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Susceptibility Patterns of Multiple Antibiotic-Resistant Bacteria from Wound and Urine Samples to the Extract of *Spondias mombin* (Linn)

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ABSTRACT

This study evaluates the antibacterial activity of *Spondias mombin* L. against multiple antibiotic-resistant bacteria isolated from wound and urine samples of patients attending five (5) selected hospitals in Akure, Ondo State, Nigeria. A total of 313 bacterial isolates were recovered from 353 samples of wound and urine using standard bacteriological procedures, with *Pseudomonas aeruginosa* and *Staphylococcus aureus* being the most predominant in urine and wound samples, respectively. The methanolic extract of *S. mombin* was the most effective against wound isolates, while the aqueous extract was the most effective against urine isolates. The results showed that the methanol extract of *S. mombin* had a zone of inhibition of 24.00±0.00, 30.67±0.33 and 19.33±0.33 mm respectively, against *S. aureus, S. epidermidis* and *P. aeruginosa* at 100 mg/ml. The aqueous extract had a zone of inhibition of 24.67±0.33, 27.33±0.33, 18.67±0.33, 24.67±0.33, 23.67±0.33, 21.33±0.33 and 21.67±0.33 mm against *Escherichia coli*, *Klebsiella pneumoniae, P. aeruginosa, Proteus mirabilis, S. aureus, S. saprophyticus* and *Trichomonas vaginalis* respectively, at 100 mg/ml. The phytochemical constituents of the extracts include alkaloids, anthraquinones, cardiac glycosides, flavonoids, phenols, saponins, steroids and tannins. These compounds may be responsible for the antibacterial activity of *S. mombin* against the multiple antibiotic-resistant bacterial isolates. The findings of this study demonstrate the potential of *S. mombin* as an alternative treatment for multiple antibiotic-resistant bacteria from wound and urine.

Keywords: Antibiotic resistance; *Spondias mombin*; Bio-active compounds.

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INTRODUCTION

Antibiotic resistance is a major global health crisis that threatens the effective treatment of infectious diseases. When bacteria acquire the ability to withstand antimicrobial medications that are meant to kill them, antibiotic resistance arises. This resistance can lead to the emergence of "superbugs" that are difficult or impossible to treat with currently available antibiotics [1]. The overuse and inappropriate use of antibiotics are major drivers of the emergence and spread of antibiotic resistance [2]. In addition, the widespread use of antibiotics in agriculture and aquaculture has also contributed to the development of antibiotic resistance in bacteria [3]. The consequences of antibiotic resistance are significant and potentially catastrophic. Antibiotic resistance causes 700,000 global deaths annually, with rising numbers expected unless urgent action is taken. Economic costs include longer hospital stays, expensive treatments, and decreased productivity [4]. In the face of this crisis, there is a pressing need to identify alternative antimicrobial agents that can effectively treat multiple drug-resistant infections.

Plant-derived compounds have been used for centuries in traditional medicine due to their antimicrobial properties [5]. Plant-based antimicrobials offer a promising alternative to synthetic ones, potentially reducing antibiotic resistance, attracting increasing scientific interest in addressing the problem [6]. One such plant is *Spondias mombin*, commonly known as the yellow mombin or hog plum in English, known as Iyeye in Yoruba, Ijikara (Igbo), and Tsardar masar (Hausa). *S. mombin,* originating from Central and South America, is a fruit-bearing tree that thrives in tropical regions. It has a long history of use in traditional medicine for the treatment of a variety of infections [7]. The fruit, leaves and bark of the tree have been used to treat a range of conditions, including stomachache, diarrhea, wound, fever and urinary tract infections [7].

Recent studies highlight the potential of *S. mombin* as an antimicrobial agent due to its phytochemicals, particularly saponins, which exhibit potent antimicrobial activity against various bacteria and fungi [7, 8]. *S. mombin* fruit, known for its antimicrobial and anti-inflammatory properties, has hypoglycemic effects in animal studies, suggesting potential as a natural diabetes treatment [9]. *S. mombin* leaf extracts have also been shown to have hypolipidemic effects in animals, meaning that they may help to lower levels of cholesterol and other lipids in the blood [9]. Despite the various medicinal properties of *S. mombin*, more research is needed to fully understand its therapeutic potential and to identify the active components responsible for its effects.

S. mombin has a long history of traditional use in various cultures. The fruit of the tree is edible and is often consumed fresh or processed into jams, jellies, and beverages [10]. In traditional medicine, different parts of the plant, including the leaves, bark and fruits, have been used for their medicinal properties. It has been employed to treat various conditions such as diarrhea, dysentery, wound healing and fever [11].

The chemical composition of *S. mombin* has been studied, revealing the presence of bioactive compounds that contribute to its medicinal properties. Phytochemical analysis of the plant revealed various constituents, including phenolic compounds, flavonoids, tannins, alkaloids, saponins and terpenoids [11, 12]. These compounds are known for their potential biological activities, including antimicrobial, antioxidant, anti-inflammatory and anticancer properties.

These findings suggest that *S. mombin* may possess broad-spectrum antimicrobial properties, making it a promising candidate for further exploration and development as a natural antimicrobial agent. The broad-spectrum antimicrobial activity of *S. mombin* extracts indicates their potential for addressing the challenges posed by antibiotic-resistant bacteria. Saha conducted a phytochemical screening of *S. mombin* leaf extracts and reported the presence of flavonoids, tannins, alkaloids, saponins, and phenolic compounds [13]. Similarly, Bossou et al. [12] performed a comprehensive phytochemical analysis of *S. mombin* and identified various bioactive components, including phenolic acids, flavonoids, tannins, terpenoids, and alkaloids. These studies provide valuable information about the chemical constituents present in *S. mombin* extracts, which may contribute to their antimicrobial properties.

Thus, the main objective of this study was to evaluate the antibacterial activity of *S. mombin* (L.) against multiple antibiotic-resistant bacteria isolated from wound and urine samples of patients attending five selected hospitals in Akure, Nigeria.

METHODOLOGY

Study Population

Seventy-one wound samples and 282 urine samples were collected from patients in five Akure hospitals, including diabetic, accident, burns, and postoperative wounds, and from patients with and without urinary tract infection history.

Wound and Urine Sample Collection

Wounds were cleansed with sterile normal saline, and wound swabs were collected from all participants using sterile moistened cotton swabs in an aseptic manner. The swabs were then placed in an icepack container and transported to the laboratory [14]. Urine samples were collected using sterile universal bottles and transferred to the research laboratory for further processing [15].

Isolation and Identification Method
Wound swab samples were immediately placed in Mueller Hinton broth, followed by streaking on Mannitol Salt Agar (MSA), Nutrient Agar, and Blood agar plates. The plates were incubated aerobically at 37°C for 24 hours. Presumptive identification of bacteria was based on their cultural characteristics on each agar plate. Representative colonies were sub-cultured on MSA plates and Nutrient agar, and further incubated at 37°C for 24 hours. The distinct, well-isolated colonies were then studied for their cultural and morphological characteristics. Gram staining and biochemical tests were conducted to confirm the identification of the isolates [16].
For urine samples, 1 µl of urine was spread quantitatively on MacConkey agar, and CLED agar, and incubated aerobically at 35°C for 24 hours. Similar procedures for presumptive identification, sub-culturing, incubation, and confirmation were followed for the urine samples [15]. Preliminary characterization of the isolates was conducted using Gram staining, morphological examination, cultural characteristics and biochemical tests according to Shoaib et al*.* [17].

Antibiotics Susceptibility Testing
The susceptibility of the isolates to antibiotics was determined using the disk diffusion method as described by Cheesbrough [18]. Gram-positive isolates were tested against eight commercially available antibiotics CAZ(30µg), CRX(30µg), GEN(10µg), CTR(30µg), ERY(5µg), CXC(5µg), OFL(5µg), AUG(30µg), while Gram-negative isolates were tested against eight different antibiotics CAZ(30µg), CRX(30µg), GEN(10µg), CXM(10µg), OFL(5µg), AUG(30µg), NIT(30µg), CPR(10µg). The zones of inhibition were compared with CLSI guidelines.

In Vitro Antibacterial Studies
The leaves were obtained from a farm in Akungba Akoko and confirmed at the department of Crop, Soil and Pest Management at Federal University of Technology, Akure. The leaves were dried and ground into coarse powder using a blender. Extraction and standardization of plant extracts were carried out following established procedures as described by Okiti and Osuntokun [19]. The agar well diffusion method was employed for the antibacterial studies. The dried plant extract was reconstituted with sterile distilled water and ethyl acetate to obtain different concentrations of 100 mg/ml, 50 mg/ml, 25 mg/ml and 12.5 mg/ml. Bacterial strains were cultured and spread on Mueller-Hinton agar plates, and wells were made in the agar. The plant extracts were introduced into the wells at different concentrations, and ciprotab (2 mg/ml) was used as control. The plates were incubated at 37°C and the zones of inhibition were measured to determine the antibacterial activity of the plant extract [20].

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)
The MIC of the extracts against the test organisms was determined using the broth dilution method described by Rankin and Coyle [21]. Serial dilutions of the extracts were prepared to obtain extract concentrations of 100 mg/ml, 50 mg/ml, 25 mg/ml and 12.5 mg/ml, and their inhibitory concentrations were recorded. The MBC was determined using the method established by the Mesbah et al. [22]. Samples that did not show visible growth after incubation were streaked on Nutrient Agar plates to determine the minimum concentration of the extract required to kill the organisms. The lowest concentration indicating a bactericidal effect was recorded as the MBC.
The qualitative phytochemical tests were carried out using the method described by the following authors: Alkaloid test [23], Anthraquinone test [24, 25], Cardiac Glycosides test [23, 25]. Flavonoid test [23, 24, 26], Phenol test [24], Saponin test [23, 27], Steroid test [23], Tannin test [24, 27].

Statistical Analysis: Data obtained were subjected to one way analysis of variance (ANOVA) and Duncan's New Multiple Range Test at 95% confidence level using SPSS 20.0 version. Differences were considered significant at $P \leq 0.05$.

RESULTS
A total of 353 samples were analyzed for the presence of multiple antibiotic-resistant bacteria. 59 bacterial isolates were recovered from 67 wound samples collected. The predominant bacteria isolated from the infected wounds were *S. aureus* 39 (66.10%) followed by *P. aeruginosa* 15 (25.42%), and *S. epidermidis* 5 (8.47%). A total of 254 bacterial isolates were recovered from 282 urine samples. The predominant bacteria isolated from the urine samples were *P. aeruginosa* 84 (33.07%), followed by *S. aureus* 71 (27.95%), *S. saprophyticus* 41 (16.14%), *E. coli* 25 (9.84%), *T. vaginalis* 19 (7.48%), *K. pneumonia* 9 (3.54%), and *Proteus* sp. 5 (1.97%).

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Table 1: Antibiotic sensitivity profile of bacteria isolated from wound

Isolates	Antibiotics to which isolates were resistant	MAR Index
S. aureus	CAZ, CRX, GEN, CTR, ERY, CXC, OFL, AUG	0.8
P. aeruginosa	CAZ, CRX, GEN, CXM, OFL, AUG, NIT, CPR	0.8
P. aeruginosa	CAZ, CRX, CXM, OFL, AUG, CPR	0.6
S. aureus	CAZ, CRX, CXC, AUG	0.4
S. epidermidis	-	0
S. epidermidis	CAZ	0.1
S. aureus	GEN, OFL	0.2

Key: CAZ – Ceftazidime (30µg), CRX - Cefuroxime (30µg), GEN - Gentamycin (10µg), CXM – Cefixime (10µg), OFL – Ofloxacin (5µg), AUG – Augmentin (30µg), NIT – Nitrofurantoin (30µg), CPR – Cefpirome (10µg).

Table 2: Antibiotic sensitivity profile of bacteria isolated from urine

Isolates	Antibiotics to which isolates were resistant	MAR Index
K. pneumoniae	-	0
P. mirabilis	CRX, OFL, AUG, CPR	0.4
E. coli	AUG	0.1
P. aeruginosa	CAZ, CRX, GEN, CXM, AUG, NIT	0.6
S. saprophyticus	CAZ, CRX, CTR, CXC, AUG	0.5
K. pneumoniae	CAZ, CRX, GEN, CTR, ERY, CXC, AUG	0.7
S. aureus	CAZ, CRX, ERY, CXC, AUG	0.5
P. mirabilis	AUG	0.1
S. aureus	CAZ, CRX, CTR, CXC, AUG	0.5

Key: CAZ – Ceftazidime (30µg), CRX - Cefuroxime (30µg), GEN - Gentamycin (10µg), CXM – Cefixime (10µg), OFL – Ofloxacin (5µg), AUG – Augmentin (30µg), NIT – Nitrofurantoin (30µg), CPR – Cefpirome (10µg).

Table 1 shows the antibiotic susceptibility patterns of several antibiotic-resistant bacteria obtained from wound samples. The MAR index was computed by dividing the number of antibiotics the bacteria were resistant to by the total number of antibiotics tested, and then multiplied by 100. The total number of antibiotics utilized per disc was 8. *S. aureus* and *P. aeruginosa* exhibited the highest MAR indices of 0.8 each, while a strain of *S. epidermidis* displayed susceptibility to all antibiotics, resulting in a MAR index of 0.

Table 2 presents the antibiotic susceptibility profiles of bacteria isolated from urine samples. *K. pneumoniae* had the highest MAR index at 0.7, followed by *P. aeruginosa* with a MAR index of 0.6. On the other hand, *P. mirabilis* exhibited a MAR index of 0.1.

Plates 1 and 2 show the sensitivity pattern of *S. aureus* against antibiotics for Gram-positive bacteria. It was observed that *S. aureus* was more susceptible to ofloxacin compared to other antibiotics.

Plates 3 and 4 show the sensitivity pattern of *P. aeruginosa* and *K. pneumoniae* against antibiotics for Gram-negative bacteria. *K. pneumoniae* was susceptible to gentamicin, ofloxacin, and cefpirome, while *P. aeruginosa* exhibited high resistance to the tested antibiotics. However, gentamicin demonstrated some level of activity against *P. aeruginosa.*

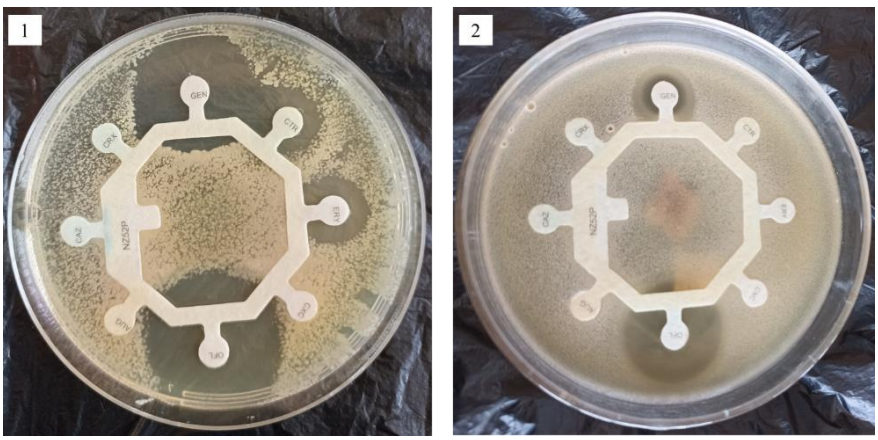

Plate 1 and 2: Antibiotic sensitivity pattern of *S. aureus*

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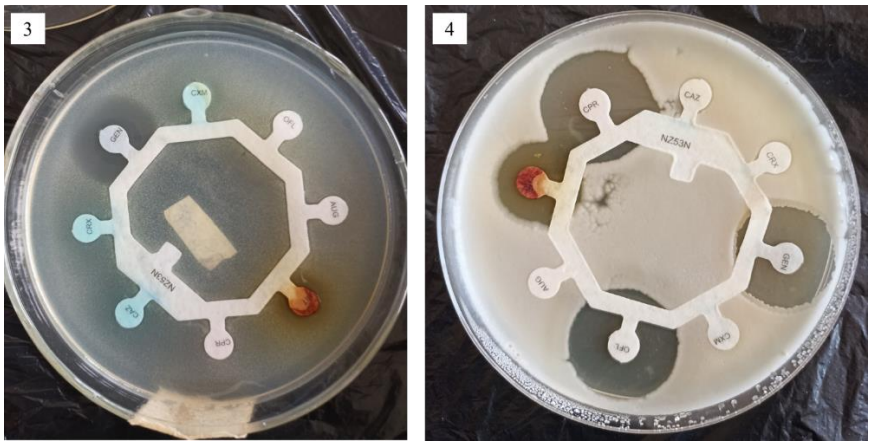

Plate 3 and 4: Antibiotic sensitivity pattern of *P. aeruginosa* and *K. pneumoniae*

Figures 1 to 3 show the susceptibility patterns of key isolates; *S.aureus* and *P. aeruginosa*, from wound and urine samples. In Figure 1, *S. aureus* was susceptible to gentamicin and ofloxacin while displaying resistance to the other antibiotics. Figure 2 illustrates the sensitivity pattern of *P. aeruginosa*, showing complete resistance to ceftazidime, cefuroxime, cefixime, augmentin, and cefpirome. Additionally, as shown in Figure 3, *P. aeruginosa* was also resistant to nitrofurantoin.

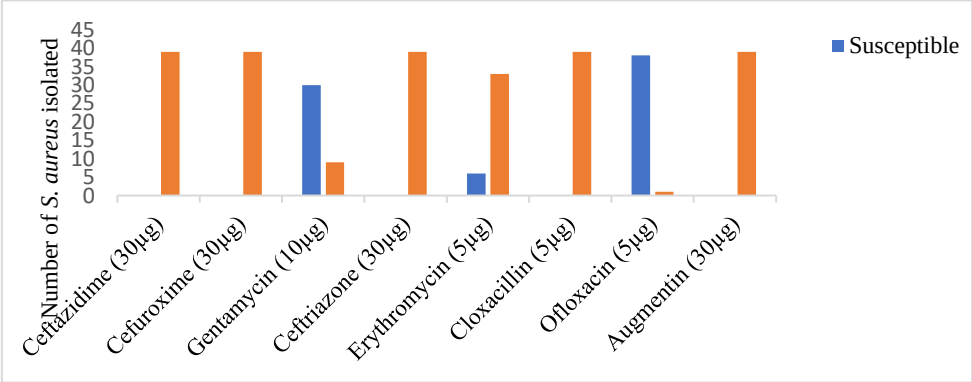

Figure 1: Susceptibility pattern of *S. aureus* isolated from wounds to conventional antibiotics

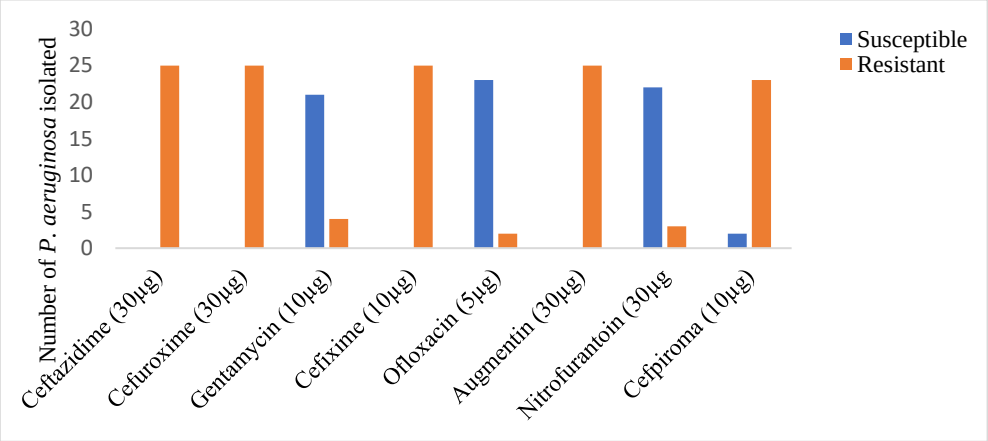

Figure 2: Susceptibility pattern of *P. aeruginosa* isolated from wounds to conventional antibiotics

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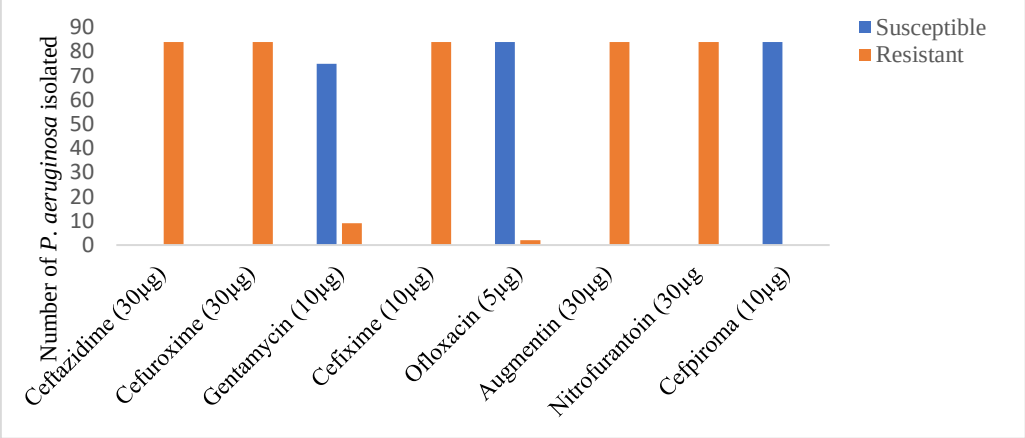

Figure 3: Susceptibility pattern of *P. aeruginosa* isolated from urine to conventional antibiotics.

Table 3-5 present the antibacterial activity of three different extracts—aqueous, methanol, and n-hexane against isolates from wound samples at various concentrations. The results are compared to positive and negative controls, where CiproTab (2 mg/m/) serves as the positive control, and water, methanol, or n-hexane are the negative controls, depending on the extract used.

In Table 3, both *S. aureus* and *S. epidermidis* showed significant antibacterial activity across all concentrations, with larger zones of inhibition at higher concentrations (100 mg/ml). *P. aeruginosa* displayed some sensitivity at higher concentrations but was completely resistant at the lowest concentration (12.5 mg/ml).

For the methanol extract in Table 4, *S. aureus* and *S. epidermidis* exhibited antibacterial activity, which decreased with decreasing concentrations, while *P. aeruginosa* showed moderate resistance, with no activity observed at the lowest concentration.

In Table 5, n-hexane extract was less effective overall, showing reduced antibacterial activity compared to the other extracts. *S. aureus* had no inhibition at lower concentrations, while *S. epidermidis* and *P. aeruginosa* showed declining activity with decreasing concentrations, with *P. aeruginosa* showing some resistance.

Table 3: Antibacterial activity of aqueous extract of on isolates from wound samples

Isolates (mg/ml)	100	50	25	12.5	C (+)	C(-)
S. aureus	24.67±0.33^{d}	21.00±0.00^{c}	17.33±0.33^{b}	11.33±0.67^{a}	23.00±1.00^{cd}	-
S. epidermidis	28.33±0.33^{d}	24.67±0.33^{c}	20.67±0.33^{b}	14.67±0.33^{a}	26.00±2.00^{cd}	-
P. aeruginosa	18.67±0.33^{d}	14.67±0.33^{c}	10.33±0.33^{b}	0.00±0.00^{a}	0.00±0.00^{a}	-

Values are presented as mean ± SE of triplicates. Values with the same superscript letter(s) along the same rows are not significantly different (P<0.05) according to Tukey's Honestly Significant Difference
KEY: C (+): Positive Control (CiproTab - 2mg/ml), C (-): Negative Control - Water.

Table 4: Antibacterial activity of methanol extract on isolates from wound samples

Isolates (mg/ml)	100	50	25	12.5	C(+)	C(-)
S. aureus	24.00±0.00^{d}	21.00±0.58^{c}	18.33±0.67^{b}	14.67±0.33^{a}	23.00±1.00^{cd}	-
S. epidermidis	30.67±0.33^{d}	26.67±0.33^{c}	22.67±0.33^{b}	18.00±0.00^{a}	26.00±2.00^{c}	-
P. aeruginosa	19.33±0.33^{d}	16.00±0.00^{c}	13.00±0.00^{b}	0.00±0.00^{a}	0.00±0.00^{a}	-

Values are presented as mean ± SE of triplicates. Values with the same superscript letter(s) along the same rows are not significantly different (P<0.05) according to Tukey's Honestly Significant Difference
KEY: C (+): Positive Control (CiproTab - 2mg/ml), C (-): Negative Control - Methanol.

Table 5: Antibacterial activity of n-Hexane extract on isolates from wound samples

Isolates (mg/ml)	100	50	25	12.5	C(+)	C(-)
S. aureus	18.67±0.33^{d}	14.00±0.58^{c}	10.33±0.33^{b}	0.00±0.33^{a}	23.00±1.00^{d}	-
S. epidermidis	26.00±0.58^{d}	23.67±0.33^{c}	18.33±0.33^{b}	11.67±0.00^{a}	26.00±2.00^{d}	-
P. aeruginosa	16.67±0.33^{d}	14.67±0.33^{c}	11.67±0.33^{b}	8.33±0.33^{a}	0.00±0.00^{a}	-

Values are presented as mean ± SE of triplicates. Values with the same superscript letter(s) along the same rows are not significantly different (P<0.05) according to Tukey's Honestly Significant Difference
KEY: C (+): Positive Control (CiproTab - 2mg/ml), C (-): Negative Control – n-Hexane.

Tables 6 – 8 show the antibacterial activity of aqueous, methanol, and n-hexane extracts against isolates from urine samples (*E. coli*, *K. pneumoniae*, *P. aeruginosa*, *P. mirabilis*, *S. aureus*, *S. saprophyticus,* and *T. vaginalis*) at concentrations of 100, 50, 25, and 12.5 mg/ml compared with positive and negative controls.

In Table 6, the aqueous extract showed notable antibacterial activity against all isolates, with larger inhibition zones at higher concentrations. *K. pneumoniae* and *P. mirabilis* were particularly susceptible, while *P. aeruginosa* displayed less sensitivity at lower concentrations, showing no activity at 12.5 mg/ml. In Table 7, similar to the aqueous extract, methanol extract displayed antibacterial activity across most isolates. The efficacy decreased as the concentration reduced, with *P. aeruginosa* and *P. mirabilis* exhibiting resistance at lower concentrations (12.5 mg/ml).

n-Hexane extract (Table 8) showed lower antibacterial activity compared to the aqueous and methanol extracts. *E. coli* and *K. pneumoniae* were inhibited at higher concentrations, while *P. aeruginosa* and *T. vaginalis* demonstrated reduced sensitivity, and several isolates including S*. aureus* and *S. saprophyticus*) showed no inhibition at the lowest concentrations.

Table 6: Antibacterial activity of aqueous extract on isolates from urine samples

Isolates (mg/ml)	100	50	25	12.5	C(+)	C(-)
E. coli	24.67±0.33^{d}	21.00±0.00^{c}	16.00±0.00^{b}	10.33±0.33^{a}	29.00±1.00^{d}	-
K. pneumoniae	27.33±0.33^{d}	24.33±0.33^{c}	18.33±0.33^{b}	12.67±0.33^{a}	31.00±1.00^{d}	-
P. aeruginosa	18.67±0.33^{d}	15.67±0.33^{c}	11.67±0.33^{b}	0.00±0.00^{a}	24.00±1.00^{d}	-
P. mirabilis	24.67±0.33^{d}	22.33±0.33^{c}	18.00±0.00^{b}	11.00±0.00^{a}	34.00±1.00^{d}	-
S. aureus	23.67±0.33^{d}	15.67±0.33^{c}	10.67±0.33^{b}	9.00±0.00^{a}	22.00±1.00^{d}	-
S. saprophyticus	21.33±0.33^{d}	17.67±0.33^{c}	11.67±0.33^{b}	9.00±0.00^{a}	28.33±0.33^{d}	-
T. vaginalis	21.67±0.33^{d}	16.67±0.33^{c}	12.33±0.33^{b}	9.00±0.00^{a}	33.33±0.33^{d}	-

Values are presented as mean ± SE of triplicates. Values with the same superscript letter(s) along the same rows are not significantly different (P<0.05) according to Tukey's Honestly Significant Difference
KEY: C (+): Positive Control (CiproTab – 2 mg/ml), C (-): Negative Control - Water.

Table 7: Antibacterial activity of methanol extract on isolates from urine samples

Isolates (mg/ml)	100	50	25	12.5	C(+)	C(-)
E. coli	22.00±0.00^{d}	18.00±0.00^{c}	13.00±0.00^{b}	7.00±0.00^{a}	29.00±1.00^{d}	-
K. pneumoniae	25.00±0.00^{d}	21.00±0.00^{c}	18.00±0.00^{b}	14.00±0.00^{a}	31.00±1.00^{d}	-
P. aeruginosa	17.00±0.00^{d}	15.00±0.00^{c}	12.00±0.00^{b}	0.00±0.00^{a}	24.00±1.00^{d}	-
P. mirabilis	20.00±0.00^{d}	17.67±0.33^{c}	13.00±0.00^{b}	0.00±0.00^{a}	34.00±1.00^{d}	-
S. aureus	20.67±0.33^{d}	18.00±0.00^{c}	14.67±0.33^{b}	11.00±0.00^{a}	22.00±1.00^{d}	-
S. saprophyticus	18.33±0.33^{d}	15.67±0.33^{c}	13.00±0.00^{b}	9.00±0.00^{a}	28.33±0.33^{d}	-
T. vaginalis	20.33±0.33^{d}	15.67±0.33^{c}	13.00±0.00^{b}	9.00±0.00^{a}	33.33±0.33^{d}	-

Values are presented as mean ± SE of triplicates. Values with the same superscript letter(s) along the same rows are not significantly different (P<0.05) according to Tukey's Honestly Significant Difference
KEY: C (+): Positive Control (CiproTab - 2mg/ml), C (-): Negative Control - Methanol.

Table 8: Antibacterial activity of n-Hexane extract on isolates from urine samples.

Isolates (mg/ml)	100	50	25	12.5	C(+)	C(-)
E. coli	18.00±0.00^{d}	15.00±0.00^{c}	13.00±0.00^{b}	7.00±0.00^{a}	29.00±1.00^{d}	-
K. pneumoniae	19.00±0.00^{d}	15.00±0.00^{c}	11.00±0.00^{b}	9.00±0.00^{a}	31.00±1.00^{d}	-
P. aeruginosa	17.00±0.00^{d}	14.00±0.00^{c}	12.00±0.00^{b}	8.00±0.00^{a}	24.00±1.00^{d}	-
P. mirabilis	19.00±0.00^{d}	17.00±0.00^{c}	14.00±0.00^{b}	9.00±0.00^{a}	34.00±1.00^{d}	-
S. aureus	16.00±0.00^{d}	14.00±0.00^{c}	11.00±0.33^{b}	0.00±0.00^{a}	22.00±1.00^{d}	-
S. saprophyticus	18.00±0.00^{d}	16.00±0.00^{c}	13.00±0.00^{b}	0.00±0.00^{a}	28.33±0.33^{d}	-
T. vaginalis	15.00±0.00^{d}	11.00±0.00^{c}	9.00±0.00^{b}	0.00±0.00^{a}	33.33±0.33^{d}	-

Values are presented as mean ± SE of triplicates. Values with the same superscript letter(s) along the same rows are not significantly different (P<0.05) according to Tukey's Honestly Significant Difference
KEY: C (+): Positive Control (CiproTab – 2 mg/ml), C (-): Negative Control - n-Hexane.

Table 9 presents the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values of the plant extracts against the isolates from wound samples. For *P. aeruginosa*, all the extracts displayed a MIC value of 50mg/ml and an MBC value of 100mg/ml. *S. epidermidis* exhibited a MIC value of 12.5mg/ml and an MBC value of 25mg/ml. In the case of *S. aureus*, both the aqueous and methanol extracts had a MIC value of 25mg/ml, while the n-hexane extract had a higher MIC value of 50 mg/ml. Additionally, the MBC value for the n-hexane extract against *S. aureus* was determined to be 100 mg/ml.

Table 10 displays the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values of the plant extracts against the isolates derived from urine samples. The isolates, including *E. coli, S. aureus, S. saprophyticus, T. vaginalis, K. pneumoniae, P. mirabilis* and *P. aeruginosa*, exhibited MIC and MBC values ranging from 25 mg/ml to 100 mg/ml. Specifically, *P. aeruginosa* displayed MIC values of 25 mg/ml, 50 mg/ml and 25 mg/ml for the aqueous, n-hexane and methanol extracts.

Table 11 highlights the presence (+) or absence (-) of various phytochemicals in aqueous, methanol, and n-hexane extracts of *S. mombin*. Alkaloids, Flavonoids, Phenols, and Tannins were Present in all extracts. Anthraquinones and Cardiac Glycosides were detected only in water and methanol extracts. Saponins were found in water and methanol extracts but absent in n-hexane. Steroids were present in water and n-hexane extracts, but absent in methanol.

Table 9: MIC and MBC values of isolates from wound samples

MIC Isolates (mg/ml)	Aqueous	N-hexane	Methanol	MBC Aqueous	N-hexane	Methanol
S. aureus	25	50	25	50	100	50
S. epidermidis	12.5	12.5	12.5	25	25	25
P. aeruginosa	50	50	50	100	100	100

Table 10: MIC and MBC values of isolate from urine samples

MIC Isolates (mg/ml)	Aqueous	N-hexane	Methanol	MBC Aqueous	N-hexane	Methanol
E. coli	50	50	50	100	100	100
K. pneumoniae	25	25	25	50	50	50
P. aeruginosa	25	50	25	50	100	50
P. mirabilis	25	25	25	50	50	50
S. aureus	50	50	50	100	100	100
S. saprophyticus	25	25	25	50	50	50
T. vaginalis	50	50	50	100	100	100

Table 11: Qualitative Phytochemical Profile of *S. mombin*

Phytochemicals	Water	Methanol	n-Hexane
Alkaloids	+	+	+
Anthraquinones	+	-	-
Cardiac Glycosides	+	+	-
Flavonoids	+	+	+
Phenols	+	+	+
Saponins	+	+	-
Steroids	+	-	+
Tannins	+	+	+

Key- +:Positive, - :Negative

DISCUSSION

This study investigated the susceptibility patterns of multiple antibiotic-resistant bacteria isolated from wound and urine samples to the extract of *S. mombin*. The findings revealed a high prevalence of multiple antibiotic-resistant bacteria in both sample types, with *P. aeruginosa* being the most commonly isolated bacterium in urine samples and *S. aureus* being the most commonly isolated bacterium in wound samples. These results align with previous research indicating the prominence of *S. aureus* and *P. aeruginosa* as common antibiotic-resistant bacteria. Liu and Qin [28] reported similar findings, identifying these two bacteria as among the top five antibiotic-resistant pathogens in recent years, reflecting their well-known association with nosocomial infections and their ability to develop antibiotic resistance. The high prevalence of antibiotic-resistant bacteria in clinical samples highlights the need for effective antimicrobial strategies. The identification of other bacterial species in wound and urine infections highlights the complexity of the microbial landscape, guiding the development of appropriate therapeutic approaches.

Antibiotic resistance profiles of isolates revealed that *S. aureus* and *P. aeruginosa* showed the highest MAR indices of 0.8, indicating resistance to 80% of tested antibiotics. A strain of *S. epidermidis* displayed susceptibility to all antibiotics, indicating a generally less resistant bacterial strain compared to other *Staphylococcus* species. These results align with previous studies that have identified *S. aureus* and *P. aeruginosa* as major contributors to antibiotic resistance [28]. Table 2 provides further insights into the antibiotic susceptibility profiles of bacteria isolated from urine samples. The results indicate that *K. pneumoniae* exhibited the highest MAR index of 0.7, reflecting resistance to 70% of the antibiotics tested. This finding is in line with previous research that has highlighted the high prevalence of multidrug-resistant *K. pneumoniae* strains [29]. Similarly, *P. aeruginosa* showed resistance to 60% and *Proteus mirabilis* had a lower resistance index, this finding does not agree with existing studies that have

recognized *Proteus* mirabilis as a high commonly encountered multidrug-resistant pathogen [30].

The aqueous extract of *S. mombin* showed susceptibility against *S. aureus, S. epidermidis*, and *P. aeruginosa,* with zone diameters greater than those produced by ciprotab (24.67±0.33d, 28.33±0.33d), suggesting that the aqueous extract of *S. mombin* may possess stronger antimicrobial activity against these bacterial isolates. The methanolic extract also showed susceptibility to these bacteria, with larger zones of inhibition than the positive control. The n-Hexane extract showed varying degrees of effectiveness against antibiotic-resistant isolates from wound samples, with zone diameters varying from 0.00±0.33a to 8.33±0.33a.

The observed antimicrobial activity of the extracts of *S. mombin* in this study aligns with existing research on the plant's medicinal properties. Previous studies have reported the presence of various bioactive compounds in *S. mombin*, including alkaloids, flavonoids, and phenols, which have been associated with antimicrobial activity [31]. Additionally, the effectiveness of *S. mombin* against antibiotic-resistant bacteria corroborates findings from other studies that have investigated the antimicrobial potential of plant extracts against multidrug-resistant pathogens [32].

Results in this study highlight the antibacterial effectiveness of different extracts of *S. mombin* against multiple antibiotic-resistant bacterial isolates obtained from urine samples. These findings contribute to the existing research on the antimicrobial properties of *S. mombin* and support its potential use as an alternative therapeutic option for treating antibiotic-resistant urinary tract infections [12].

The study reveals the antibacterial properties of *S. mombin* extracts against various antibiotic-resistant bacteria from urine samples. The aqueous extract showed the highest inhibition zone against *K. pneumoniae* (27.33±0.33d), while the methanolic extract showed the highest inhibition zone against all isolates, with zone diameters ranging from 17.00±0.00 to 25.00±0.00 at a concentration of 100 mg/ml. The n-Hexane extract also showed the highest inhibition zone against all isolates, with smaller zones of inhibition than the positive control. These findings support the potential of *S. mombin* as an alternative therapeutic option for treating antibiotic-resistant urinary tract infections. The results support the potential of *S. mombin* as a potential therapeutic option.

The findings of this study are consistent with previous research on the antimicrobial properties of *S. mombin* extracts. Other studies have reported the presence of bioactive compounds in *S. mombin*, such as flavonoids, tannins, and alkaloids, which are known for their antimicrobial activities [32]. Furthermore, studies investigating the antimicrobial potential of *S. mombin* extracts against antibiotic-resistant bacteria have shown promising results [7]. These findings support the notion that *S. mombin* extracts may serve as valuable resources for the development of new therapeutic agents against antibiotic-resistant urinary tract infections.

The higher effectiveness of the aqueous extract compared to the methanol and n-hexane extracts suggests that the water-soluble components of *S. mombin* may play a significant role in its antimicrobial activity. This finding aligns with previous studies that have reported the antimicrobial properties of aqueous extracts of *S. mombin* against various bacterial strains [33]. The presence of bioactive compounds such as alkaloids, flavonoids, phenols, and tannins in the aqueous extract, as identified in previous phytochemical analyses [13], may contribute to its observed antibacterial activity. The observed lower zones of inhibition for the n-hexane extract suggest that the non-polar compounds present in *S. mombin* may have limited effectiveness against the tested isolates. This aligns with the work of Trusheva et al. [34] who have reported that the varying antimicrobial activities of different solvent extracts from *S. mombin*, indicating that the choice of solvent can significantly influence the extraction of bioactive compounds with antimicrobial properties. In contrast, the aqueous extract of *S. mombin* showed the highest effectiveness against tested isolates, with larger zone diameters of inhibition. This suggests the extract contains water-soluble bioactive compounds, such as alkaloids, flavonoids, phenols, and tannins, which have antibacterial properties [13].

The MIC and MBC values of S. mbin extracts against wound sample isolates was explored tonassay for antimicrobial potential and effective concentration for specific bacterial strain. The extracts showed a MIC value of 50 mg/ml against *P. aeruginosa*, with a MBC value of 100 mg/ml, suggesting a higher concentration is needed for bactericidal effects, aligning with previous studies on *S. mombin* extracts [35]. *S. mombin* extracts showed lower MIC and MBC values against *S. epidermidis* compared to *P. aeruginosa*, indicating lower antibiotic resistance. These results suggest lower concentrations are needed for effective inhibitory and bactericidal effects. The study found that *S. mombin* extracts have moderate antimicrobial activity against *S. aureus* strains, with a MIC value of 25 mg/ml for aqueous and methanol extracts, and 50mg/ml for n-hexane extract. Table 10 provides insights into the MIC and MBC values of *S. mombin* extracts against isolates derived from urine samples, including *E. coli*, *S. aureus*, *S. saprophyticus*, *T. vaginalis*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa*. The MIC and MBC values ranged from 25 mg/ml to 100 mg/ml, indicating varying levels of susceptibility among the tested isolates. The range of MIC and MBC values

suggests variations in the effectiveness of the extracts against different pathogens. These findings are consistent with previous studies that have reported variable susceptibility patterns of bacterial isolates to *S. mombin* extracts [35, 12].

The qualitative analysis of *S. mombin* extracts revealed various phytochemical classes, including alkaloids, anthraquinones, cardiac glycosides, flavonoids, phenols, saponins, steroids, and tannins, which are known for their antimicrobial and antioxidant properties against antibiotic-resistant bacterial isolates. These compounds can contribute to the observed antimicrobial effects of *S. mombin* extracts against antibiotic-resistant bacterial isolates [13]. Anthraquinones were not detected in methanol and n-hexane extracts, suggesting they may not be the main antimicrobial constituents, possibly due to the extraction process and solvents. Saponins were also detected in the aqueous and methanol extracts but were not found in the n-hexane extract. Saponins are known for their diverse biological activities, including antimicrobial, anti-inflammatory, and anticancer properties [36]. The presence of saponins in *S. mombin* extracts further supports their potential as antimicrobial agents against multidrug-resistant bacterial isolates. The comprehensive analysis of bacterial isolates obtained from wound and urine samples, as well as the evaluation of *S. mombin* extracts, provided valuable insights into the prevalence of antibiotic-resistant bacteria and the potential antibacterial properties of the plant.

CONCLUSION

Antibiotic-resistant bacteria pose a global health challenge, necessitating novel therapeutic approaches. *S. mombin* plant extracts offer potential for developing new antimicrobial agents, highlighting the importance of exploring natural products and their bioactive compounds to combat antibiotic resistance and improve public health.

RECOMMENDATION

Further research is needed to identify bioactive compounds responsible for antimicrobial activity, understand their mechanisms, and conduct in vivo experiments and clinical trials for potential therapeutic applications.

LIST OF ABBREVIATIONS

CAZ- Ceftazidime
CRX - Cefuroxime
GEN - Gentamycin
CTR - Ceftriazone
ERY - Erythromycin
CX - Cloxacillin
OFL - Ofloxacin
AUG - Augmentin
CXM - Cefixime
NIT- Nitrofurantoin
CPR - Cefpirome
MSA - Mannitol salt agar
CLED - Cysteine lactose electrolyte-deficient.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

ETHICAL APPROVAL

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all authors. Author AFO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author MKO and AOO managed the analyses of the study and were in charge of direction and planning.

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