Antibacterial effect of the hydroalcoholic extract of *Mauritia flexuosa* leaves on gram-negative and gram-positive bacteria

[version 1; peer review: awaiting peer review]

Ana Sandoval Vergara¹, Marlon Farfán Córdova¹, Marco Leoncio Salazar Castillo², Icela Marissa Rodríguez Haro², Ana Paula Vizconde Rodríguez²

¹Universidad César Vallejo, Carretera Marginal Norte Fernando Belaúnde Terry Km. 8.5, Sector Maronilla Mishquiyacu, Cacatachi, San Martín, Peru
²Universidad Nacional de Trujillo, Av. Juan Pablo II, Trujillo, 13011, Peru

Abstract

**Background:** Plant-derived compounds are sometimes used as substitutes for pharmaceuticals. *Mauritia flexuosa* is a palm tree that is widely distributed in South America, especially in the Amazon region. The San Martín region of Peru, in which this species of the Arecaceae family is found, has great biological diversity and there is economic potential in the utilization of natural resources in the region.

**Methods:** In this study, the antibacterial effect of the hydroalcoholic extract of *Mauritia flexuosa* leaves was evaluated for gram-positive bacteria *Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 6633 and gram-negative *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Salmonella enterica* subsp. *enterica* ser. *Typhi* ATCC 11011. *Mauritia flexuosa* leaves were used to prepare concentrations of 10, 20, 40 and 60mg/ml. Phytochemical analysis was performed to identify secondary metabolites in the plants. For the experiment, 10 Mueller-Hinton agar plates were prepared and 1ml of bacterial inoculum, standardized to 0.5 McFarland, was added to each plate. The hydroalcoholic extract was added via the diffusion method, making five holes of 5mm each (four with extract concentrations and one with distilled water as a control group), and the plates were incubated for 24 hours at 36°C. The inhibition halo was measured in mm using a digital vernier caliper.

**Results:** For gram-negative bacteria, an antibacterial effect was demonstrated for *Pseudomonas aeruginosa* only, at an extract concentration of 60mg/ml, with an inhibition halo of 14.8 mm. For gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*, an antibacterial effect was demonstrated at an extract concentration of 60mg/ml, with inhibition halos of 13.2mm and 15.4mm in diameter, respectively.

**Conclusion:** It can be concluded that the hydroalcoholic extract of *Mauritia flexuosa* does not inhibit bacterial growth for gram-negative bacteria *Salmonella Typhi* and *Escherichia coli*.
Keywords
Antibacterial, hydro-alcoholic extract, Mauritia flexuosa, gram negative bacteria, gram positive bacteria

Corresponding author: Ana Sandoval Vergara (asandoval@ucv.edu.pe)

Author roles: Sandoval Vergara A: Conceptualization, Investigation, Methodology, Project Administration, Supervision, Validation, Writing – Original Draft Preparation, Writing – Review & Editing; Farfán Córdova M: Investigation, Methodology, Writing – Original Draft Preparation; Salazar Castillo ML: Investigation, Methodology, Writing – Original Draft Preparation; Rodríguez Haro IM: Investigation, Methodology, Writing – Original Draft Preparation; Vizconde Rodríguez AP: Investigation, Methodology, Writing – Original Draft Preparation

Competing interests: No competing interests were disclosed.

Grant information: The author(s) declared that no grants were involved in supporting this work.

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How to cite this article: Sandoval Vergara A, Farfán Córdova M, Salazar Castillo ML et al. Antibacterial effect of the hydroalcoholic extract of Mauritia flexuosa leaves on gram-negative and gram-positive bacteria [version 1; peer review: awaiting peer review] F1000Research 2019, 8:1487 (https://doi.org/10.12688/f1000research.19151.1)

First published: 23 Aug 2019, 8:1487 (https://doi.org/10.12688/f1000research.19151.1)
Introduction

Flora is important, because of its diversity, for the production of phytochemical compounds, used as a natural alternative to pharmaceuticals for the treatment of diseases caused by fungi, bacteria and other organisms. Plant-derived compounds have been used as substitutes for pharmaceuticals; for example, one out of three people use medicinal plants for healing purposes in Europe. A study conducted in the United States found that 25% of drugs are derived from plants.

*Mauritia flexuosa* is a palm tree that is widely distributed in South America, especially in the Amazon region. The San Martín region of Peru, in which this species of the Arecaceae family is found, has great biological diversity and there is economic potential in the utilization of natural resources in the region. In the food industry, the shell and the endocarp of the *Mauritia flexuosa* fruit are usually discarded or underused for the preparation of sweets, ice creams, juices, jams, infant food and oils. Some studies have emphasized the pharmacological potential of these parts, which contain bioactive compounds, to play a role in antimicrobial defense, lipid metabolism, hypoglycaemia and curative activities. *Mauritia flexuosa* also produces secondary metabolites belonging to chemical groups, such as alkaloids and cyanogenic glycosides, and non-nitrogenous compounds, such as tannins, flavonoids, terpenes and anthocyanin.

Bacteria have become resistant to many medicines; thus, infections have been disseminating quickly between people and animals. Gram-positive bacteria, including *Staphylococcus aureus* (causes a variety of infectious diseases, including skin infections, wound infections and pneumonia) and *Bacillus subtilis* (which is not considered a human pathogen but can cause intoxication and food contamination), as well as gram-negative bacteria, including *Salmonella enterica* subsp. *enterica* ser. Typhi (*Salmonella Typhi*, causes typhoid fever), *Pseudomonas aeruginosa* (resistant to multiple drugs and responsible for healthcare-associated infections) and *Escherichia coli* (causes diarrhea and renal insufficiency, which can lead to death) are involved in human bacterial infection.

Medicinal plants are considered an alternative solution for controlling bacterial diseases; thus, there is a requirement for research with the aim of finding new natural alternatives. The World Health Organization has shown that they can be effective and their percentage of health risk may be minimal. Research shows that the hydroalcoholic extracts of leaves have an antibacterial effect on gram-positive and gram-negative bacteria because of their active principles, which play an important role in the development of new therapeutic agents.

The objective of this research was to evaluate the antibacterial effect of the hydroalcoholic extract of *Mauritia flexuosa* leaves on gram-positive and gram-negative bacteria.

Methods

Source of plant material

Leaves of *Mauritia flexuosa* were collected by researchers (ANSV, MFC, MLSC, IMRH and APVR) in the district of Cacatachi, San Martín at 295 meters above sea level, 12 km to the north of Tarapoto (6°29'40" of South latitude and 76°27'57" of West longitude). The specimen was transferred to the Herbarium Truxillense of Universidad Nacional de Trujillo for identification by specialists, obtaining a registration code. The sample was transported in a wooden press inside a labelled vacuum bag and kept at an ambient temperature of 37°C.

Preparation of the extract

Whole leaves were selected and those with signs of deterioration discarded. Leaves were washed with distilled water and disinfected with cotton dipped in 96% ethanol. Leaves were fragmented to an approximate size of 3 mm and the maceration method was carried out as follows. Fresh leaves, with a weight of 200g, were washed with distilled water to remove impurities, wrapped in kraft paper and dried in a universal oven (Memmert GmbH + Co. KG) at 25°C for approximately 12 hours. The dry sample was cut with scissors to obtain small pieces, placed in an amber glass jar and 500mL of 96% ethanol was added, followed by agitation using a vertical rotavapor (Scilogex RE-100) at 70 rpm for 10 minutes every four hours, except overnight from 10 pm to 7 am, for 15 days. The sample was filtered four times with Whatman N°1 and then N°2 filter paper. Then, the vertical rotavapor (Scilogex RE-100) was used for two hours at 70rpm to obtain a dry extract, which was dissolved in alcohol at 96°C and used to prepare concentrations of 10, 20, 40 and 60mg/mL.

Phytochemical analysis

The phytochemical analysis of *Mauritia flexuosa* leaves was qualitative and was carried out according to the method described by Miranda and Cuellar. Each sample was subjected to solvents of increasing polarity in order to obtain secondary metabolites according to their solubility, using reagents and dyes to determine presence or absence of active components such as terpenes, flavonoids, reducing sugars, among others. The assays used to determine the presence of each type of secondary metabolite are listed in Table 1. The results of the color change were judged by eye according to the method described by Miranda and Cuellar and classified as light, moderate or strong.

| Table 1. Phytochemical analysis of hydroalcoholic extracts of *Mauritia flexuosa*. |
|---------------------------|-----------|--------------|
| Assay                     | Metabolites          | Identification |
| Liebermann-Burchard      | Steroids and triterpenes | (+)         |
| Ferric chloride           | Phenolic compounds    | (+++)       |
| Shinoda                   | Flavonoids           | (+++)       |
| Baljet                    | Lactones             | (+)         |
| Fehling                   | Reducing sugar       | (+)         |
| Gelatin                   | Tannins              | (+)         |

(+), light; (++), moderate; (+++), strong.
Source of bacterial strains

Standard bacterial strains (American Type Collection Culture (ATCC) were provided by the Bacteriology Laboratory of Universidad Nacional de Trujillo. Gram positive bacteria Staphylococcus aureus ATCC 25923 and Bacillus subtilis ATCC 6633 were used. Gram-negative Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, and Salmonella Typhi ATCC 11011 were used.

Inhibition experiment protocol

Bacteria were stored at a temperature below 5°C. From there, they were removed and reactivated in brain heart infusion medium at 37°C for 18 days. Mueller-Hinton agar (Merck) was prepared in biosafety level one conditions according to the manufacturer’s specifications. 8ml of Mueller-Hinton agar was poured into 100mm Petri dishes and left to dry at 37 °C for 30 minutes. A suspension of 5×10^8 colony forming units (CFU) of each bacterial culture was prepared in a test tube of 10ml isotonic sodium chloride solution, equivalent to 0.5 MacFarland. For the experiment, 1ml of each suspension was spread onto Mueller-Hinton plates and was left to dry for 30 minutes. For the application of the hydroalcoholic extract, the diffusion method in agar was used and five holes of 5mm were made in each Petri dish; four to add 70μl of the prepared extract concentrations (10, 20, 40 and 60mg/ml) and one to add 70μl of the control (distilled water). In total, the experiment involved 50 plaques, with two Petri dishes of five plaques each used for each of the five species of bacteria. The plates were incubated at 36°C for 24 hours. The results were determined using a digital vernier caliper (CALDI-6MP, Truper), giving the diameter of the halo in mm.

Statistical analysis

SPSS (version 22) software was used for the summation, averages, tables and graphs. For the calculation of the inhibition halo, the mean average of the two repetitions for each extract concentration for each bacterium was calculated.

Results

It was observed that Mauritia flexuosa has a large number of phenolic compounds and flavonoids (Table 1).

As shown in Figure 1, no inhibition halos were formed in the control group (distilled water) around any of the gram-negative bacteria. For Pseudomonas aeruginosa, there was an inhibition halo of 14.8mm of diameter at a Mauritia flexuosa extract concentration of 60mg/ml, a halo of 12.4mm at 40mg/ml, a halo of 10.2mm at 20mg/ml and a halow of 8.0mm at 10mg/ml. However, for Salmonella Typhi and Escherichia coli, no inhibition halos were observed.

As shown in Figure 2, the control (distilled water) did not produce inhibition halos around any of the gram-positive bacteria. For Bacillus subtilis, there was an inhibition halo with a diameter of 13.2mm at a Mauritia flexuosa extract concentration of 60mg/ml, a halo of 11.1mm at 40mg/ml, a halo of 9.4mm at 20mg/ml and a halo of 6.6mm at 10mg/ml. For Staphylococcus aureus, diameter of the inhibition halo was 15.4mm at an
extract concentration of 60mg/ml, a halo of 13.2mm at 40mg/ml, a halo of 11.3mm at 20mg/ml and a halo of 8.1mm at 10mg/ml.

**Discussion**

Medicinal plants have become common in today’s research, analyzing different parts of the plant, in order to ensure the diversity, and evaluating and their potential as therapeutic agents\(^2^3\)–\(^2^5\). Therefore, the increase in bacterial genetic mutations conferring bacterial resistance to antibiotics and the increase in difficulty in treating these infections has led to the investigation of new antibacterial agents, especially those of natural origin\(^2^6\).

As shown in Table 1, secondary metabolites were qualitatively identified in *Mauritia flexuosa* leaves, such as steroids, triterpenes, phenolic compounds, flavonoids, lactones, reducing sugars and tannins. The qualitative analysis of leaves of *Mauritia flexuosa* is important, since the evidence that there is a large presence of secondary metabolites, such as steroids, triterpenes, phenolic compounds and flavonoids, allows comparison of its potential against growth of bacteria with other research.

As shown in Figure 1, three gram-negative bacteria were studied, of which only *Pseudomonas aeruginosa* was inhibited by the hydroalcoholic extract, at a concentration of 60mg/ml and producing an inhibition halo of 14.8 mm in diameter. There was no inhibitory effect on *Salmonella Typhi* and *Escherichia coli*. In a study carried out in Brazil, in which *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used, no sensitivity to the extract of *Mauritia flexuosa* was evidenced\(^2^7\). Therefore, it should be pointed out that the chemical composition of the soil and the climatic conditions may result in variation of the active constituents; therefore, differences in the results may be observed despite the use of the same bacterial species. Furthermore, it has been found previously that the hydroalcoholic extract inhibited the bacterial growth of the same species used for this study\(^2^8\)–\(^2^9\). Therefore, the chemical composition of the leaves plays an essential role in the antibacterial effect, since the effect is composed of a set of active principles that work synergistically.

As shown in Figure 2, gram-positive strains of *Bacillus subtilis* and *Staphylococcus aureus* had inhibition halos of 13.2mm and 15.4mm in diameter, respectively, at a hydroalcoholic extract concentration of 60mg/ml, identifying that at a higher concentration there is lower bacterial growth. It is known that hydroalcoholic extracts of plants release a large quantity of phenols and flavonoids and other compounds, including great diversity of secondary metabolites, which may explain this antibacterial action\(^3^0\)–\(^3^2\). Similar results were identified in other studies that tested hydroalcoholic extracts on gram-positive bacteria, finding that the extract had an antibacterial effect\(^3^3\)–\(^3^4\). Therefore, it is important to emphasize the importance of each active principle since act synergistically to obtain better results, being more efficient and effective\(^3^5\). Finally, gram-positive and gram-negative bacteria can be pathogenic; hence, the importance of identifying new alternatives to mitigate the pathologies caused by these bacteria\(^3^6\). Therefore, it is necessary to study regional plants as a treatment alternative and develop new plant products with protective effects.
In conclusion, the hydroalcoholic extract of *Mauritia flexuosa* does not inhibit bacterial growth of gram-negative bacteria *Salmonella Typhi* and *Escherichia coli*. However, in *Pseudomonas aeruginosa* the extract inhibits growth at a concentration of 60mg/ml, forming an inhibition halo of 14.8 mm. For gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*, bacterial inhibition is observed at a concentration of 60mg/ml, with halos of 13.2mm and 15.4mm of diameter, respectively, demonstrating an antibacterial effect in these species.

**Data availability**

Figshare: Supporting data for Figure 1 and Figure 2. https://doi.org/10.6084/m9.figshare.8051870.v3

Figshare: Photographs of each agar plate. https://doi.org/10.6084/m9.figshare.8312777.v3

Figshare: Photographs of the phytochemical analyses. https://doi.org/10.6084/m9.figshare.8862431.v1

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

**Grant information**

The author(s) declared that no grants were involved in supporting this work.

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