

Comparison of the Antibacterial Activity of the Ethanol Extract vs Hydroalcoholic Extract of the Leaves of *Mangifera indica* L. (Mango) in Different Concentrations: An *In Vitro* Study

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ABSTRACT

Aim: To compare the *in vitro* antibacterial activity of different types of hydroalcoholic extracts of the leaves of the *Mangifera indica* L. (mango) plant on the strain of *Staphylococcus aureus* ATCC 6538™.

Materials and methods: This study was experimental *in vitro* and determined the antibacterial activity of four dilutions: *Mangifera indica* L. ethanol extract (MEE) and *Mangifera indica* L. hydroalcoholic extract (MHE) at 50% and 100% on cultures of *S. aureus* ATCC 6538™ comparing with the positive control (chlorhexidine 0.12%) and negative (alcohol 96°) in Mueller Hinton agar cultures using the Kirby-Bauer diffusion method for each study group and incubating the cultures at 37°C for 24 hours.

Results: It was found that the 50% and 100% MEE had a smaller size of the inhibitory halo of 21.3 ± 0.5 and 24.1 ± 0.8 mm, respectively. In addition, with respect to the 50% and 100% MHE, it was found that they had a higher antibacterial activity of 24.6 ± 0.5 and 33.5 ± 1.2 mm, respectively.

Conclusion: Mango leaf extracts are potent antibacterial, proving 100% MHE to be more effective, thus confirming the presence of active constituents in medicinal plants.

Clinical significance: This research has a great clinical applicability due to the opening of research lines that prove the usefulness of these extracts in the therapeutic control of certain oral diseases.

Keywords: Antibacterial activity, Ethanol extract, Hydroalcoholic extract, *Mangifera indica* L.

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INTRODUCTION

Bacterial resistance is a public health problem that involves the world's population. This is because bacteria that cause infectious diseases become resistant to antibiotics, so science has been in a position to take measures such as the development of new antibacterial that can cope with these multidrug-resistant bacteria. On the other hand, traditional medicine and phytotherapy has been gaining increasing strength since a wide variety of plants show that they have biological properties for the different conditions that man presents, so there is a great empirical source of active ingredients, it is convenient to clarify those properties.¹⁻⁴

Currently, bacterial resistance against antibiotics has been increasing, resulting in a worldwide concern since mild infections cannot be controlled. This caused by the inappropriate and excessive use of drugs in addition to deficiencies in the prevention and control of infections. Since it is not possible to use first-line antibiotics, it is necessary to use more expensive medicines, which increases the costs for the family and society.⁵⁻⁸

In health sciences, for example, the use of antibiotics is justified in the presence of odontogenic infections and as prophylaxis in at-risk patients. Among stomatological infections, bacteremia and osteomyelitis are common as a result of invasive dental procedures whose multifactorial origin includes a very varied microflora, finding opportunistic microorganisms such as *S. aureus*, which is related to the onset of these diseases.^{9,10}

In recent years, research shows various medicinal plants of stomatological interest, including *Mangifera indica* L., to

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which antibacterial properties are attributed due to its active substance that is mangiferin. As the phenolic compound is one of the most abundant chemical components in this plant, it is considered that it gives it the ability to inhibit or block the growth of certain microorganisms. Some bacteria that cause

oral diseases such as *S. mutans*, *E. faecalis*, *C. albicans*, *S. aureus* and *E. coli* among others that have proven to be susceptible to *Mangifera indica* L.¹¹⁻¹⁵ In addition, according to the literature, the leaves of this plant have certain antibacterial properties due to the presence of saponins, steroids, alkaloids, anthracenocides, coumarins, flavonones, among other chemical compounds that are considered responsible for providing antimicrobial properties to this natural resource.^{4,11}

Therefore, the main objective of this research was to compare the *in vitro* antibacterial effect of two types of extracts (ethanol and hydroalcoholic) based on the *Mangifera indica* L. (mango) leaf of Northern Peru against strains of *Staphylococcus aureus* ATCC 6538™ so that it can provide scientific evidence to the profile of this plant and incorporate it as a possible source of antibacterial agent.

MATERIALS AND METHODS

The study was experimental *in vitro*, the execution of the present investigation was carried out in the Pharmaceutical Production Center (CENPROFARMA), in the Microbiology Laboratory of the Faculty of Pharmacy and Biochemistry of the National University of San Marcos (UNMSM). The sample was obtained using the means comparison formula with the Stata® 12.0 software with a 95% confidence level, with a statistical power of 0.80 from the results of the pilot test, determining a sample size of $n = 48$ discs embedded with the experimental substances in each of the six groups which was made up of $n = 8$ discs.

Group I: 50% MEE facing *S. aureus*

Group II: 100% MEE facing *S. aureus*

Group III: 50% MHE facing *S. aureus*

Group IV: 100% MHE facing *S. aureus*

Group V: chlorhexidine 0.12% vs *S. aureus* as a positive control

Group VI: alcohol 96% facing *S. aureus* as a negative control

Collection of *Mangifera indica* L. (Mango)

The collection of mango leaves was made in the hamlet "Encuentros de Romero," on the banks of the Quiroz River, located in the province of Sullana, in the Department of Piura in Northern Peru. The hamlet is approximately 250 m.a.s.l. and 62 km from the district of "Lancones" that is between 80° 28'40" Longitude West and 4° 40'50" Latitude South (Regional Government of Piura, 2007). The average annual temperature for this area was

23°C and the temperature reaches its maximum end in the months of November and December (34.2°C) and its minimum end in June (15°C) (Fig. 1).

Preparation of the Extracts and Taxonomic Classification

A sample of mango leaves was taken to the Natural History Museum of the UNMSM for taxonomic determination, which certified the authenticity of the plant under study (Constancy No. 302-USM-2018). Determining the following classification:

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Rosidae

Order: Sapindales

Family: Anacardiaceae

Genus: *Mangifera*

Species: *Mangifera indica* L.

In this investigation, the *Mangifera indica* L. leaves of the same species were used in all the tests performed, however, different preparations were made of the same plant (ethanol and hydroalcoholic extract). Subsequently, the leaves were washed with distilled water and allowed to dry at room temperature for a period of 5 days, then the physical chemical stabilization of the plant was started by putting it to dry 3 days in the oven at 40°C. The leaves were crumbled and passed through a manual mill where it was pulverized for the preparation of the extracts (ethanolic and hydroalcoholic), 400 mL of the solvent was used for every 50 g of the dried plant, all this was stored in an amber bottle and it was macerated during 15 minutes movements once a day for 10 days. After maceration, it was filtered with a vacuum pump with no. 40 Whatman filter paper and the extract was poured into a glass source for further drying and taken to an incubation oven at 40°C for 4 days. Then, it was poured into a vial using 2 g in 4 mL of solvent. Finally, the same protocol was performed for both solvents.

In Vitro Antimicrobial Susceptibility Testing

The strain of *S. aureus* ATCC 6538 was obtained from the Gen Lab laboratory of Peru SAC and refrigerated between 4°C and 8°C on a plate with TSA agar, for the activation of the strain a colony with the bacteriological handle was taken and seeded in a tube with sterile TSB broth, then it was taken to the incubator at 37°C for 24 hours where turbidity showed growth of the suspension. The TSB broth was seeded on a plate with TSA agar and taken back to the incubator at 37°C for 24 hours. For the preparation of the inoculum, a certain amount of colonies was taken from pure colonies of the *S. aureus* microorganism and diluted in a test tube containing 10 mL of sterile physiological serum (0.9% sodium chloride) such that the resulting solution had a turbidity corresponding to tube no. 1 of the MacFarland scale (turbidimetric scale consisting of a series of tubes with increasing turbidity that allows to find the approximate concentration of a bacterial solution) which corresponds to a concentration of 3×10^8 CFU/mL. From this last solution a dilution of 1 in 3 was made, for this of this prepared solution 3 mL was taken and diluted to a total volume of 9 mL with physiological serum in a tube with sterile screw cap, the resulting solution had a concentration of 1×10^8 CFU/mL. For inoculation of the plates, 100 µL of the prepared bacterial inoculum (1×10^8 CFU/mL) was added to 12 plates with Mueller Hinton agar and with the help of a Drigalski spatula the inoculum was spread throughout the plate in such a way that it was obtained a homogeneous growth, for which



Fig. 1: Leaves of *Mangifera indica* L.

the handle slid in the plate in parallel and well compact covering the entire surface of the plate then the procedure was repeated rotating the plate 60° in two more opportunities.

Measurement of Inhibition Halos

After 24 hours of incubation each plate was examined, the diameters of the entire inhibition zone were measured in millimeters passing through the center of each well. The measurement was performed in triplicate for each well with a Caliper Model: DC-515 digital vernier that measures up to one hundredth of a millimeter (Kirby–Bauer technique). Triplicate measurement values were averaged and rounded to report as a natural number (Fig. 2).

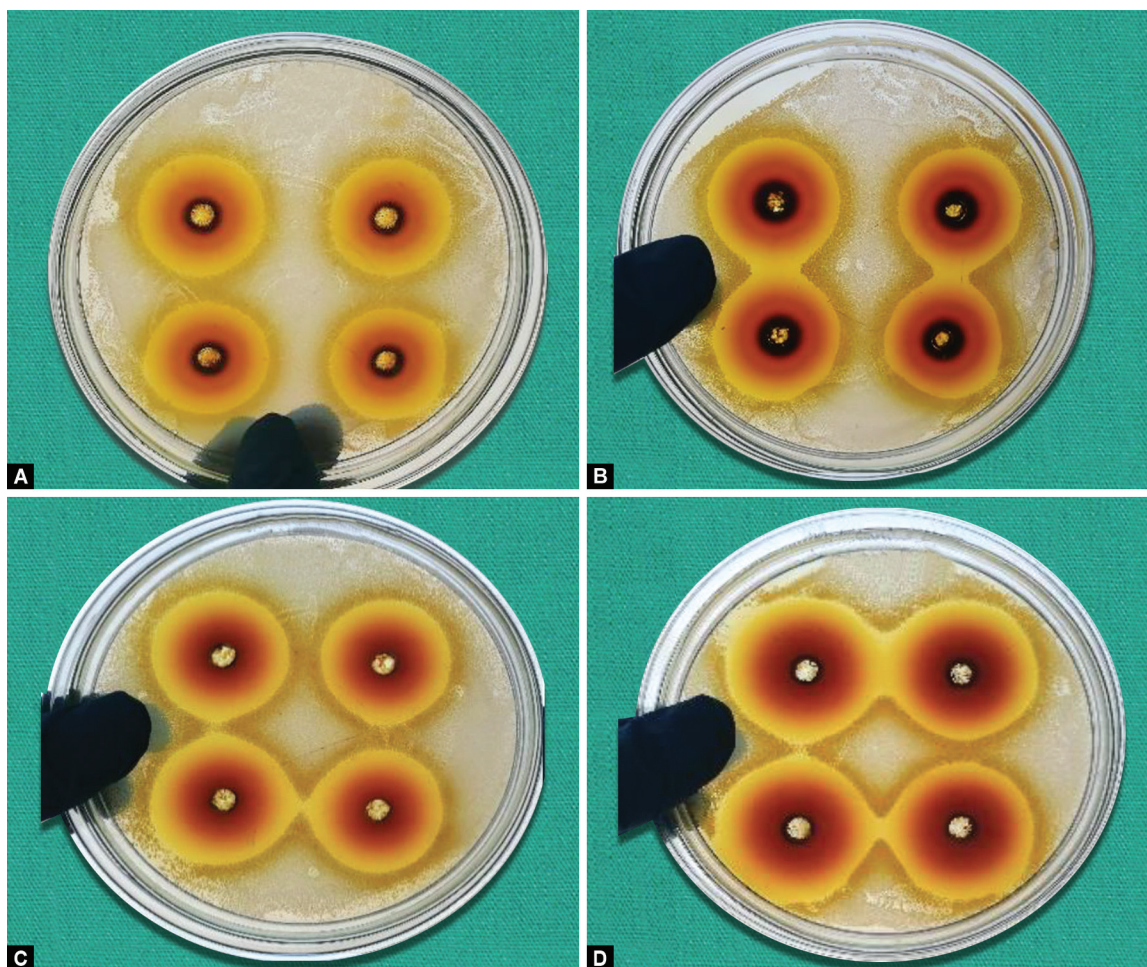
Statistical Analysis

A database was created in Excel 2010 and for the statistical analysis the statistical program Stata® 15.1 was used. Descriptive analysis: the measures of central tendency (arithmetic mean) and dispersion (standard deviation, minimum and maximum) were calculated to describe the behavior of the dependent variable studied and double entry tables and bar graphs were drawn up with their lines of error. Inferential analysis: the hypothesis test and the Student's *t* parametric tests were used for the comparison between two groups. The *F* test through the variance analysis technique (ANOVA) was used to compare the four extracts. The level of significance that was used was 0.05 with a 95% confidence level.

RESULTS

When evaluating the antibacterial activity of the *Mangifera indica* L. (mango), it was shown that between 50% and 100% MEE concentrations they presented an inhibitory halo at 21.3 ± 0.5 and 24.1 ± 0.8 mm, respectively. In addition, with respect to the 50% and 100% MHE an activity of 24.6 ± 0.5 and 33.5 mm was found, respectively. Finally, in relation to the hydroalcoholic extracts with the positive (chlorhexidine 0.12%) and negative (alcohol 96%) control group, an antimicrobial activity of 25.3 ± 1.5 and 6.0 ± 0.0 mm was found. Finally, all groups presented normal distribution with a $p > 0.05$ (Table 1 and Fig. 3).

Table 1 shows that when performing the inferential statistics between the antibacterial activities of the 50% and 100% MEE, differences were found to be significantly significant with a $p < 0.001$. Likewise, when comparing the MHE at 50% and 100%, it was also evident that there were statistically significant differences with a $p < 0.001$. Finally, when comparing the ethanol and hydroalcoholic extracts with the control group (chlorhexidine 0.12%), statistically significant differences were found ($p < 0.001$). In relation to the comparison of the negative control group (alcohol 96%) and positive control group (chlorhexidine 0.12%), there were also statistically significant differences between these groups. The latter being the one that had the greatest antimicrobial effect $p < 0.001$.



Figs 2A to D: Antibacterial activity: (A) *Mangifera indica* L. ethanol extract (MEE) 50%; (B) MEE 100%; (C) *Mangifera indica* L. hydroalcoholic extract (MHE) 50%; (D) MHE 100%

Table 1: Comparison of the antimicrobial activity of *Mangifera indica* L.

Groups	24 hours				<i>p</i> *	<i>p</i> **	<i>p</i> ***
	Mean	SD	Min.	Max.			
MEE 50%	21.3	0.5	21.0	22.0	>0.05	0.001	<0.001
MEE 100%	24.1	0.8	23.0	25.0			
MHE 50%	24.6	0.5	24.0	25.0	>0.05	0.001	
MHE 100%	33.5	1.2	32.0	35.0			
Clorhexidine 0.12%	25.3	1.5	24.0	27.0	>0.05	0.001	
Alcohol 96°	6.0	0.0	6.0	6.0			

All values were recorded in mm

*Shapiro–Wilk test

**Student *t* test

***ANOVA test

Level of significance (*p* < 0.05)

MEE, *Mangifera indica* L. ethanolic extract; MHE, *Mangifera indica* L. hydroalcoholic extract

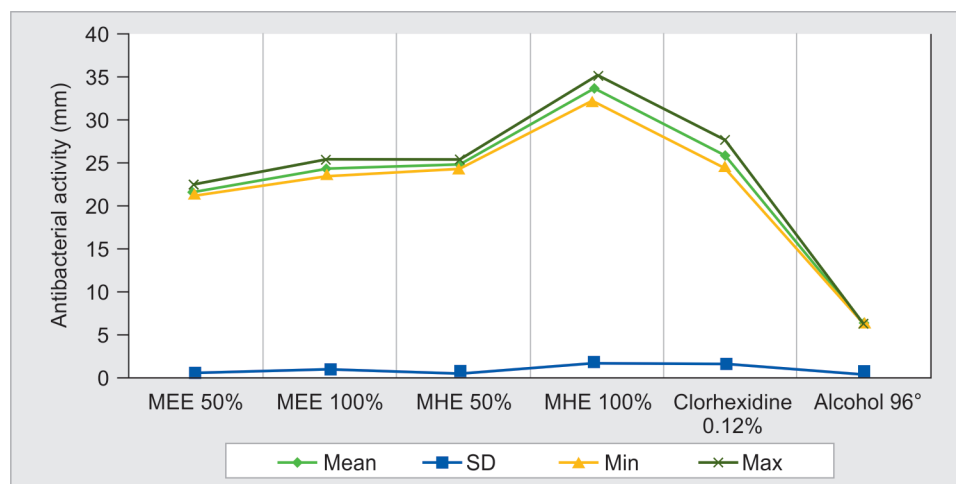
DISCUSSION

Biofilm is a layer of homologous extracellular polysaccharides produced by some strains of *S. aureus*. This extracellular network serves as an aid for the adhesion of the bacterial community to different surfaces. It is also involved in the persistence and colonization of this bacterium in probes, prostheses and catheters. This biofilm can prolong the time of colonization and infection, as well as the dispersion to different parts of the human body.^{4,5,7} Infections develop from the deepening of this bacterium by a solution of tissue continuity due to trauma, skin or surgical lesions. These infections generate purulent material forming abscesses and can cause minor infections of the skin and soft tissues and invasive infections such as: infections of the central nervous system, respiratory, gastrointestinal and urinary tract, bacteraemia and osteomyelitis.^{16–20}

In the present investigation the antibacterial activity of different mango leaf extracts on *S. aureus* strain ATCC 6538 was evaluated, the ethanolic and hydroalcoholic extracts were used at 50% and 100% dilutions on bacterial cultures, using the diffusion method from Kirby–Bauer wells and it was found that in effect the four dilutions have extremely sensitive activity, there are significant differences between them and in comparison with the positive control (chlorhexidine 0.12%). The results obtained in this research agree with similar studies carried out by other authors.

For example, in the study conducted by Madduluri et al.²¹ the antibacterial activity of the leaves of five native plants was determined, including *Mangifera indica* L., comparing the effects of methanol and ethanol extracts and using the diffusion agar disk method in five different strains, including *S. aureus*. It was found that the five strains showed sensitivity to the extracts of the five plants being one of the most susceptible *S. aureus* with halos of 11.5 mm for the ethanolic extract and 15 mm for the methanolic extract of *Mangifera indica* L. concluding an antibacterial activity positive of the mango leaves coinciding with the present study. However, smaller halos were obtained here and a concentration of 10 mg/mL was used with native leaves of India while in the present study concentrations of 50% and 100% were used with native leaves of Peru. Similarly, Diso et al.²² evaluated the antibacterial activity of leaves and bark of mango tree native to Nigeria with aqueous extracts and chloroform using the diffusion method on methicillin-resistant *S. aureus* and verified that the extracts possess antimicrobial activity since they formed inhibition halos between 11 mm and 17 mm around the wells at 120 mg/mL which classifies this activity as very sensitive coinciding with the present work since that a positive antibacterial activity was found, although concentrations of 50% and 100% were used in the present study and the inhibition halos were between 21.38 mm and 33.5 mm, these results suggest a sensitivity of these microorganisms to the evaluated plant. And further Diso et al. used a strain resistant to methicillin. Another study that found similar results was that described by Anand et al.²³ who made an ethanolic extract of walnut and mango leaves at 20 mg/mL in India and applied it on oral bacterial strains including *S. aureus* with the diffusion method, which showed inhibition halos with an average of 14.67 mm that according to the Duraffourd scale, the bacterium is very sensitive to the antibacterial applied, thus verifying the effectiveness of the extract, possibly being attributed to the presence of bioactive compounds. This is consistent with the present study since it also used ethanol as a solvent although finding halos larger than 21.38 mm and 24.13 mm at 50% and 100%, respectively being interpreted as highly sensitive. All these results are consistent with those described in the present study since we also found an antibacterial activity of the mango based ethanol and hydroalcoholic extract.

Within the main limitations of this research, we have found that when performing two types of extracts and several concentrations, the demand for supplies and reagents was extremely considerable,

**Fig. 3:** *In vitro* comparison of *Mangifera indica* L. based ethanol and hydroalcoholic extracts

so this effect could not be proven against other oral germs. In addition, the low availability of strains pure bacterial, limited this study. On the other hand, another limitation was the need to travel to the North of Peru to get this natural resource and transfer it to the capital (Lima), so time and logistics resources had to be invested to guarantee the correct transport of this plant to the microbiology laboratory.

The importance of this study is that it was verified that the leaves of the mango tree are found in large quantities in Northern Peru, and these have the ability to act as an antibacterial agent against a strain found in greater percentage in certain diseases such as osteomyelitis and other bacteremia. In addition, it had a methodological justification because it identified pharmacological effects and contributed scientific evidence to the profile of the mango plant on the *S. aureus* ATCC 6538, so that it motivates the elaboration of subsequent investigations. Finally, this plant can be used as a possible alternative source for the production of new antibacterial agents of natural origin and accessible to the population.

CONCLUSION

It was determined that the ethanolic and hydroalcoholic extracts of the 50% and 100% mango leaves formed halos of inhibition at 24 hours against the strains of *S. aureus* ATCC 6538. In addition, when comparing the extracts, the 100% hydroalcoholic evidenced a halo of greater inhibition with statistically significant difference compared to the other concentrations of the extracts evaluated.

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