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Efficacy of Ziziphus Mauritania Stem Bark Extract Against Clinical Isolate of Multidrug Resistant Pseudomonâs Aeroginosa and Methicillin Resistant Staphylococcus Aureus

Abduljalil, Nafisa Adamu[1*], Zainab Musa Danchuwa[2], Saleh, Ali[3], Basiru, Shamsu[4], Bello Aliyu[5], Abdulhalim, Musa Kabo[6] & Muhammad, Abdullahi Sharif[7]
[1,2,3]Department of Microbiology, Faculty of Life Sciences, Ahmadu Bello University Zaria, Kaduna State.
[4]Department of Microbiology, Federal University of Medicine and Medical Sciences, Abeakuta, Ogun State.
[5]Department of Science Laboratory Technology, School of Pure and Applied Sciences, Federal Polytechnic Ilaro. Abeakuta, Ogun State
[6,7]Department of Science Laboratory Technology, Faculty of Health Sciences, Federal University of Science and Technology, Kabo, Kano State
[*]Corresponding Author Email: nafabduljalil@gmail.com

ABSTRACT
This study investigated the antibacterial efficacy of *Ziziphus mauritiana* stem bark extracts against clinical isolates of multidrug-resistant (*MDR*) *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus* (*MRSA*). The isolates were re-confirmed by sub-culturing into freshly prepared Cetrimide agar for *Pseudomonas aeruginosa* and Mannitol Salt Agar for *Staphylococcus aureus*. The MDR status of each of the isolates was also established. Stem bark of *Z. mauritiana* was obtained, identified, authenticated and subsequently extracted prior to phytoconstituents analysis. An Antibacterial susceptibility assay was conducted testing using agar well diffusion. The Phytoconstituents analysis revealed the presence of alkaloids, flavonoids, saponins, tannins, glycosides, phenols, and steroids, while anthraquinones were absent. Antibiotic susceptibility profiling confirmed multidrug resistance among the isolates, with *P. aeruginosa* resisting several antibiotics including levofloxacin, cefotaxime, sparfloxacin, and amoxicillin, while MRSA isolates exhibited resistance to cefoxitin with inhibition zones of **15 mm and 20 mm**, confirming methicillin resistance. The antibacterial activity of the extracts demonstrated that the ethanolic extract exhibited greater efficacy against MRSA, producing zones of inhibition ranging from **10–26 mm** across concentrations of **25–200 mg/mL**, whereas the aqueous extract showed comparatively lower activity (**0–24 mm**). Both extracts revealed no antibacterial activity against MDR *P. aeruginosa*. However, upon testing the MIC and MBC values of the extract against both extracts revealed bacterial growth inhibitory properties and bactericidal activity against same isolates. An MIC value of **12.5 mg/mL** and MBC value of **25 mg/mL** were recorded. Both extracts exhibited moderate antibacterial activity against MRSA, although relatively high concentrations are required.

Keywords:
MRSA, MDR P. Aeruginosa, Z. mauritiana, Phytoconstituents Analysis

INTRODUCTION

Antimicrobial resistance is one of the greatest public health challenges, several bacterial pathogens are increasingly resisting commonly used antimicrobial agents previously used to treat the infections they cause. More worrisome are bacterial pathogens that are Multidrug-resistant (MDR) or extensively drug resistant (XDR) as they pose and even greater risk to public health leading to high morbidity and mortality rates (WHO, 2021).

Pseudomonas aeruginosa and Methicillin-resistant *Staphylococcus aureus* (MRSA) are among such pathogens of high priority because, they are associated with hospital and community-acquired infections that are increasingly difficult to manage (CDC, 2022)

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a gram-positive bacterium responsible for a wide range of infections including skin and soft tissue infections, pneumonia, and septicemia.

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MRSA has developed resistance to multiple classes of antibiotics, including beta-lactams, thereby limiting treatment options (Tong, *et al*., 2015). Similarly, *Pseudomonas aeruginosa*, is a gram-negative opportunistic pathogen commonly associated with pneumonia, wound and urinary tract infections. The resistance mechanisms adopted by this bacterium is due to its biofilm formation and efflux pumps mechanisms (Lister, *et al*., 2009). Given these challenges, researchers have turned attention towards medicinal plants as alternative sources of antimicrobial compounds.

Different parts of medicinal plants have been in use traditionally for centuries in treating infectious diseases with usage spanning across several cultures, nations and regions (Abduljalil, *et al*., 2025). According to WHO, there are more than 20,000 different plant species that have been documented and verified for use as therapeutic agents *Ziziphus mauritiana* is an important indigenous medicinal and economic plant widely distributed across the savannah and semi-arid regions of Nigeria, studies have confirmed that this plant contains bioactive compounds with antibacterial activity (Newman & Cragg, 2020). The plant belong to the family *Rhamnaceae*, it is commonly known as Indian jujube or Magarya in Hausa. The various plant parts bear varying antimicrobial efficacy including its leaves, fruits, roots, and stem barks. Apart from its antimicrobial activity, it has an antioxidant, anti-inflammatory, and hepatoprotective activities (Kumar, *et al*., 2022). The stem bark of *Ziziphus mauritiana* has been reported to contain secondary metabolites such as flavonoids, alkaloids, tannins, saponins, and phenolic compounds, which contribute to its antimicrobial properties (Goyal, *et al.*, 2012). Several studies have demonstrated that extracts from the plant exhibit inhibitory activity against a variety of pathogenic bacteria including *Escherichia coli, Staphylococcus aureus*, and *Klebsiella pneumoniae* (Akinmoladun, *et al*., 2020).

However, there is limited research specifically targeting the activity of *Z. mauritiana* stem bark extract against clinical isolates of multidrug-resistant *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus*, despite their clinical relevance. This study therefore seeks to investigate the efficacy of *Ziziphus mauritiana* stem bark extract against clinical isolates of multidrug-resistant *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus*. Such findings could contribute to the continuing search for more effective, less toxic and more affordable therapeutic agents derived from natural sources for better the management of antimicrobial resistance.

MATERIALS AND METHODS

Study area

This study was conducted in Ahmadu Bello University, Zaria at the Departmental of Microbiology, Faculty of Life Sciences. Clinical isolates were obtained at the Department of Public Health, Faculty of Veterinary Medicine.

Study design:

The study a cross-sectional design to assess the efficacy of autochthonous Nigerian plants against multi-drug-resistant bacterial isolates.

Plant Collection and Processing

The *Ziziphus mauritiana* stem bark was collected from healthy and matured plant, free from blemishes or damage. The sample was taken to the Herbarium of the Department of Botany, Ahmadu Bello University (ABU), Zaria, for authentication with the voucher number ABUH01841. The authenticated stem bark was transported to the Department of Microbiology Laboratory for further processing. The plant material was air-dried in the laboratory for two weeks. The dried stem bark was pounded using a mortar and pestle, and the resulting fine powder was stored in a clean, sterile container.

Extraction of Plant Material

Aqueous Extraction

Preparation of the aqueous extract was done following the method of (Owolara, *et al*., 2022). Twenty-five grams (25 g) of powdered *Z. mauritiana* stem bark was macerated in 250 mL of distilled water in a sterile conical flask. The mixture was kept at room temperature for 24 hours with intermittent shaking. It was then filtered using Whatman No. 1 filter paper into a sterile flask. The filtrate was evaporated to dryness in a water bath at 40 °C, and the dried extract was stored in an airtight bottle until use.

Ethanolic Extraction

Two hundred and fifty milliliters of 95% ethanol was measured and poured in a conical flask, 25g of powdered stem bark was weighed and poured in to the solvent. The mixture was placed on a shaker for 72 hours. Whatman No. 1 filter paper was used to separate the marc from the filtrate, the filtrate was placed in a moderate heat oven to evaporate the solvent. The extract was obtained after the solvent has been completely dried, it was thereafter stored in airtight bottles until further use.

Phytoconstituents Screening

Phytoconstituents screening of the crude extracts was conducted following the methods of (Abduljalil, *et al*., 2025).

Test for Alkaloids

About 0.5 g of the extract was mixed with 2 mL of 1% HCl and gently heated. Mayer's reagent was then added to the mixture. Appearance of whitish precipitate was considered to be positive for alkaloids.

Test for Flavonoids

About 0.5 g of the extract was dissolved in 2 mL of 10% aqueous NaOH solution and filtered. A color change from yellow to colorless upon the addition of dilute hydrochloric acid indicated the presence of flavonoids.

Test for Saponins

The extract was mixed with 5 mL of distilled water in a test tube and shaken vigorously for 30 seconds. The formation of stable foam was taken as an indication of the presence of saponins.

Test for Steroids

Five drops of concentrated H_2SO_4 were added to 0.5 g of the extract. A color change from violet to green or blue confirmed the presence of steroids.

Test for Glycosides

About 0.5 g of the extract was diluted with 5 mL of distilled water in a test tube, and three drops of ferric chloride solution were added. The presence of a green to black precipitate indicated the hydrolysis of glycosides.

Test for Tannins

To 1 mL of the extract, 1 mL of water and 1–2 drops of 0.1% ferric chloride solution were added. A blue color indicated the presence of gallic tannins, while a green-black color indicated the presence of tannins.

Test for Phenol

Five drops of neutral ferric chloride solution were added to 0.5 gram of the extract. A deep violet or blue color indicated the presence of phenol.

Test for Anthraquinones

Half gram of the extract was boiled with dilute sulfuric acid, filtered, shaken with chloroform, and treated with ammonia solution. A pink, red, or violet color indicated the presence of anthraquinones.

Collection of Clinical Isolates

Clinical isolates of *Pseudomnas aeruginosa* and *Staphylococcus aureus* were collected from the Bacteriology Laboratory at the Microbiology Unit, of the Department of Public health, Faculty of Veterinary medicine, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

Identification of the Clinical Isolates

The isolates were sub-cultured on selective and differential media (Cetrimide agar for *P. aeruginosa* and Mannitol Salt Agar for *S. aureus*) and re-confirmed by observing the colonies macroscopically, Gram staining, and biochemical tests were also conducted according (Cheesbrough, 2018).

Microscopic Observations of colonies on plates

The overnight growth culture was observed for the characteristic colonial morphology, appearance, colouration of the media as well as characteristic scents/odour.

Gram Staining

A thin smear of each isolate was prepared on a grease-free glass slide, air-dried, and
heat-fixed. The smear was stained with crystal violet for 60 seconds, rinsed, and treated with Lugol's iodine for 30 seconds. It was decolorized with 70% alcohol, rinsed, and counterstained with neutral red for 60 seconds. The slide was washed, air-dried, and examined under oil immersion (Fawole & Oso, 2004).

Biochemical Tests

Catalase Test

A loop full of an overnight culture was placed on a clean grease-free slide. The culture was emulsified with a loop full of freshly prepared 3% hydrogen peroxide (H_2O_2) on the slide, and the reaction was observed immediately for effervescence, indicating catalase-positive organisms

Oxidase Test

A colony from an overnight culture was picked with a sterile wireloop and placed on on a piece of filter paper. Two drops of oxidase reagent were added, and the reaction was observed within 1–2 minutes for a deep purple coloration, indicating oxidase positivity

Citrate Utilization Test

Simmons citrate agar slants were prepared and inoculated by streaking the slope with a sterile loop containing the test organism. The slants were incubated at 37°C for 24 hours. A color change from green to blue indicated positive citrate utilization

Motility Test

Motility was tested using sulphide indole motility medium. A straight stab inoculation was made with a sterile needle into the center of the medium and incubated at 37°C for 24 hours. Diffuse growth radiating from the stab line indicated a motile organism, while growth confined to the stab line indicated a non-motile organism

Coagulase Test

This test was performed by emulsifying a colony of the bacterium in rabbit plasma on a clean slide. The slide was observed immediately for agglutination. Formation of visible clumping within 10 seconds was recorded as a positive coagulase reaction.

Indole Test

A loop-full of the test organism was inoculated into two test tubes containing 5 mL of Tryptone broth, leaving one test tube uninoculated to serve as a control. The tubes

were incubated for 48 hours at 37°C. After incubation, 0.5 mL of Kovac's reagent was added and shaken gently. It was allowed to stand for 20 minutes, allowing the reagent to rise. A red ring color indicated a positive result

Voges-Proskauer Test

MR VP medium was prepared and sterilized as the growth medium and allowed to cool to room temperature for 24 hours. Then, 1 mL of 40% potassium hydroxide and 3 drops of alpha-naphthol were added. The tube was shaken well and allowed to stand for 5 minutes
before observation. A pink-red color indicated the presence of acetoin, meaning the bacterium could ferment glucose

Methyl Red Test

Methyl red indicator solution was added to inoculated culture media and incubated at 37°C for up to 24 hours. A color change to red indicated an MR test positive appearance of the tested bacteria

Antibiotic Susceptibility Test

Mueller–Hinton agar was prepared according to the manufacturer's instructions, sterilized, and poured into plates to a uniform depth, then allowed to solidify. Colonies of *P. aeruginosa* from an overnight culture were suspended in sterile saline and adjusted to a 0.5 McFarland standard. A sterile swab was used to spread the standardized inoculum evenly over the surface of the agar. Antibiotic discs (Levofloxacin 20 µg, Cefotaxime 10 µg, Sparfloxacin 10 µg, Ciprofloxacin 30 µg, Amoxicillin 30 µg, Augmentin 10 µg, Gentamicin 30 µg, Pefloxacin 30 µg, Ofloxacin 10 µg, Azithromycin 12 µg) were aseptically placed on the inoculated plate and pressed lightly to ensure contact. The Plates were incubated at 37 °C for 24 hours. After incubation, the diameters of zones inhibition were measured in mm and interpreted using CLSI M100 breakpoints (CLSI, 2023). An isolate was classified as multi-drug resistant if it was resistant to at least one agent in three or more different antibiotic classes.

For methicillin-resistant *Staphylococcus aureus* (MRSA), colonies from an overnight culture were suspended in sterile saline and adjusted to a 0.5 McFarland standard. A sterile swab was used to spread the standardized inoculum evenly over the agar surface. A cefoxitin disc (30 µg) was aseptically placed on the inoculated plate and pressed lightly. Plates were incubated at 37
°C for 24 hours.

Preparation of Extract Concentrations

Two grams (2 g) of the extract was weighed and dissolved in 10 mL of 10% Dimethyl Sulfoxide (DMSO) to obtain a stock concentration of 200 mg/mL. Serial dilutions were prepared to obtain 100 mg/mL, 50 mg/mL, and 25 mg/mL concentrations.

Antibacterial Susceptibility of Extracts (Agar Well Diffusion)

Agar well diffusion method was adopted following the work of (Olutiola, *et al*., 2016) to determine the inhibitory activity of aqueous and ethanolic extracts of *Z. mauritiana* against MDR-*P. aeruginosa* and MRSA. Four wells of 6 mm diameter were bored using sterilized 6mm cork borer and inoculated into Mueller–Hinton agar plates, and 0.1 mL of the extract at different concentrations (200, 100, 50, 25 mg/mL) was introduced. The Plates were incubated at 37 °C for 24 hours, and inhibition zones were measured with a meter ruler.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the extracts was determined using the broth dilution method as described by *(Ali, et al*., 2017), with slight modifications. Two-fold serial dilutions of the extract were prepared in test tubes containing Mueller Hinton Broth (MHB). Two milliters (2 mL) of MHB was dispensed into each of four test tubes, and 2 mL of the extract at 100 mg/mL concentration was added to the first tube to give a concentration of 50 mg/mL. Serial two-fold dilutions were then carried out to obtain concentrations of 25 mg/mL, 12.5 mg/mL, and 6.25 mg/mL. Each tube was inoculated with standardized bacterial suspensions of *Pseudomonas aeruginosa* and *Staphylococcus aureus* and incubated at 37 °C for 24 hours. The lowest concentration that showed no visible turbidity was recorded as the MIC.

Determination of Minimum Bactericidal Concentration (MBC)

The MBC was determined as described by (Ali & Ibrahim, 2017), with modifications. Aliquots from the tubes showing no visible growth in the MIC test were sub-cultured onto Mueller Hinton Agar (MHA) plates. Each plate was divided into four equal sections, and one aliquot was streaked onto each section to minimize plate usage and allow comparison. The plates were incubated at 37 °C for 24 hours. The lowest concentration at which no bacterial growth was observed in the streaked sections was taken as the MBC.

RESULTS AND DISCUSSION

Phytoconstituents Analysis of Plant Extract

The qualitative Phytoconstituents screening of the ethanolic stem bark extracts of *Ziziphus mauritiana* revealed the presence of various bioactive compounds, the extract was found to contain alkaloids, saponins, tannins, glycosides, flavonoids, steroids, and phenols, while anthraquinones were absent.

Table 1: Phytoconstituents analysis of Ethanolic Extract of *Ziziphus mauritiana* Stem Bark

S/N	Phytoconstituents test conducted	Result
1	Flavonoids	+
2	Glycosides	+
3	Saponins	+
4	Alkaloids	+
5	Phenols	+
6	Tannins	+
7	Steroids	+
8	Anthraquinones	–

Key: + = Present; – = Absent

Macroscopic observation, Cell morphology, Gram's Reaction and Biochemical Characteristics of the Isolates

The clinical isolates were presumptively identified by their macroscopic characteristics, cell morphology, Gram staining and biochemical tests.

Table 2: Macroscopic observation, Gram's Reaction, Cell morphology and Biochemical Characteristics of clinical isolate of *P. aeruginosa*

Isolate	Macroscopic observation of plate	Gram reaction	Cell morphology	Biochemical tests					
				Catalase	Oxidase	Indole	MR	VP	Motility

P. aeruginosa I	Large, smooth, greenish colonies with grape like smell on cetrimide agar	-	Rod	+	+	-	-	-	+
P. aeruginosa II	Large, smooth, greenish colonies with grape like smell on cetrimide agar	-	Rod	+	+	-	-	-	+

Key: += positive, -= negative

Table 3: Macroscopic observation, Gram's Reaction, Cell morphology and Biochemical Characteristics of clinical isolate of *S. aureus*

Isolate	Macroscopic observation on plate Gram	Gram reaction	Cell morphology	Biochemical test	
				Catalase	Coagulase

S. aureus I	Smooth, opaque, golden yellow and change mannitol salt agar from pink to yellow.	+	Cocci in clusters	+	+
S. aureus II	Smooth, opaque, golden yellow and change mannitol salt agar from pink to yellow	+	Cocci in clusters	+	+

Key: += positive, -= negative

Antibiotic Susceptibility Testing of Isolates

The antibiotic susceptibility of the clinical isolates of presumptively *Pseudomonas aeruginosa* and *Staphylococcus aureus* was determined using the Kirby–Bauer disc diffusion method according to CLSI 2023 guidelines. Ten antibiotics were tested at different disc potencies.

Table 4a: Antibiotic Susceptibility Profile of *Pseudomonas aeruginosa* isolates (Using Disc Diffusion, CLSI 2023)

Antibiotic (Disc, µg)	Isolate 1 (mm)	Interpretation	Isolate 2 (mm)	Interpretation
(LEV, 20)	0	R	20	S
(CF, 10)	0	R	30	S
(SP, 10)	0	R	34	S
(CPX, 30)	20	S	30	S
(AM, 30)	0	R	26	R

(AU, 10)	0	R	36	S
(CN, 30)	30	S	28	S
(PEF, 30)	0	R	30	S
(OFX, 10)	0	R	41	S
(AZ, 12)	15	R	29	S

Key: R = Resistant; S = Susceptible; LEV= Levofloxacin; CF= Cephalothin; SP=Sparfloxacin; CPX= Ciprofloxacin; AM=Amoxicillin; AU=Augmentin; CN=Gentamicin; PEF=Pefloxacin; OFX=Ofloxacin; AZ=Azithromycin.

For MRSA, cefoxitin (30 µg) was used as the marker antibiotic. The results showed inhibition zones of **15 mm (MRSA I)** and **20 mm (MRSA II)**, both confirming methicillin resistance.

Table 4b: Cefoxitin Susceptibility Test for MRSA Isolates

Isolate	**Antibiotic (Disc, µg)**	**Zone of Inhibition (mm)**	**CLSI Interpretation**
MRSA I	Cefoxitin (30)	15	R
MRSA II	Cefoxitin (30)	20	R

Key: R=Resistant;

Antibacterial Activity of Ethanolic and Aqueous Extracts of *Ziziphus mauritiana* Stem Bark

The antibacterial activity of the ethanolic and aqueous extracts was evaluated against MRSA and MDR *P. aeruginosa* isolates using agar well diffusion. The ethanolic extract demonstrated higher activity against MRSA compared to the aqueous extract, while both extracts showed no inhibitory effect on *P. aeruginosa*.

Table 6: Antibacterial Activity of Ethanolic and Aqueous Extracts of *Ziziphus mauritiana* stem bark against clinical bacterial isolates

Isolate	**Extract type**	**Plant Extract Concentration**					
		200mg/ml	100mg/ml	50mg/ml	25mgml	Antibiotic used as control (CPX)	Interpretation
MRSA I	Ethanolic	26	22	16	11	15	R
	Aqueous	24	16	14	0		
MRSA II	Ethanolic	23	15	12	10	10	R
	Aqueous	19	16	12	0		
P aeruginosa I	Ethanolic	NA	NA	NA	NA	20	S
	Aqueous	NA	NA	NA	NA		
P. aeruginosa II	Ethanolic	NA	NA	NA	NA	30	S
	Ethanolic	NA	NA	NA	NA		

Key: NA = No Activity

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC and MBC of the ethanolic and aqueous extracts were determined by broth dilution. For all four isolates (MDR *P. aeruginosa* and MRSA), the MIC was found to be **12.5 mg/ml**, while the MBC was **25 mg/ml**. This indicates that while the extracts inhibit bacterial growth at lower concentrations, complete bactericidal activity requires higher concentrations.

Table 7: Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of *Ziziphus mauritiana* Stem Bark Extracts against MDR-Clinical isolates of *P. aeruginosa and MRSA*

Isolate	**Extract**	**MIC (mg/ml)**	**MBC (mg/ml)**
MRSA I	Ethanolic/Aqueous	12.5	25
MRSA II	Ethanolic/Aqueous	12.5	25

P. aeruginosa I	Ethanolic/Aqueous	12.5	25
P. aeruginosa II	Ethanolic/Aqueous	12.5	25

This study evaluated the antibacterial efficacy of ethanolic and aqueous stem bark extracts of *Ziziphus mauritiana* against clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) and multidrug-resistant (MDR) *Pseudomonas aeruginosa*. Phytoconstituents analysis detected numerous bioactive compounds encompassing flavonoids, glycosides, saponins, alkaloids, phenols, tannins, and steroids, while anthraquinones were absent. These results are consistent with recent studies reporting similar Phytoconstituents profiles for *Z. mauritiana* extracts, confirming their richness in polyphenols and alkaloids (Zouine *et al.*, 2024). Such secondary metabolites are known to exert antimicrobial effects through mechanisms such as membrane disruption, inhibition of nucleic acid synthesis, enzyme inactivation, and metal ion chelation (Tariq, *et al.*, 2022).

The *P. aeruginosa* isolates exhibited classic Gram-negative features and multidrug resistance. Specifically, isolate 1 was resistant to levofloxacin, cefotaxime, sparfloxacin, amoxicillin, Augmentin, and pefloxacin, but remained susceptible to ciprofloxacin and gentamicin. Isolate 2 was largely susceptible to most antibiotics except amoxicillin. Similarly, the MRSA isolates were resistant to cefoxitin, confirming methicillin resistance. These findings align with global reports by the World Health Organization (2024) which highlight the growing threat of MDR *P. aeruginosa* and MRSA strains that compromise the efficacy of commonly used antibiotics.

The agar well diffusion assay demontrated that the ethanolic extract produced measurable inhibition zones against MRSA at higher concentrations, whereas the aqueous extract exhibited weaker activity. Neither extract inhibited *P. aeruginosa* in agar diffusion tests. This pattern is corroborates with a previous research that also demonstrates that Gram-positive bacteria are generally more susceptible to plant-derived antimicrobials than Gram-negative bacteria. The outer membrane of Gram-negative bacteria and efflux pumps act as barriers to the permeability of hydrophobic compounds (Khameneh, 2019; Oyeleke, 2021). Similar results were reported by Adegboyega (2022), who found that ethanolic extracts of Z. mauritiana significantly inhibited S. aureus but had minimal activity against *P. aeruginosa*. Earlier studies by Mainasara, & Yakubu, (2012); Oladunmoye, (2020). Also reported stronger antibacterial effects on *S. aureus* than on *P. aeruginosa*. Although the diffusion test did not detect growth inhibition of *P. aeruginosa*, the broth dilution assay revealed that both ethanolic and aqueous extracts demonstrated inhibitory (MIC = 12.5 mg/mL) and bactericidal (MBC = 25 mg/mL) activities against all isolates. A similar observation was reported by Larbenno & Mbah (2025) where *Z. mauritiana* extracts displayed significant antibacterial activity in broth assays despite weak agar diffusion results. This discrepancy may be attributed to the limited diffusion of some Phytoconstituents of this plant in the media (Balouiri & Ibnsouda, 2016). Therefore, the broth dilution method provided a more accurate representation of the antibacterial potential of the extracts. These high MIC and MBC values indicate that the crude extracts are less potent than conventional antibiotics, which typically act in the microgram range. Nevertheless, the inhibitory effects observed against MDR isolates are noteworthy. Shariati, & Hosseini, (2024) also concluded that crude plant extracts can exhibit considerable inhibitory activity against resistant pathogens, though higher concentrations may be required. These findings highlight the potential of *Z. mauritiana* as a source of natural antibacterial agent.

CONCLUSION

This study revealed that the stem bark extracts of *Ziziphus mauritiana* contain diverse phytochemical constituents, including alkaloids, flavonoids, tannins, saponins, phenolic compounds, terpenoids, and glycosides, all of which bear a great antibacterial compound. This vast phytochemical constituent affirms the plant's usage in traditional medicinal. Both *Staphylococcus aureus* and *Pseudomonas aeruginosa* were confirmed as Methicillin-resistant and multidrug-resistant respectively. This finding underscores the growing public health concern associated with antimicrobial resistance and highlights the urgent need for alternative antimicrobial agents capable of combating resistant pathogens.

Assessment of the antibacterial activity of the plant extracts revealed that the ethanolic stem bark extract exhibited greater antibacterial efficacy than the aqueous extract. Moderate zones of inhibition were observed against MRSA isolates, whereas no visible inhibition was produced against multidrug-resistant *P. aeruginosa* in agar diffusion assays. Nevertheless, broth dilution assays revealed both bacteriostatic and bactericidal activities of

the ethanolic extract against MRSA and MDR *P. aeruginosa*, with a minimum inhibitory concentration (MIC) of 12.5 mg/mL and a minimum bactericidal concentration (MBC) of 25 mg/l. These findings indicate that the extract possesses appreciable antibacterial activity, particularly against Gram-positive organisms, while the lower susceptibility of *P. aeruginosa* may be associated with the intrinsic resistance mechanisms characteristic of Gram-negative bacteria.

Conflict of interest

No conflict of interest declared

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