus aureus* (causing infectious diseases), *Bacillus subtilis* (which is not considered a human pathogen, but can cause food poisoning and contamination).

Likewise, gram-negative bacteria, including *Salmonella typhi* (causes typhoid fever, gastrointestinal diseases), *Pseudomonas aeruginosa* (multidrug resistant and responsible for healthcare-associated infections) and *Escherichia coli* (causes diarrhea and kidney failure, which can lead to death) are involved in human bacterial infection (Sika et al, 2014). Medicinal plants are considered an alternative for the control of bacterial diseases. It has been shown that they can be effective and their health risk percentage can be minimal (Afsar et al, 2015). Studies show that hydroalcoholic extracts of the leaves have an antibacterial effect; effect on gram-positive and gram-negative bacteria due to their secondary metabolites, which play an important role in the development of new therapeutic agents (Rezende et al., 2019). The objective of the research was to evaluate the antibacterial potential of the hydroalcoholic extract of *Mauritia flexuosa* leaves on gram-positive and gram-negative bacteria.

2. Method

2.1 Vegetal sample

Mauritia flexuosa leaves were selected and collected in the district of Cacatachi, San Martin at 295 meters above sea level, 12 km north of Tarapoto (6°29'40" south latitude and 76° 27'57" west longitude). The sample was transferred in a wooden press labeled to maintain an ambient temperature between 20 to 25 °C. Finally, for its identification, it was taken to the *Truxillense Herbarium* of the National University of Trujillo obtaining as registration code COD. 59597.

2.2 Preparation of the hydroalcoholic extract.

The leaves were selected discarding those with signs of deterioration, washed with distilled water and disinfected with 96% cotton, wrapped in kraft paper for drying in a universal oven (Memmert GmbH + Co. KG) at 25 ° C for 12 hrs. Afterwards, the leaves were cut with scissors to an approximate size of 3 mm. For the preparation of the hydroalcoholic extract, the maceration method was used: 500 mL of 96 ° ethanol was placed in an amber-colored glass bottle with 400 g of *Mauritia flexuosa* leaves and left to macerate for 15 days. The solution obtained was taken to a vertical rotary evaporator (Scilogex RE-100) at 80 rpm for 60 minutes; the sample was filtered four times with Whatman No. 1 paper to obtain a dry extract, which was dissolved in alcohol at 96°C to prepare concentrations of, 20, 50 and 80 mg/mL (Alvarado et al., 2018).

2.3. Phytochemical analysis

The phytochemical analysis of *Mauritia flexuosa* leaves was qualitative and was performed by the method referred by Lock (2016). The sample was subjected to solvents of increasing polarity using reagents and dyes to identify the presence or absence of active compounds such as tannins, triterpenes, flavonoids, phenolic compounds, reducing sugars, among others. The sample was classified as light, moderate or strong. The results are identified in Table 1.

Table 1: Phytochemical analysis of the hydroalcoholic extract of *Mauritia flexuosa* leaves.

Assay	Secondary metabolites	<i>Mauritia flexuosa</i>
Lieberman-Bouchard	Steroids and triterpenes	(++)
Borntrager	Quinones	(-)
Ferric chloride	Phenolic compounds	(+++)
Shinoda	Flavonoids	(+++)
Baljet	lactones	(+)
Dragendorff	Alkaloids	(-)
Mayer	Alkaloids	(-)
Fehling	Sugar reducers	(++)
Gelatin	Tannins	(++)
Foam	Saponins	(-)

Note: Color changes of secondary metabolites in (+) = slight, (++) = moderate and (+++) = strong.

2.4. Biological material: Bacterial strains

Standard bacterial strains American Type Collection Culture (ATCC) were provided by Bacteriology, Trujillo National University Laboratory. Gram-positive bacteria *Staphylococcus aureus* (ATCC 25923) *Staphylococcus*

epidermidis (ATCC 25524) and *Bacillus subtilis* (ATCC 6633); gram-negative bacteria *Escherichia coli* (ATCC 25922), *Salmonella typhi* (ATCC 11011) and *Pseudomonas aeruginosa* (ATCC 27853).

2.5. Bacterial inhibition protocol

The bacteria were at a lower temperature of 5 °C and heart infusion culture medium (BHI) at 37 °C for 8 hrs was used to reactivate them. Subsequently, another culture medium, Mueller-Hinton Agar (Merck), was prepared under biosafety level one conditions according to the manufacturer's specifications. 10 mL of Mueller-Hinton agar was poured into 100 mm Petri dish and allowed to dry for 45 minutes. A suspension of 5×10^8 colony forming units (CFU) of each bacterium was prepared in a test tube with 10 mL of isotonic sodium chloride solution equivalent to 0.5 MacFarland; 1 mL of each suspension was spread on the plates containing Mueller-Hinton Agar and allowed to dry for 30 minutes. For the application of the hydroalcoholic extract, the agar diffusion method was used (Sanchez and Muhammad, 2016; Bandara et al., 2018), making four 5 mm holes in each Petri dish; three with 70 μ L of the prepared extract at concentrations (20, 50 and 80 mg/mL) and one of 70 μ L with erythromycin at a concentration of 50 mg/mL (control group). The plates were incubated at 37 °C from 24 h to 48 h, the analyses were performed in triplicate, with a total of 18 petri dishes, the results were determined with the diameter in mm of the bacterial growth inhibition halos using a Digital Vernier (CALDI-6MP, Truper) (Garcia and Angeles, 2020).

Table 2. Degree of inhibition of bacterial growth

Degree of inhibition	Diameter range (mm)
No antimicrobial activity	Less than 7 mm.
Low antimicrobial activity	8 - 10 mm
Medium antimicrobial activity	11 - 13 mm
High antimicrobial activity	14 – 17 mm

(Azuero et al, 2016).

3. Results

Figure 1 shows that gram-positive bacteria, *Bacillus subtilis* (13mm) and *Staphylococcus aureus* (12mm) have medium antimicrobial activity, and *Staphylococcus epidermidis* (14mm) high antimicrobial activity. Gram negative bacteria; *Pseudomona aeruginosa* (5mm) and *Salmonella typhi* (7mm) there is no antimicrobial activity; *Escherichia coli* (10mm) has low antimicrobial activity.

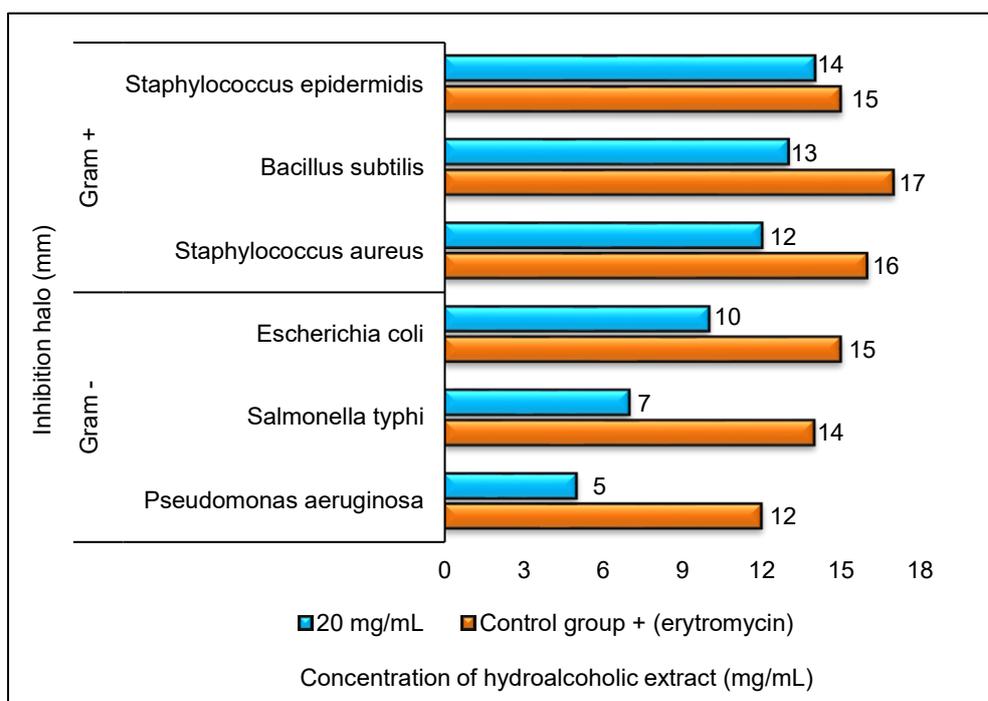


Figure 1. Inhibition halo diameter (mm) in gram-negative bacteria and positive de 20 mg/mL

However, comparing the concentration of the hydroalcoholic extract of *Mauritia flexuosa* 20 mg/mL and the control group, the latter has greater antimicrobial activity, therefore greater effectiveness is expected.

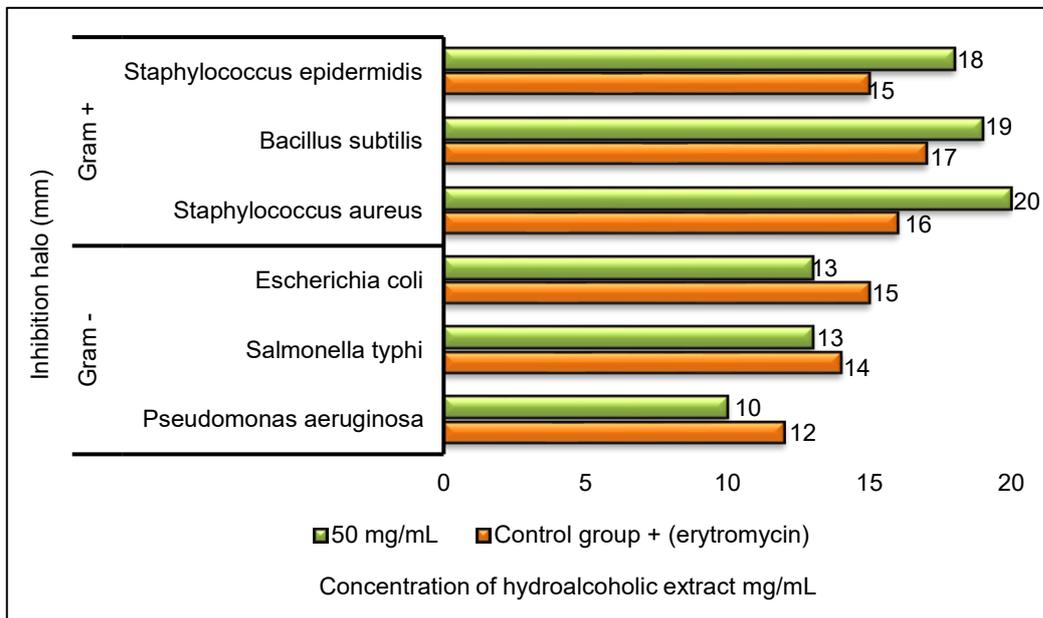


Figure 2. Inhibition halo diameter (mm) in gram-negative bacteria and positive de 50 mg/mL

Figure 2 shows that gram-positive bacteria, *Staphylococcus epidermidis* (18mm) have high antimicrobial activity, *Bacillus subtilis* (19mm) high antimicrobial activity and *Staphylococcus aureus* (20mm) high antimicrobial activity. Gram negative bacteria; *Pseudomona aeruginosa* (10mm) has medium antimicrobial activity, *Salmonella typhi* (13mm) medium antimicrobial activity y *Escherichia coli* (13mm) medium antimicrobial activity. When comparing the control group with the hydroalcoholic extract of *Mauritia flexuosa* at a concentration of 50 mg/mL, the latter has high antimicrobial activity against gram-positive bacteria; however, the control group (erythromycin) has high antimicrobial activity against gram-negative bacteria.

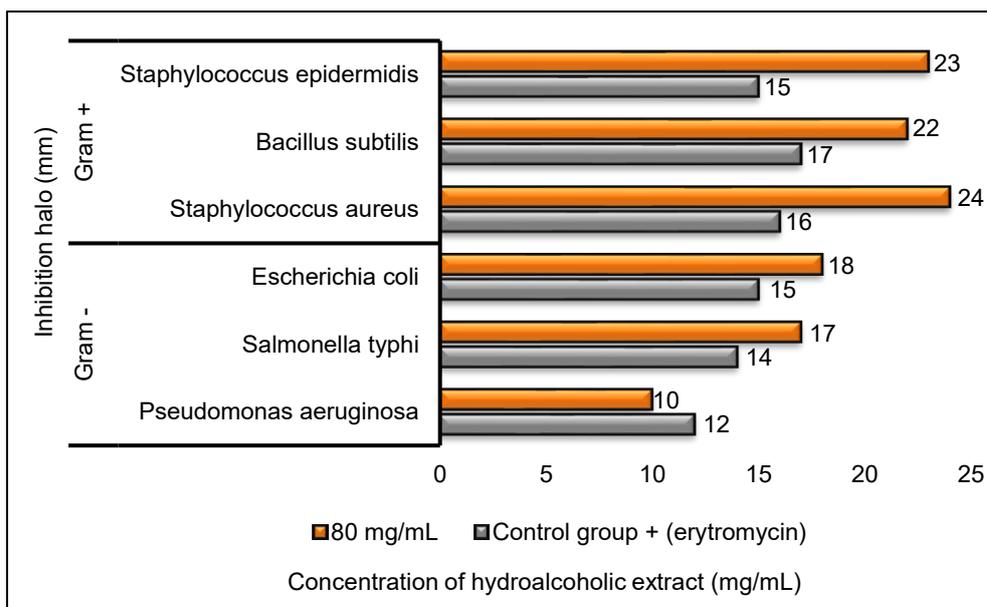


Figure 3. Inhibition halo diameter (mm) in gram-negative bacteria and positive de 80 mg/mL

Figure 3 shows that gram-positive bacteria, *Bacillus subtilis* (22mm) have high antimicrobial activity, *Staphylococcus epidermidis* (23mm) high antimicrobial activity and *Staphylococcus aureus* (24mm) high antimicrobial activity. Gram negative bacteria; *Pseudomonas aeruginosa* (10mm) has medium antimicrobial activity, *Salmonella typhi* (17mm) high antimicrobial activity y *Escherichia coli* (18mm) high antimicrobial activity. When comparing the control group with the hydroalcoholic extract of *Mauritia flexuosa* at a concentration of 80 mg/mL, the latter has high antimicrobial activity against gram-positive and gram-negative bacteria: except in *Pseudomonas aeruginosa* where the control group (erythromycin) has high antimicrobial activity.

4. Conclusions

Phytochemical analysis of *Mauritia flexuosa* leaves was performed, which evidenced the presence of secondary metabolites, such as steroids, triterpenes, phenolic compounds and flavonoids. Six bacteria were studied, three gram-negative bacteria, of which only *Pseudomonas aeruginosa* does not present antimicrobial activity by the hydroalcoholic extract of *Mauritia flexuosa*, while *Salmolla typhi* and *Echerichia coli* have high microbial activity at a concentration of 80 mg/mL with a greater inhibition halo compared to the control group. Regarding the gram-positive bacteria *Staphylococcus aureus*, *Bacillus subtilis* and *Staphylococcus epidermidis*, it is evident that the concentrations of 50 and 80 mg/mL are high in antimicrobial activity compared to the control group. Therefore, it is important to note that the chemical composition of the leaves has a high antimicrobial potential since the effect is constituted by a set of active principles that work synergistically. Hydroalcoholic extracts of plants are known to release a large amount of phenols and flavonoids explaining their antibacterial action. It is important to identify new alternatives to mitigate the pathologies caused by these bacteria; the increase in genetic mutations confers resistance to antibiotics. Therefore, it is necessary to study regional plants as treatment options to help develop new products of natural origin with protective effects.

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