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ANTIBACTERIAL EFFICACY OF FROZEN AND UNFROZEN *Ziziphus mauritiana* LEAF EXTRACTS ON SELECTED CLINICAL BACTERIA

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ABSTRACT
Ziziphus mauritiana, commonly known as Magarya, is a widely recognized traditional medicinal plant believed to have antimicrobial properties. This study aimed to evaluate the efficacy of both frozen and unfrozen leaf extracts of Z. mauritiana against clinical bacterial isolates, including Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae. Fresh leaves were collected, dried, and subjected to extraction using ethanol and distilled water via the percolation technique. The resulting extracts were analyzed for phytochemical content and antimicrobial activity. Antimicrobial activity was assessed using minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) tests through broth microdilution. Phytochemical screening confirmed the presence of alkaloids, tannins, saponins, steroids, and flavonoids in both ethanolic and aqueous extracts. The extracts exhibited dose-dependent antibacterial activity, with zones of inhibition ranging from 0 to 15mm, indicating moderate to strong effects. Both freeze-dried and unfreeze-dried extracts showed substantial effectiveness, particularly at higher concentrations. Additionally, a toxicity assessment using healthy laboratory rats demonstrated that the extracts are safe at doses up to ≤5000 mg/kg, supporting their safe use in traditional medicine. The study confirms the long-standing traditional use of Z. mauritiana for treating infections and highlights its potential as a natural source of antimicrobial agents, warranting further investigation for pharmaceutical development.
Keywords: Antimicrobial activity, Herbal therapy, Phytochemical screening, Phytochemicals, Ziziphus mauritiana

INTRODUCTION

The plant kingdom has proven to be the most useful in the treatment of diseases and they provide important source of all the world's pharmaceuticals. Plants in all facets of life have served as valuable starting materials for drug development (Ajibesin, 2011). Phytochemicals are compounds that occur naturally in plants. They contribute to the colour, flavour and perfumery/scent/smell of plants. In addition, they form part of a plant's natural defense mechanism against diseases and competition. Their therapeutic values to human health and disease prevention have been reported (Okwu, 2004). The phytochemicals are grouped into two main categories namely primary and secondary constituents, according to their functions in plant metabolism. Primary constituents comprise amino acids, common sugars, proteins and chlorophyll. The secondary constituents consist of alkaloids, essential oils, flavonoids, terpenoids, tannins, saponins, and phenolic compounds. Terpenoids exhibit various important pharmacological activities such as anti-inflammatory, anti-cancer, anti- malarial, and inhibition of cholesterol synthesis, anti-viral and anti-bacterial activities (Krishnaiah *et al.*, 2009).

Ziziphus plants are traditionally used as medicine for the treatment of various diseases such as digestive disorders, urinary troubles, diabetes, skin infections, diarrhoea, fever, bronchitis, liver complaints, anaemia, etc (Goyal *et al.*, 2012; Mishra and Bhatia, 2014). *Ziziphus mauritiana* (Magarya in Hausa) consists of dried ripe fruits, leaf, roots and seeds. It is a medium-sized shrub that grows vigorously and has a rapidly developing taproot that is well adapted to drought conditions. The species varies widely in height,

from a bushy shrub of 1.5 to 2m tall, to a tree of 10 to 12m tall with a trunk diameter of about 30cm. *Z. mauritiana* may be erect or wide-spreading, with gracefully drooping thorny branches, zigzag branchlets, thornless or set with short, sharp straight or hooked spines (Folaranmi, 2009). The study aim to evaluate the antibacterial activity of both frozen and unfrozen leaf extracts of *Zizipus mauritiana* against selected clinical bacterial isolates.

MATERIALS AND METHODS

Plant Material

Fresh leaves of *Ziziphus mauritiana* (Magarya) were collected from a from Kuido and Labi areas in Shanono Local Government in Kano state. The leaves were authenticated at the Department of Plant Biology herbarium at Bayero University, Kano, Nigeria and issued voucher number (BUKHAN 0233). The leaves were thoroughly washed with distilled water to remove any debris and air-dried at room temperature. After drying, the leaves were ground into a fine powder using a pestle and mortar.

Extraction of Plant Material

Two extraction methods were employed: aqueous extraction and ethanol extraction.

A total of 200 grams of powdered *Ziziphus mauritiana* leaf material was used for both aqueous and ethanol extractions. For the aqueous extraction, the plant powder was mixed with 200 mL of distilled water in a 500 mL conical flask, covered with aluminum foil, and shaken at 30-minute intervals for 6 hours. The mixture was then left to stand at room temperature for 72 hours. After maceration, it was filtered using sterile muslin cloth, and the resulting filtrate was evaporated to dryness using a steam bath. The dried extract was weighed, transferred into a sterile universal sampling bottle, and stored at 4°C for further analysis, following the method of Yusha'u *et al.* (2010). Similarly, for the ethanol extraction, 200 grams of the powdered plant material was soaked in 200 mL of ethanol in a 500 mL conical flask. The mixture was shaken every 30 minutes and allowed to stand for 72 hours. It was then filtered, and the filtrate was evaporated to dryness. The dried ethanol extract was weighed, stored in a sterile universal sampling bottle, and refrigerated at 4°C, as described by Sofowora (2006).

The freeze-dried extracts were then stored in airtight containers at 4°C until use. In addition, fresh (unfrozen) extracts were prepared by using the same process, but the extracts were not subjected to freezing prior to testing.

Determination of Some Physical Properties and Phytochemical Composition of the Leaf Extract

The color, weight, odor and texture were assessed immediately after the removal of the solvents by evaporation process using water bath. The texture was felt manually using glass rod and feeling of the particulate nature of the resultant fraction between fingers as described by Yusha'u *et al.* (2010).

Both aqueous and ethanol extracts, both frozen and unfrozen, were screened for the presence of various phytochemicals, including Saponin, Flavonoid, Glycoside, carbohydrate, Alkaloid, Tannin, Terpenoid and Steroid was carried out using standard quality method as described by (Hamisu *et al.*, 2019).

Collection of Microbial Strains

Test isolates including of *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 10031), *Pseudomonas aeruginosa* (ATCC 27853) and *Staphylococcus aureus* (ATCC 29213) were obtained from Microbiology laboratory of Aminu Kano Teaching Hospital Kano, transported and processed in the Microbiology Department, Bayero University, Kano and subjected to further identification and reconfirmation tests. The isolates were maintained on freshly-prepared nutrient agar slants and kept in a refrigerator at 4ºC until required for use.

The media used was prepared according to the Manufacturer's instructions, where by Mannitol Salt agar (MSA), and MacConkey agar were used for the presumptive identification of the test organisms and confirmed by morphological characteristics (colony appearance), some of these presumptive characteristics include the size, shape, odor and consistency of the colonies). Gram staining, microscopy and biochemical reactions using biochemical tests such as Catalase, Oxidase, Gas production test, Sugar fermentation and motility test were also carried out Cheesbrough (2010).

Preparation of Different Concentration of Extract

To prepare varying concentrations of the plant extract (320 mg/mL, 160 mg/mL, 80 mg/mL, 40 mg/mL, and 20 mg/mL), a serial dilution method was employed. A stock solution of 320 mg/mL was first prepared by dissolving 1600 mg (1.6 g) of the plant extract in 5 mL of the appropriate solvent. This stock served as the starting point for all subsequent dilutions. From the 320 mg/mL stock, a two-fold serial dilution was performed. Specifically, 2.5 mL of the stock solution was mixed with 2.5 mL of solvent to

obtain a 160 mg/mL solution. This process was repeated sequentially: 2.5 mL of the 160 mg/mL solution was diluted with an equal volume of solvent to yield an 80 mg/mL solution; the same procedure was followed to produce 40 mg/mL and 20 mg/mL concentrations, respectively.

At each stage, the solutions were thoroughly mixed to ensure uniformity. A final volume of 5 mL was maintained for each dilution. All preparations were labelled accordingly and stored under appropriate conditions until use.

Antibacterial Activity Testing

The antibacterial activity of the extracts was evaluated using the agar well diffusion method. Nutrient agar plates were prepared and inoculated with each bacterial strain by spreading 100 µL of the bacterial suspension over the surface. Wells of 6 mm diameter were created in the agar, into which varying concentrations (320mg/mL, 160mg/mL, 80mg/mL, 40mg/mL, and 20mg/mL) of the aqueous and ethanol extracts were introduced. The plates were incubated at 37°C for 24 hours, and the diameters of the inhibition zones were measured in millimeters.

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

The MIC of the extracts was determined by the broth dilution method. Briefly, two-fold serial dilutions of each extract were prepared in nutrient broth, and 100 µL of the standardized bacterial suspension was added to each well. The plates were incubated at 37°C for 24 hours, and the lowest concentration of extract that prevented visible growth was recorded as the MIC.

The MBC was determined by subculturing the contents of the wells that showed no visible growth onto fresh nutrient agar plates. The MBC was the lowest concentration that resulted in no growth after incubation (Kawo *et al.,* 2011).

Toxicity Testing

To assess the safety of the *Ziziphus mauritiana* extracts, toxicity testing was performed using adult albino rats. The rats were divided into five groups, with each group receiving different doses of the extracts (20mg/mL, 40mg/mL, 80mg/mL, 160mg/mL, and 320mg/mL) via oral administration. The rats were observed for 14 days for any signs of toxicity, behavioral changes, or adverse effects. Body weight and overall health were monitored throughout the study period (Magashi *et al.*, 2019).

Statistical Analysis

The data generated in this study was analysed using the Statistical Package for Social Sciences (SPSS) for windows version 25.0 used for statistical analysis and data interpretation. All the presented in a simple descriptive statistics.

RESULTS

The results of aqueous and ethanol extraction experiments conducted on *Z. mauritiana* plant material are shown in Table 1. More of the higher extract (47.8g) was obtained from the aqueous extract compared to the ethanol extract (39.2%). The water-based extract showed a greater concentration (23.9%) than the ethanol-based extract (19.6%). The aqueous extract and ethanol extract had distinct color and texture characteristics, with the aqueous extract appearing greenish-black and the ethanol extract being reddish-brown, in both cases taking the form of a fine powder.

The aqueous extracts, as shown in Table 2, comprised flavonoids, tannins, and alkaloids but lacked saponins, steroids, and glycosides, with this chemical composition observed in both the freeze-dried and unfrozen extracts. The ethanol extracts were also discovered to comprise flavonoids, tannins, alkaloids, saponins and steroids with no glycosides present in both the frozen and unfrozen extracts (Table 2).

Higher concentrations of *Z. mauritiana* extracts generally lead to greater levels of inhibition for all bacterial strains, as shown in Table 3. Extracts from unfrozen ethanol tend to have the largest zone of inhibition values compared to all other extract types and concentrations. The freeze extracts typically exhibit smaller zones of inhibition compared to the unfreeze extracts. The various bacterial strains exhibit different degrees of sensitivity to the extracts. Larger zones of inhibition were seen in *E. coli* and *K. pneumoniae*, compared to *P. aeruginosa* and *S. aureus*.

Table 1: Physical Properties of *Zizipus mauritiana* Leaf Extracts

Solvent of extraction	Leaf extract weighed (g)	Extracts recovered (g)	(%) yield	Color/ texture
Distilled water	200	47.8	23.9	Greenish black
Ethanol	200	39.2	19.6	Reddish brown

Table 2: Phytochemical analysis of *Zizipus mauritiana* leaf extracts

Phytochemicals	Unfreeze Aqueous extracts	Freeze Aqueous extracts	Unfreeze Ethanol extracts	Freeze ethanol extracts
Flavonoids	+	+	+	+
Tannins	+	+	+	+
Alkaloids	+	+	+	+
Saponins	-	-	+	+
Steroids	-	-	+	+
Glycosides	-	-	-	-

Keys: + = Present - = Absent

Table 3: Antibacterial activity of *Z. mauritiana* leaf extracts against *E. coli, K. pneumoniae*, *P. aeruginosa* and *S. aureus*

Z. mauritiana Extract	Concentrations	*E. coli*	*K. penumoniae*	*P. aeruginosa*	*S. aureus*
UAE	320	12	13	9	13
	160	11	12	8	11
	80	7	8	6	9
	40	7	7	6	6
	20	0	6	0	4
FAE	320	9	12	9	13
	160	9	8	7	10
	80	6	7	4	6
	40	4	4	4	4
	20	0	4	0	0
UEE	320	14	14	11	15
	160	14	14	9	12
	80	11	11	8	10
	40	9	8	7	7
	20	6	6	4	4
FEE	320	12	13	9	12
	160	10	12	9	8
	80	7	7	7	8
	40	6	7	4	4
	20	6	6	4	4
Control Ciprofloxacin	5µg	15	21	14	17

Key: UAE: Unfreeze Aqueous Extracts, FAE: Freeze Aqueous Extracts, UEE: Unfreeze Ethanol Extracts, FEE: Freeze Ethanol Extracts

Table 4 lists the Minimum Inhibitory Concentration (MIC) values for aqueous and ethanol extracts of *Z. mauritiana* when tested against various bacterial isolates. The MIC values of the aqueous extracts were measured against *E. coli, K. pneumoniae*, and *P. aeruginosa*, which were found to be 320, 160, and 320mg/ml, respectively. The minimum inhibitory concentration (MIC) value against *S. aureus* was 160mg/ml. The aqueous extracts from the freeze showed MIC values that were similar to one another, with the exception of *S. aureus*, which exhibited a higher MIC value of 640.

The MIC values for the extracts of ethanol were 160, 160, 320, and 160mg/ml against *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and S. aureus,

respectively. MIC values for freeze ethanol extracts were similar across all bacterial isolates evaluated, with concentrations ranging from 320 to 640mg/ml. The statistically significant difference in MIC values between aqueous extracts that were not frozen and those that were frozen against *E. coli* is indicated by a *P-value* of 0.0000.

The minimum bactericidal concentrations of ethanol extracts against *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *S. aureus* were 1280, 640, 640, and 320mg/ml, respectively. The MBC values for the aqueous extracts were measured against *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *S. aureus* bacteria, with results of 2560, 2560, 1280, and 1280mg/ml, respectively. Aqueous extracts that were frozen displayed slightly decreased MBC values for *E. coli* and *S. aureus* at concentrations of 2500 and 640mg/ml, respectively. In contrast, the MBC values for *K. pneumoniae* and *P. aeruginosa* did not change. In terms of ethanol extracts, the MBC values against *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *S. aureus* were 1280, 640, 640, and 320mg/ml, respectively. The freeze ethanol extracts showed comparable Minimum Bactericidal Concentration (MBC) values for all bacterial isolates, which fell within the range of 640 to 1280mg/ml. The *P-value* of 0.0000 signifies a statistically significant disparity in MBC values between unfrozen aqueous extracts and frozen aqueous extracts, as well as between unfrozen ethanol extracts and frozen ethanol extracts, in comparison to *E. coli*.

Table 6 shows the reactions of the treated rats to different doses of the extracts of the *Z. mauritiana* leaf. None of the rats reacted negatively to the extract at any of the tested concentrations, ranging from 10mg/m to 5000mg/ml. This suggests that the administration of *Z. mauritiana* at these specified doses did not result in any observed adverse effects or behaviors in the test subjects. Thus could be safe and effective at these concentrations (≤5000mg/ml).

Table 4: Minimum Inhibitory Concentration (MIC) for Aqueous and ethanol extracts of *Z. mauritiana*

	MIC for Aqueous and ethanol extracts of *Z. mauritiana*(mg/ml)		***P-value***
Isolate	UAE	FAE	
E. coli	320	640	0.0000
K. pneumonia	160	640	
P. aeruginosa	320	640	
S. aureus	320	160	
	UEE	FEE	
E. coli	160	320	0.0000
K. pneumonia	160	320	
P. aeruginosa	320	320	
S. aureus	160	320	

Keys: UAE: Unfreeze Aqueous Extracts, FAE: Freeze Aqueous Extracts, UEE: Unfreeze Ethanol Extracts, FEE: Freeze Ethanol Extracts

Table 5: Minimum Bactericidal Concentration (MBC) for Aqueous and ethanol extracts of *Z. mauritiana*

	MBC for Aqueous and ethanol extracts of *Z. mauritiana* (mg/ml)		***P-value***
Isolate	UAE	FAE	
E. coli	2560	2500	0.0000
K. pneumoniae	2560	2560	
P. aeruginosa	1280	2560	
S. aureus	1280	640	
	UEE	FEE	
E. coli	1280	1280	0.0000
K. pneumoniae	640	1280	
P. aeruginosa	640	640	
S. aureus	320	640	

Keys: UAE: Unfreeze Aqueous Extracts, FAE: Freeze Aqueous Extracts, UEE: Unfreeze Ethanol Extracts, FEE: Freeze Ethanol Extracts

Table 6: Toxicity Test Reactions of the rats to different doses of *Z. mauritiana*

Behavioural/Anatomial features	***Z. mauritiana* Doses (mg/ml)**					
	10	100	1000	1600	2900	5000
Unfreeze Aqueous extracts						
Salivation	Nil	Nil	Nil	Nil	Nil	Nil
Stretching of the body	Nil	Nil	Nil	Nil	Nil	Nil
Weakness	Nil	Nil	Nil	Nil	Nil	Nil
Brushing of nose on the floor	Nil	Nil	Nil	Nil	Nil	Nil
Restlessness	Nil	Nil	Nil	Nil	Nil	Nil
Feeding habit	Nil	Nil	Nil	Nil	Nil	Nil
Piloerection of the Fur	Nil	Nil	Nil	Nil	Nil	Nil
Swelling and reddening of eyes	Nil	Nil	Nil	Nil	Nil	Nil
Coma	Nil	Nil	Nil	Nil	Nil	Nil
Death	Nil	Nil	Nil	Nil	Nil	Nil
Freeze Aqueous extracts						
Salivation	Nil	Nil	Nil	Nil	Nil	Nil
Stretching of the body	Nil	Nil	Nil	Nil	Nil	Nil
Weakness	Nil	Nil	Nil	Nil	Nil	Nil
Brushing of nose on the floor	Nil	Nil	Nil	Nil	Nil	Nil
Restlessness	Nil	Nil	Nil	Nil	Nil	Nil
Feeding habit	Nil	Nil	Nil	Nil	Nil	Nil
Piloerection of the Fur	Nil	Nil	Nil	Nil	Nil	Nil
Swelling and reddening of eyes	Nil	Nil	Nil	Nil	Nil	Nil
Coma	Nil	Nil	Nil	Nil	Nil	Nil
Death	Nil	Nil	Nil	Nil	Nil	Nil
Unfreeze Ethanol Extracts						
Salivation	Nil	Nil	Nil	Nil	Nil	Nil
Stretching of the body	Nil	Nil	Nil	Nil	Nil	Nil
Weakness	Nil	Nil	Nil	Nil	Nil	Nil
Brushing of nose on the floor	Nil	Nil	Nil	Nil	Nil	Nil
Restlessness	Nil	Nil	Nil	Nil	Nil	Nil
Feeding habit	Nil	Nil	Nil	Nil	Nil	Nil
Piloerection of the Fur	Nil	Nil	Nil	Nil	Nil	Nil
Swelling and reddening of eyes	Nil	Nil	Nil	Nil	Nil	Nil
Coma	Nil	Nil	Nil	Nil	Nil	Nil
Death	Nil	Nil	Nil	Nil	Nil	Nil
Freeze Ethanol Extracts						
Salivation	Nil	Nil	Nil	Nil	Nil	Nil
Stretching of the body	Nil	Nil	Nil	Nil	Nil	Nil
Weakness	Nil	Nil	Nil	Nil	Nil	Nil
Brushing of nose on the floor	Nil	Nil	Nil	Nil	Nil	Nil
Restlessness	Nil	Nil	Nil	Nil	Nil	Nil
Feeding habit	Nil	Nil	Nil	Nil	Nil	Nil
Piloerection of the Fur	Nil	Nil	Nil	Nil	Nil	Nil
Swelling and reddening of eyes	Nil	Nil	Nil	Nil	Nil	Nil
Coma	Nil	Nil	Nil	Nil	Nil	Nil
Death	Nil	Nil	Nil	Nil	Nil	Nil

DISCUSSION

In this study, both extracts (ethanolic and aqueous) were found to show the presence of alkaloids, tannins, and flavonoids, with saponins and steroids found only in the ethanolic extract. This confirms the phytomedicinal value of the leaves. The findings of the present study agreed with those of previous studies conducted by Yahaya *et al.* (2019), who reported that leaves of *Z. mauritiana* possess alkaloids, tannins, saponins, flavonoids, and glycosides. *Al* Ghasham *et al.* (2017) reported that leaves of *Z. mauritiana* possess alkaloids, flavonoids, tannins, saponins, and steroids. Kenneth *et al.* (2017) reported that the methanolic extract of *Z. mauritiana* leaves contains saponins, tannins, alkaloids, terpenoids, and flavonoids.

The results of the study show that *Z. mauritiana* extracts have antibacterial activity against *E. coli*, *K. pneumoniae, P. aeruginosa*, and *S. aureus*. The extracts categorized as unfreeze aqueous, freeze aqueous, unfreeze ethanol, and freeze ethanol exhibit varying degrees of effectiveness against all tested bacteria. The aqueous extracts exhibited an increased inhibition zone at higher concentrations, indicating a dose-dependent effect on bacterial growth. *S. aureus* was most sensitive to the extracts, with the largest inhibition zones. However, at the lowest concentration (20mg), there is little to no effect on *E. coli* or *P. aeruginosa*. The ethanolic extracts, both freeze and unfreeze, demonstrated strong antibacterial activity against all tested bacteria. *S. aureus* was the most susceptible, with the largest inhibition zones, and even at the lowest concentration (20mg), there was some antibacterial effect, particularly against *K. pneumoniae* and *S. aureus*. Some published studies have supported the results of this study; Priyanka *et al.* (2015) and Ali *et al.* (2015) reported that the *Z. mauritiana* leaves extract exhibits a significant level of antibacterial activity.

Minimum Inhibitory Concentration (MIC) values are crucial in determining the potency of an antimicrobial substance and can guide its use in potential therapeutic applications (Belanger and Hancock, 2021). In this study, MIC values have been determined for both the aqueous and ethanol extracts of *Z. mauritiana* against four selected bacterial isolates. Among the tested bacterial isolates, *S. aureus* appeared to be the most susceptible to these extracts, with lower MIC values indicating higher sensitivity. For some extracts, such as unfreeze ethanol extract (UEE) and freeze ethanol extract (FEE), the MIC values are generally lower than their aqueous counterparts, suggesting that ethanol extracts may have higher antibacterial potency. *K. pneumoniae* and *P. aeruginosa* exhibit similar MIC values for both extracts, indicating comparable sensitivity to these extracts.

The Minimum Bactericidal Concentration (MBC) is the lowest concentration of an antimicrobial agent that not only inhibits bacteria but also kills them (Parvekar *et al*., 2020). In the context of *Z. mauritiana* extracts, MBC values provide insights into the concentrations required to not only inhibit but also effectively eradicate the tested bacterial strains. *S. aureus* appears to be the most susceptible of the tested bacterial strains because it generally has lower MBC values across different extract types. Ethanol extracts (UEE and FEE) tend to have lower MBC values than their aqueous counterparts, suggesting that ethanol extracts may be more effective in killing bacteria. *K. pneumoniae* and *P. aeruginosa* exhibit varying MBC values across different extracts, indicating differences in their susceptibility to the extracts. MBC values provide valuable information about the bactericidal efficacy of *Z. mauritiana* extracts against the tested bacterial strains.

The toxicity of *Ziziphus mauritiana* leaf extract in this study represents a vital contribution to public health and safety, especially for users of medicinal plants in Nigeria and other regions. Medicinal plants, including *Z. mauritiana*, are used for traditional medicinal purposes. However, without a clear understanding of their safety profile, unintended fatalities or adverse health effects. By determining the toxicity of leaf extract, this study helps mitigate the risk of harm to users. This study provides evidence-based safety information on the *Z. mauritiana* leaf extract. Such information is crucial for healthcare practitioners, traditional healers, and individuals reliant on herbal treatments. It enables decision-makers to make informed decisions about the use of this plant in medical or therapeutic applications. The findings of this study are consistent with those of Yahaya *et al.* (2019).

CONCLUSION

The study revealed that *Ziziphus mauritiana* leaf extracts contain key bioactive compounds alkaloids, flavonoids, tannins, saponins, and steroids indicating notable medicinal potential. The extracts demonstrated strong antibacterial activity, particularly against *S. aureus*, with ethanol extracts showing greater efficacy. MIC and MBC results confirmed their bactericidal strength, while toxicity testing established the extracts as safe at concentrations up to ≤5000 mg/mL, supporting their potential use in traditional and alternative medicine.

Recommendations

Based on the findings of this comprehensive study on *Z. mauritiana* leaf extracts, the following recommendations are made

1. *Zizipus mauritiana* leaf extracts have demonstrated a rich phytochemical composition, including alkaloids, flavonoids, tannins, saponins, and steroids. Given the known biological activities of these phytochemicals, further research into the medicinal potential of *Z. mauritiana* should be encouraged. This could involve investigations into specific health benefits, such as antimicrobial, antioxidant, or anti-inflammatory effects.

2. The antibacterial effects of *Z. mauritiana* extracts should be further validated through clinical trials. This will provide more robust evidence of their efficacy and safety in human applications.
3. The study's determination of the toxicity profile of *Z. mauritiana* leaf extracts is a crucial step in ensuring safe use in traditional and alternative medicine. Further toxicological studies, including acute and chronic toxicity assessments, are necessary to establish a comprehensive safety profile.
4. Understanding the traditional uses of *Z. mauritiana* within local cultures can provide valuable insights into its historical and contemporary applications. Collaborative research involving ethnobotanists can help bridge the gap between traditional knowledge and scientific investigation.

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