

Anti-bacterial activity of mango (*Mangifera indica*) leaf extracts against *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

A cross-sectional study.

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Abstract

Background.

This study evaluated the antibacterial activity of *M. indica* leaf extracts against clinical isolates of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Methodology.

Mature *M. indica* leaves were collected from the University of Kisubi compound, authenticated, dried, pulverized, and extracted by maceration in a 1:1 ethanol-acetone solvent mixture (20% w/v). Phytochemical screening was performed using standard qualitative tests. Antibacterial activity was assessed by the agar well diffusion method. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by broth microdilution with resazurin dye, and MBC/MIC ratios were calculated to classify activity as bactericidal (≤ 4) or bacteriostatic (> 4).

Results.

Phytochemical screening confirmed the presence of flavonoids, tannins, and mangiferin in the extract. The extract exhibited antibacterial activity against both test organisms, with significantly greater activity against *Staphylococcus aureus* (mean inhibition zone = 16.93 ± 1.83 mm) than *Pseudomonas aeruginosa* (10.53 ± 3.07 mm) ($p < 0.001$). The predominant MIC values were 1/128 for *Staphylococcus aureus* and 1/16 for *Pseudomonas aeruginosa*. Bactericidal activity was observed in all *Staphylococcus aureus* isolates and in 93.3% of *Pseudomonas aeruginosa* isolates.

Conclusion.

Mangifera indica leaf ethanol-acetone extract possesses significant antibacterial activity, particularly against *S. aureus*, and predominantly bactericidal effects against both tested pathogens.

Recommendation.

Further bioassay-guided fractionation is recommended to isolate and characterize the active compounds, followed by in vivo toxicity, pharmacokinetic, and efficacy studies to evaluate clinical potential as an alternative antibacterial agent.

Keywords: *Mangifera indica*, mango leaf extract, antibacterial activity, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, phytochemicals, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), antimicrobial resistance, agar well diffusion, broth microdilution.

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Background.

The rise of antibiotic resistance (ABR) can be traced back to the widespread introduction of antibiotics in the mid-20th century, a period marked by breakthroughs in the treatment of infectious diseases. (Hutchings, Truman et al. 2019). Over time, the usefulness of these drugs has been undermined by the growing ability of bacteria to survive antimicrobial therapy. (WHO 2019) Today, AMR is recognized as a major global health concern with an estimated 1.27 million deaths directly linked to it. The highest mortality rates occur in sub-Saharan Africa and Asia, with Western sub-Saharan Africa experiencing the highest all-age AMR-attributable death rate at 27.3 per

100,000 (Mayito, Kibombo et al. 2024).

In Uganda, factors such as unregulated access to antibiotics and self-medication are among the drivers of AMR. (Namubiru, Migisha et al. 2024). District-level evidence from Wakiso shows that antibiotics are commonly sold and consumed without prescriptions, and both healthcare providers and community members demonstrated inconsistent knowledge of AMR (Muleme, Ssempebwa et al. 2023). These patterns highlight the need for affordable and locally accessible alternatives that can complement or substitute conventional antibiotic therapy. Phytochemical theory states that medicinal plants contain healing properties. (Claro, Palanza et al. 2024). They offer a practical

option, as plant-derived remedies such as extracts, oils, and gums have long been used for therapeutic purposes and continue to provide primary healthcare support for over 80% of the global population. The WHO has also emphasized the importance of integrating traditional remedies into national health systems due to their therapeutic value and availability (Diso, Ali et al. 2017).

Among promising medicinal plants, Mango leaf extracts (*Mangifera indica*) have received growing scientific attention due to their rich phytochemical profile. Mango leaves contain compounds such as mangiferin, flavonoids, tannins, phenols, and saponins, which contribute to antioxidant, anti-inflammatory, antimicrobial, and immunomodulatory properties (Alaiya & Odeniyi, 2023). Studies show that mango leaf extracts exhibit antibacterial activity against pathogens including *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, achieved through mechanisms such as membrane disruption, enzyme inhibition, and oxidative stress modulation (Alaiya and Odeniyi 2023). Despite extensive global evidence, research on the antibacterial effectiveness of *M. indica* leaf extracts within Uganda remains limited, and given the escalating AMR burden and the documented therapeutic potential of plant-based agents, this study evaluates the antimicrobial activity of Mango leaf extracts (*M. indica*) against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, which are common resistant strains to antibiotics or conventional drugs.

Methodology.

Research Design

This study employed an experimental laboratory-based design. Antibacterial activity was assessed using the agar well diffusion method; MIC and MBC were determined via broth dilution.

Study Site/Location.

The extraction was conducted at the University of Kisubi, Faculty of Health Sciences in the Main laboratory, under the guidance of the Practical lecturer and the supervision of my site supervisor.

Clinical isolates of *Staphylococcus aureus* and *Pseudomonas aeruginosa* were prepared from the Makerere University Clinical Microbiology Lab, as well as the Culture and Sensitivity Lab.

Sample Size determination.

A standard formula by Kish -Leslie (1965) was used to calculate the sample size.

The sample size was calculated based on a percentage resistance of 1%.

$$n = \frac{Z^2 P(1-P)}{d^2}$$

d^2
 n = study sample size required
 Z = critical value associated with a 95% confidence interval = 1.96
 P = estimated percentage resistance (1% for each species).
 d = margin of error = 0.05

$$n = \frac{1.96^2 * 0.01(1 - 0.01)}{0.05^2}$$

n = 15 isolates.

A total of 15 isolates of *Staphylococcus aureus* and *P. aeruginosa*, respectively, were obtained from Makerere University Clinical Microbiology Lab for this study.

Sampling techniques.

Mature leaves of *Mangifera indica* were collected from the University compound using purposive sampling. The leaves were selected based on their characteristic morphological features, including a glossy, deep dark green upper surface and a lighter green underside,

Mature leaves of the *M. indica* were collected, packed in polythene bags, labelled, and transported to the University Laboratory for further processing (Kaggwa, Nakayita et al. 2023).

To ensure accurate plant identification, the collected specimens were verified by a qualified botanist from the Uganda Wildlife Education Centre (UWEC), who confirmed that the plant species were *Mangifera indica*.

Data Analysis.

Data were analysed using descriptive and inferential statistics. The diameters of inhibition zones were recorded in millimetres and expressed as mean $\pm$ standard deviation from triplicate experiments. Results were presented in tables. One-way ANOVA and independent t-test were used to compare differences in the response of the two bacteria to the extract. A p-value of ≤ 0.05 will be considered statistically significant. Data analysis was performed using SPSS.

Extract Preparation Procedures.

Data were collected using laboratory-based experimental procedures suitable for evaluating antibacterial activity. The following methods were applied;

Mango Leaf Extract preparation.

The preparation of the mango leaf extract followed a standardized maceration procedure adapted from previously published methods, with modifications to suit the experimental requirements.

Sample Collection and Cleaning.

Fresh *Mangifera indica* leaves were collected, washed thoroughly with tap water to remove dirt and surface contaminants, and air-dried to eliminate residual moisture.

Drying and Pulverization.

The cleaned leaves were then dried in a ventilated oven at 50 °C for 72 h to avoid thermal degradation of sensitive phytochemicals. Once completely dried, the leaves were ground using a blender, and the resulting powder was sieved through a 40-mesh sieve to obtain uniform particle size suitable for extraction.

Solvent Addition and Maceration Setup

80 g of dried mango leaf powder was weighed and transferred into a sterile bottle.

Four hundred millilitres (400 mL) of a 1:1 ethanol-acetone solvent mixture were added to obtain a 1:5 (w/v) solid-to-solvent ratio, equivalent to a 20% extract concentration.

The bottle was tightly sealed and kept in a dark room to limit exposure to light during the extraction process. A negative control consisting of 50 mL of 70% ethanol and 50 mL of 50% acetone without plant extract was prepared separately and used during the antimicrobial assays to assess any inhibitory effects attributable to the solvents alone. (Sari, Amanah et al. 2022)

Maceration Process

The sterile bottle was kept in a dark room and left to macerate for 72 hours. During this period, the mixture was gently shaken every 8 hours to promote effective contact between the solvent and the plant material, thereby enhancing the extraction of bioactive compounds (Sari, Amanah et al. 2022)

Filtration and Collection of Extracts

Following maceration, the mixture was filtered through Whatman No. 1 filter paper, and the filtrates were transferred into a large sterile beaker to facilitate evaporation by increasing the exposed surface area.

Evaporation Process

The filtrate was evaporated to dryness using a water bath maintained at 40°C. The resulting crude extract was weighed, yielding 0.5 g. The dried extract was then dissolved in 100 mL of 10% dimethyl sulfoxide (DMSO) to ensure complete solubilisation.

Storage Conditions

The final extract was stored at -20 degrees Celsius until further use to maintain stability and prevent oxidation or photodegradation.

Phytochemical Screening of Mango Leaf Extracts (Qualitative)

Qualitative phytochemical tests were carried out to detect flavonoids, tannins, and mangiferin using standard procedures described in established photochemistry manuals.

All tests were performed in duplicate, alongside appropriate positive and negative controls.

Test for Flavonoids (Shinoda Test)

Procedure

I. 1 mL of the mango leaf extract was pipetted into a clean test tube.

II. A few drops of 10% lead acetate.

Negative Control:

The procedure was repeated using a solvent blank (ethanol/acetone without extract).

Conclusion

The solution remained colourless.

Test for Tannins (Lead acetate Test)

Procedure

I. 1 mL of the mango leaf extract was transferred into a test tube.

II. A few drops of 1% lead acetate solution. (Shaikh and Patil 2020).

Negative Control:

The same procedure was applied to the solvent blank. No white precipitate was observed.

Test for Mangiferin (Ferric Chloride Test)

Procedure

I. To 1 mL of the mango leaf extract, add 3 drops of 5% ferric chloride solution and mix gently (Shaikh and Patil 2020)

Negative Control:

Carried out the same steps using distilled water. Only the pale yellow tint of ferric chloride was visible (Shaikh and Patil 2020).

Bacterial Culture procedures

Preparation of bacterial cultures

Source of Bacterial Strains

15 clinical isolates of *Staphylococcus aureus* and *Pseudomonas aeruginosa* were obtained from Makerere University Clinical Microbiology Laboratory.

Primary Culture on Selective Media

The clinical isolates of *S. aureus* and *P. aeruginosa* suspensions were streaked on to Blood Agar, respectively,

following the manufacturer's instructions for media preparation.

Incubation

Inoculated plates were incubated at 37 °C in humid 5- 10% CO₂ incubator to obtain fresh colonies.

Preparation of Standardized Inoculum

Fresh colonies were emulsified in sterile normal saline, and the turbidity adjusted to match a 0.5 McFarland standard ($\approx 1.5 \times 10^8$ CFU/mL) using a Barium sulfate solution reference.

Preparation of Bacterial Lawns

Standardized inocula were aseptically spread onto sterile Mueller-Hinton Agar plates using the streak plating method to obtain uniform lawns of growth (Rukundo, Omara et al. 2023).

Assessment of antibacterial activity

Preparation of Agar Wells

After preparing the bacterial lawns, the plates were left at room temperature for approximately 15 minutes to allow excess surface moisture to settle.

A cork borer of width 6 mm in diameter was flamed using a Bunsen burner and was used to bore wells on the inoculated plates.

The wells were aseptically created in each Mueller-Hinton agar plate using a sterile cork borer (Mustapha, Enemali et al. 2014)

Introduction of Extracts and Controls

The wells were then filled with 100 μ L of the extract at a concentration of 1:5. (Mustapha, Enemali et al. 2014), while Vancomycin (5 μ g/disc) was included as the positive control for *Staphylococcus aureus*, and levofloxacin (5 μ g/disc) as the positive control for *Pseudomonas aeruginosa*. All extracts were tested against *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Pre-diffusion and Incubation

Following sample loading, the plates were allowed a pre-diffusion period of about five minutes at room temperature. They were then incubated at 37 °C for 24 hours to permit adequate diffusion of the test agents and subsequent bacterial inhibition.

Measurement of Antibacterial Activity

At the end of the incubation period, the diameters of the inhibition zones were measured in millimetres using a standard zone-measuring ruler. All measurements were carried out in triplicate, and the mean values were recorded to ensure reliability and accuracy.

Determining Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) (Rukundo, Omara et al. 2023)

The minimum inhibitory concentration (MIC) of the mango extract against the test microorganisms was determined

using sterile tissue-treated microtiter plates. A stock solution of the extract was used at a concentration of 500 mg/mL, from which the subsequent dilutions were generated.

Procedure

- Standardised the inoculum of *Staphylococcus aureus* and *Pseudomonas aeruginosa* to 0.5 McFarland standard ($\approx 1.5 \times 10^8$ CFU/ML).
- Using sterile tissue treated microtiter plates, 100 μ L of Mueller Hinton Broth were added in each well of the microtiter plate.
- Added 100 μ L of extract in each well of column 1 of the microtiter plate.
- Performed two-fold serial dilution up to column 10 (e.g. 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256).
- Added 100 μ L of ethanol and acetone solvent in MHB for sterility check in column 11.
- Experimented duplicate
- Added 50 μ L of the standardised inoculum in each well except in column 11.
- Sealed the plate with aluminium foil and incubated at 37°C for 24hrs.
- Added 30 μ L of resazurin dye in each well at a concentration of 0.1mg/mL.
- Incubated the plate for 3hrs at 37°C.

Observation

- Change of the resazurin dye from blue to pink indicated presence of viable organisms present whereas persistence of the blue colour of the resazurin dye indicated the absence of viable organisms.

Conclusion

MIC was defined as the lowest extract concentration preventing macroscopically visible bacterial growth.

Determination of the MBC

To determine the minimum bactericidal concentration (MBC), a loopful of broth from each blue coloured well was subcultured onto Mueller-Hinton Agar and incubated again. The MBC was identified as the lowest extract concentration at which no bacterial colonies grew, indicating bactericidal activity.

Procedure

- Subcultured all the wells that had resazurin dye color blue on the blood agar plate.
- Mixed each content by pipetting up and down three times.
- Transferred 10 μ L of well content on blood agar.
- Incubated at 37°C in a humid 5-10% CO₂ incubator.
- Read the plates for growth.

Replication of Experiments

All MIC and MBC determinations will be performed in triplicate to ensure accuracy, reproducibility, and statistical validity.

Note: Acetone and ethanol were tested separately against clinical isolates of *Staphylococcus aureus* and *Pseudomonas aeruginosa* as negative controls. No zones of inhibition were observed, indicating that the solvents had neither bactericidal nor bacteriostatic effects on the test organisms. Therefore, the antimicrobial activity observed was attributed to the *Mangifera indica* leaf extracts rather than the solvents.

Ethical Considerations.

In this study, ethical considerations were addressed through alignment with an already approved research protocol under the supervision of Dr. Jacqueline Kyosiimire, who holds

valid ethical clearance for the extraction and laboratory evaluation of herbal materials.

The current research operated strictly within the scope of her approved protocol, and all procedures involving sample collection, plant extraction, and microbiological testing followed the ethical, safety, and biosafety standards established therein.

Results

Demographic features of the bacterial isolates.

Bacterial isolates from the different types of clinical samples were obtained for this study, as shown in table 1 and their resistance profiles were well described, as shown in table 2 and 3.

Table 1; Demographic features of the bacterial isolates

Sample type	Number of <i>S.aureus</i> isolates	Number of <i>P.aeruginosa</i> isolates	Total	Percentage (%)
Nasal swab	2	0	2	6.67
Pus swab	5	6	11	36.67
Ear swab	1	2	3	10
Pus aspirate	1	0	1	3.3
Blood	3	0	3	10
Sputum	2	2	4	13.33
Catheter tip	0	1	1	3.3
Tracheal aspirate	0	2	2	6.67
Urine	0	1	1	3.3
Control	1	1	2	6.67
Total	15	15	30	100

A total of 30 bacterial isolates were obtained for this study of which 2/30 (6.67%) isolates were from nasal swabs, 11/30 (36.67%) isolates were from pus swabs, 3/30 (10%) isolates were from Ear swabs, 1/30 were from Pus aspirates

(3.3%), 3/30 were from Blood samples (10%), 4/30 were from Sputum (13.33%), 1/30 were from catheter tip (3.3%), 2/30 were from Tracheal aspirate (6.67%), 1/30 were from Urine (3.3%) and 2/30 (6.67%) isolates were controls.

Table 2: Resistance profile of *Staphylococcus aureus* isolates (n=14)

Antibiotic	Resistance (%)	Intermediate (%)	Susceptible (%)	Total
Chloramphenicol	6(42.9%)	0	8(57.1%)	14
Ciprofloxacin	5(35.7%)	0	9(64.2%)	14
Clindamycin	6(42.9%)	1(7.14%)	7(50%)	14
Erythromycin	7(50%)	0	7(50%)	14
Linezolid	0(0%)	0	14(100%)	14
Oxacilin	6(42.9%)	0	8(57.1%)	14
Trimethoprim-sulfamethoxazole (SXT)	10(71.4%)	1(7.14%)	3(21.4%)	14

Table 2 shows the resistance profile of 14 *Staphylococcus aureus* isolates against seven antibiotics.

The highest resistance was observed against Trimethoprim-sulfamethoxazole (SXT), with 10 (71.4%) isolates resistant, 1 (7.14%) intermediate, and 3 (21.4%) susceptible.

Erythromycin showed 50% resistance, with 7 isolates resistant and 7 susceptible. Chloramphenicol, Clindamycin, and Oxacillin each showed resistance in 6 (42.9%) isolates. Ciprofloxacin exhibited the lowest resistance among the commonly used antibiotics, with 5 (35.7%) isolates resistant

and 9 (64.2%) susceptible. Linezolid demonstrated the highest effectiveness, with all 14 (100%) isolates being susceptible and no resistant isolates detected. One isolate (7.14%) showed intermediate susceptibility to Clindamycin.

Table 3: Resistance profile of *Pseudomonas aeruginosa* isolates (n=14)

Antibiotic	Resistance (%)	Intermediate (%)	Susceptible (%)	Total
Amikacin	3(21.4%)	3(21.4%)	8(57.1%)	14
Cefepime	3(21.4%)	2(14.3%)	9(64.2%)	14
Ceftazidime	3(21.4%)	2(14.3%)	9(64.2%)	14
Ciprofloxacin	6(42.9%)	2(14.3%)	6(42.9%)	14
Gentamicin	5(35.7%)	3(21.4%)	6(42.9%)	14
Imipenem	3(21.4%)	1(7.14%)	1(7.14%)	14
Piperacillin	4(28.6%)	4(28.6%)	6(42.9%)	14
Piperacillin-tazobactam	3(21.4%)	2(14.3%)	9(64.2%)	14

Table 3 shows the antibiotic resistance profile of *Pseudomonas aeruginosa* isolates.

The highest resistance was observed against Ciprofloxacin, with 6 (42.9%) isolates resistant, 2 (14.3%) intermediate, and 6 (42.9%) susceptible. Gentamicin showed resistance in 5 (35.7%) isolates, while Piperacillin showed resistance in 4 (28.6%) isolates. Amikacin, Cefepime, Ceftazidime, Imipenem, and Piperacillin-tazobactam each recorded resistance in 3 (21.4%) isolates. Susceptibility was highest for Imipenem, with 10 (71.4%) isolates susceptible, followed by Cefepime, Ceftazidime, and Piperacillin-tazobactam, each with 9 (64.2%) susceptible isolates.

Mango (*Mangifera indica*) leaf extract.

The first specific objective was to determine the phytochemical composition of mangifera indica leaf ethanol-acetone solvent maceration extract from University of Kisubi. 100 mls of filtered evaporated extract was obtained from 400mls of 70% ethanol and 50% acetone. 80g of the homogenized mango leaves were also obtained; the concentration of the finished extract was then 80g/400mls (20% w/v). Qualitative tests were done on the leaf extract to confirm for the presence of different phytochemicals in the extract, as shown in table 4.

Table 4: Qualitative tests for phytochemicals.

TESTS	OBSERVATION	INFERENCE
10% Lead acetate test	A yellow precipitate colour was observed.	Flavonoids present +++
1% Lead acetate test	A white precipitate was observed, indicating the presence of tannins	Tannins present ++
5% Ferric Chloride	A deep green to brownish-black color was formed.	Mangiferin present ++



Figure 1; Positive 10% lead acetate test



Figure 2; Positive 1% lead acetate test

Table 5 details the standardized laboratory observations across all 30 evaluated isolates.

SN	Lab ID	Organism	Agar Well Diffusion	MIC	MBC	MBC/MIC RATIO
1	5095	S.aureus	20 mm	1/128	1/064	2
2	5550	S.aureus	19 mm	1/128	1/064	2
3	4176	S.aureus	16 mm	1/128	1/064	2
4	2502	S.aureus	15 mm	1/128	1/064	2
5	3226	S.aureus	16 mm	1/128	1/064	2
6	6876	S.aureus	15 mm	1/128	1/064	2
7	967	Pus swab	16 mm	1/128	1/128	1
8	5139	S.aureus	17 mm	1/256	1/064	4
9	5752	S.aureus	20 mm	1/128	1/032	4
10	2747	S.aureus	18 mm	1/128	1/064	2
11	1922	S.aureus	16 mm	1/128	1/032	4
12	4178	S.aureus	18 mm	1/128	1/064	2
13	3202	S.aureus	16 mm	1/128	1/064	2
14	5678-2	S.aureus	14 mm	1/016	1/016	1
15	ATCC 25923	S.aureus	18 mm	1/128	1/064	2
16	6871	P.aeruginosa	16 mm	1/016	1/016	1
17	969	P.aeruginosa	08 mm	1/016	1/016	1
18	2930	P.aeruginosa	10 mm	1/016	1/016	1
19	6146	P.aeruginosa	10 mm	1/016	1/016	1
20	2256	P.aeruginosa	12 mm	1/016	1/008	2
21	7191-2	P.aeruginosa	12 mm	1/016	1/008	2
22	3010	P.aeruginosa	10 mm	1/016	1/016	1
23	2125-1	P.aeruginosa	10 mm	1/016	1/016	1
24	1964	P.aeruginosa	06 mm	1/016	1/008	2
25	2125-2	P.aeruginosa	08 mm	1/016	1/016	1
26	7191-1	P.aeruginosa	08 mm	1/016	1/016	1
27	4261	P.aeruginosa	10 mm	1/016	1/016	1
28	3292	P.aeruginosa	10 mm	1/016	1/016	1
29	2116	P.aeruginosa	10 mm	1/016	>1/2	8
30	ATCC 25923	P.aeruginosa	18 mm	1/016	1/016	1

Evaluation of the MBC/MIC ratio demonstrated that:

The MBC/MIC ratio was used to assess whether the extract killed bacteria or only inhibited their growth. An MBC/MIC ratio of ≤ 4 indicated bactericidal activity, while a ratio > 4 indicated bacteriostatic activity. All *Staphylococcus aureus* isolates (15/15) had MBC/MIC ratios of ≤ 4 , showing that

the extract was bactericidal against all tested isolates. The most common MIC and MBC values were 1/128 and 1/64, respectively.

Similarly, 14 of the 15 *Pseudomonas aeruginosa* isolates (93.3%) had MBC/MIC ratios of ≤ 4 , whereas only one isolate (6.7%) had a ratio of 8, indicating a bacteriostatic response. Overall, the extract showed predominantly bactericidal activity, killing 96.7% of the isolates tested.

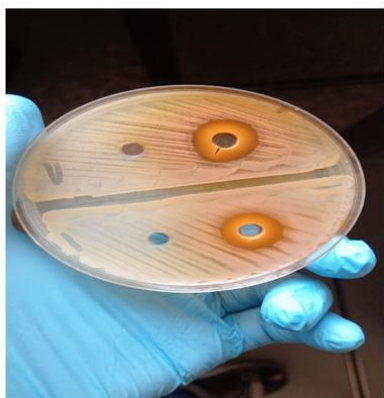


Figure3; Zones of Clearance of isolate 2502 by *Mangifera indica* leaf extract, with no inhibition by ethanol

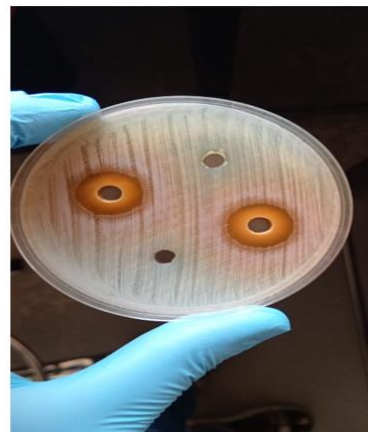


Figure 4; Zones of Clearance of isolate 25923 by *Mangifera indica* leaf extract, with no inhibition by ethanol and acetone controls.

Quantitative computations according to standard biostatistical formulations:

Sample Mean ($\bar{x}$): $\bar{x} = (\sum x_i) / n$ | Sample Standard Deviation (SD): $s = \sqrt{\sum (x_i - \bar{x})^2 / (n - 1)}$

Standard Error (SE): $SE = s / \sqrt{n}$ | 95% Confidence Interval (CI): $CI_{95\%} = \bar{x} \pm (t_{0.025, df} \times SE)$

Independent Samples t-test (t): $t = (\bar{x}_1 - \bar{x}_2) / \sqrt{s_p^2 (1/n_1 + 1/n_2)}$, where $s_p^2 = [(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2] / (n_1 + n_2 - 2)$

Effect Size (Cohen's d): $d = (\bar{x}_1 - \bar{x}_2) / s_p$ | Spearman's Rank Correlation (ρ): $\rho = 1 - [(6 \sum d_i^2) / (n(n^2 - 1))]$

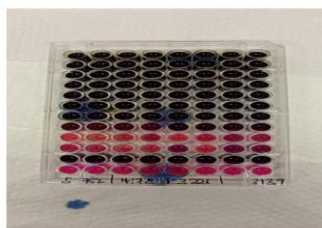


Figure 5; MIC of *S. aureus* isolates 5752, 4178, 3226 and 5139

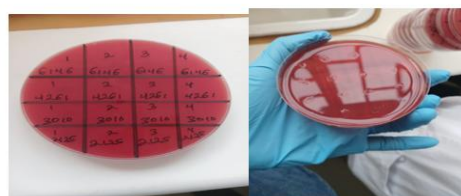


Figure 6; MBC of *P. aeruginosa* isolates 6146, 4261, 3010 and 2125

Inhibition Zone Comparison: For *S. aureus* ($n_1 = 15$), mean zone $\bar{x}_1 = 16.93$ mm, $s_1 = 1.83$ mm. For *P. aeruginosa* ($n_2 = 15$), mean zone $\bar{x}_2 = 10.53$ mm, $s_2 = 3.07$ mm. Pooled variance $s_p^2 = [(14 \times 3.352) + (14 \times 9.409)] / 28 = 6.381$ ($s_p = 2.526$ mm).

Calculated $t = (16.933 - 10.533) / [2.526 \times \sqrt{(2/15)}] = 6.400 / 0.922 = 6.938$. With $df = 28$, two-tailed $p = 0.00000018$ ($<$

0.001). Cohen's $d = 6.400 / 2.526 = 2.53$, demonstrating an exceptionally strong effect size.

Non-Parametric Verification (Mann-Whitney U): Due to skewness in *P. aeruginosa* zones (ATCC strain outlier at 18 mm), the Mann-Whitney U test was computed: $U = 209.0$, $p = 0.000056$ (< 0.001), confirming that differences remain highly significant regardless of distribution assumptions.

Graphical Representations

Figure 7; Boxplot and distribution overlay of inhibition zone diameters (mm) for *S. aureus* vs. *P. aeruginosa*. Horizontal bar represents median; box limits denote IQR.

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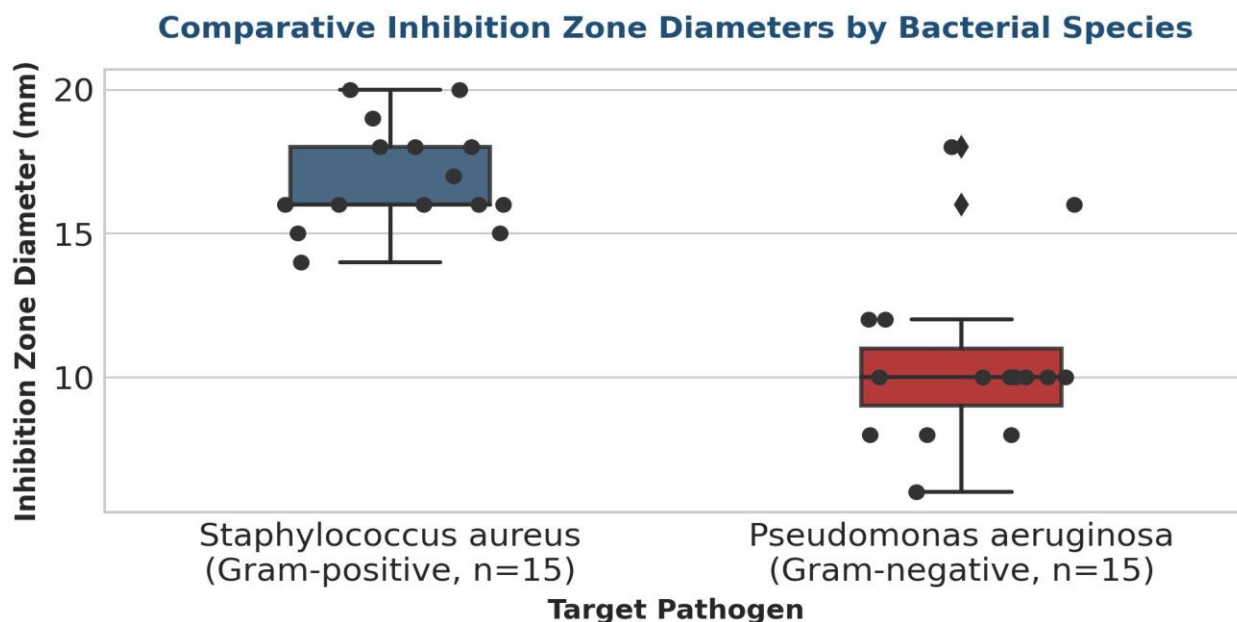


Figure 7 presents a boxplot comparing the inhibition zone diameters produced by *Mangifera indica* leaf extract against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The graph shows that *S. aureus* had generally larger inhibition zones, with values clustered around 16-18 mm, indicating greater susceptibility to the extract. In contrast, *P. aeruginosa* exhibited smaller inhibition zones, mostly ranging from 8-12 mm, suggesting lower susceptibility. The

median inhibition zone was higher for *S. aureus* than for *P. aeruginosa*, and there was little overlap between the two groups. A few higher values were observed among *P. aeruginosa* isolates, but overall, the distribution confirms that the extract was more effective against *S. aureus* than *P. aeruginosa*. These findings support the statistical results, which showed a significantly greater antibacterial effect of the extract against the Gram-positive organism.

Table 6: Comparison of susceptibility of bacterial isolates to conventional antibiotics and *Mangifera indica* leaf extract

Organism	Antimicrobial Agent	Susceptibility (%)
<i>S. aureus</i>	Mango leaf extract	100.0
<i>S. aureus</i>	Linezolid	100.0
<i>S. aureus</i>	Ciprofloxacin	64.2
<i>S. aureus</i>	Chloramphenicol	57.1
<i>S. aureus</i>	Trimethoprim-SXT	21.4
<i>P. aeruginosa</i>	Mango leaf extract	93.3
<i>P. aeruginosa</i>	Imipenem	71.4
<i>P. aeruginosa</i>	Cefepime	64.2
<i>P. aeruginosa</i>	Piperacillin-tazobactam	64.2
<i>P. aeruginosa</i>	Ciprofloxacin	42.9

Determine the Minimum Inhibitory Concentration (MIC) of *Mangifera indica* leaf extracts against *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

The MIC results showed that the mango leaf extract was capable of stopping the growth of both *Staphylococcus aureus* and *Pseudomonas aeruginosa*, although the two organisms responded differently. *S. aureus* was inhibited at lower extract concentrations than *P. aeruginosa*, indicating that it was more sensitive to the extract. This was reflected by the lower MIC value observed for *S. aureus* (1/128) compared to *P. aeruginosa* (1/16). The difference in susceptibility may be linked to structural variations between the two bacteria. *S. aureus*, a Gram-positive bacterium, lacks the outer membrane present in Gram-negative bacteria, making it easier for antimicrobial compounds to reach their target sites. In contrast, *P. aeruginosa* possesses an additional outer membrane that can limit the entry of antimicrobial agents and contribute to its resistance. These findings suggest that the active compounds present in *Mangifera indica* leaves remain effective even when used at relatively low concentrations, particularly against *S. aureus*. The ability of the extract to inhibit both organisms demonstrates its potential as a source of antibacterial agents. Given the increasing challenge of antimicrobial resistance, plant-based products such as mango leaf extracts may provide useful alternatives for future antimicrobial development. This observation is consistent with previous studies that reported greater effectiveness of mango leaf extracts against Gram-positive bacteria than Gram-negative bacteria. The lower MIC values obtained against *S. aureus* therefore reinforce the view that mango leaves contain bioactive compounds with promising antibacterial properties.

Determine the Minimum Bactericidal Concentration (MBC) of *Mangifera indica* leaf extracts against *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Beyond inhibiting bacterial growth, the extract demonstrated considerable bactericidal activity. All *S. aureus* isolates and the majority of *P. aeruginosa* isolates produced MBC/MIC ratios indicative of a bactericidal effect. This means that the extract was not merely slowing bacterial multiplication but was capable of killing the organisms at appropriate concentrations. The strong bactericidal activity observed may be linked to the combined action of the phytochemicals identified during screening. Plant-derived compounds such as flavonoids and tannins have been reported to damage bacterial cell membranes, disrupt essential metabolic pathways, and alter membrane permeability, ultimately leading to cell death.

The susceptibility of *Mangifera indica* leaf extract was compared descriptively with historical susceptibility data for conventional antibiotics. Against *Staphylococcus aureus*, the extract demonstrated susceptibility in all isolates (100%), a performance comparable to Linezolid (100%) and superior to Ciprofloxacin (64.2%), Chloramphenicol (57.1%), and Trimethoprim-sulfamethoxazole (21.4%). These findings suggest that the extract retained activity against isolates that exhibited considerable resistance to commonly used antibiotics.

Similarly, against *Pseudomonas aeruginosa*, the mango leaf extract showed susceptibility in 93.3% of isolates, exceeding the activities of Imipenem (71.4%), Cefepime (64.2%), Piperacillin-tazobactam (64.2%), and Ciprofloxacin (42.9%). Although *P. aeruginosa* is known for its intrinsic resistance mechanisms, the extract remained effective against the majority of isolates tested.

Overall, the results indicate that *Mangifera indica* leaf extract exhibited antibacterial activity that was comparable to the most effective antibiotics and superior to several commonly used antibiotics against both pathogens. This highlights its potential as a source of alternative antibacterial compounds for managing infections associated with antimicrobial resistance. These comparisons are descriptive, as the antibiotic susceptibility data were obtained from historical records rather than generated concurrently with the mango leaf extract experiments.

Discussion of findings.

Qualitatively screen mango leaf extracts for the presence of flavonoids, tannins, and mangiferin.

The phytochemical analysis confirmed the presence of flavonoids, tannins, and mangiferin in the ethanol-acetone extract of *Mangifera indica* leaves. These findings agree with previous reports that mango leaves contain a variety of bioactive compounds associated with antimicrobial activity. The presence of these phytochemicals provides a possible explanation for the antibacterial effects observed in this study. Flavonoids are known to interfere with bacterial cell functions, tannins can inactivate microbial proteins and enzymes, while mangiferin has been widely reported for its antimicrobial properties. Together, these compounds may act either individually or synergistically to inhibit bacterial growth.

The detection of these phytochemicals also supports the traditional use of mango leaves in the management of infectious conditions. Their presence suggests that *M. indica* leaves remain a valuable source of naturally occurring compounds that could be explored further for pharmaceutical development.

The predominance of bactericidal activity in this study therefore suggests that *M. indica* leaf extracts possess genuine therapeutic potential rather than only temporary growth-inhibitory effects (Alaiya & Odeniyi, 2023).

Overall, the study provides evidence that *Mangifera indica* leaf extracts possess significant antibacterial activity against both *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The presence of key phytochemicals, together with the demonstrated inhibitory and bactericidal effects, supports the potential use of mango leaves as a source of alternative antibacterial agents. This is particularly relevant in the context of increasing antimicrobial resistance, where the search for effective and affordable alternatives has become increasingly important.

Conclusion.

Mangifera indica leaf extract is a potent natural bactericidal agent, particularly against *Staphylococcus aureus*, and shows moderate potency against *Pseudomonas aeruginosa*.

Recommendations

Bioassay-Guided Fractionation: Isolated chromatographic fractions should be investigated to identify active pure compounds (e.g., mangiferin).
 In Vivo Toxicity & Pharmacokinetics: Further study should evaluate cell cytotoxicity and animal model bioefficacy.

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List of abbreviations.

ABR – Antibiotic resistance
 AMR – Antimicrobial resistance
 ATCC – American Type Culture Collection
 CFU – Colony-forming units
 DMSO – Dimethyl sulfoxide
 MBC – Minimum bactericidal concentration
 MIC – Minimum inhibitory concentration
 SPSS – Statistical Package for the Social Sciences
 SXT – Trimethoprim-sulfamethoxazole
 UWEC – Uganda Wildlife Education Centre
 WHO – World Health Organization

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Conflicts of interest.

The author declares no conflict of interest in this study.

Data availability.

The data sets generated and analyzed during this study are available from the corresponding author upon reasonable request.

Author contribution

Linda Frances Ajambo generated and analysed the datasets during this study, and these can be obtained from the corresponding author upon reasonable request. Data are not publicly available because they contain information related to laboratory isolates and research materials that may require institutional authorization for access.

Dr. Jacqueline Kyosiimire: supervised research proposal development, guided study design and methodology, critically reviewed the report, and revised the manuscript.

Mr. Fortunate Lujjimbirwa: Co-supervised proposal development, guided literature review and methodology, reviewed manuscript

Dr. Priscilla Balungi: Reviewed research report, provided feedback on data presentation and corrections, reviewed manuscript

Mr James Kasozi: Co-supervised proposal development, provided guidance on literature review and methodology.

Mr. Talemwa Majidu Walusimbi: Helped in the modification of the Research methodology in line with up-to-date practice and performed and interpreted some experiments.

Derrick Napokoli: Helped in the analysis and interpretation of results.

Michael Amooti: supervised the research development process

David Serunjogi: served as final editor. edited manuscript for language, clarity, and formatting, and ensured compliance with academic standards.

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