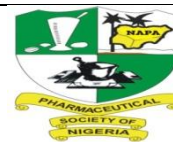


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# Comparative Antibacterial Efficacy of Aqueous and Ethanolic Extracts of *Acacia nilotica* Fruit against Strains of Methicillin-Resistant *Staphylococcus aureus* (MRSA)

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article.

### Abstract

**Background:** Methicillin-resistant *Staphylococcus aureus* (MRSA) remains a formidable public health threat, with the World Health Organization recognizing it as a high-priority pathogen demanding urgent global attention.

**Objectives:** This study investigates the antimicrobial activity of ethanol and aqueous extracts of *Acacia nilotica* fruit, a plant traditionally used in Northern Nigeria for treatment of infections.

**Methods:** The antimicrobial efficacy of the extracts was evaluated against clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) and reference strains using the agar well diffusion method on Mueller-Hinton agar. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values for susceptible strains were determined using the solid agar dilution method.

**Results:** Both extracts demonstrated concentration-dependent antimicrobial activity, with the ethanol extract exhibiting superior efficacy. All tested MRSA clinical isolates and the reference strain were susceptible to both ethanol and aqueous extracts, each producing appreciable zones of inhibition. The zones of inhibition for the ethanol extract ranged from 21.0 to 35.5 mm, while those for the aqueous extract ranged from 20.5 to 32.0 mm. MIC values ranged from 1–4 mg/mL for ethanol extracts and 2–4 mg/mL for aqueous extracts. MBC values ranged from 2–8 mg/mL for ethanol extracts and 4–8 mg/mL for aqueous extracts.

**Conclusion:** These findings suggest that *Acacia nilotica* fruit extracts, particularly the ethanol extract, possess significant antimicrobial potential and may serve as natural alternatives for managing MRSA infections. Further studies are warranted to isolate and characterize the active phytoconstituents, elucidate their mode of action, and evaluate their pharmaceutical potential *in vivo*.

**Keywords:** *Acacia nilotica*, ethanol and aqueous extracts, Antibacterial activity, Methicillin-resistant *Staphylococcus aureus* (MRSA).

### INTRODUCTION

The escalating burden of antimicrobial resistance (AMR) presents a profound challenge to global health, food security, and sustainable development (Salam *et al.*, 2023). Among the most notorious drug-resistant pathogens is *Staphylococcus aureus*, particularly methicillin-resistant *Staphylococcus aureus* (MRSA), which has emerged as a major cause of morbidity and mortality worldwide (Abebe

and Birhanu, 2023; Ezeobiora and Okore, 2020). The World Health Organization (WHO) has classified MRSA as a high-priority pathogen, emphasizing the need for accelerated research into novel antimicrobial agents. MRSA is responsible for a wide range of infections, including skin and soft tissue infections, endocarditis, osteomyelitis, and life-threatening sepsis (Abdullah *et al.*, 2022) Its ability to acquire and disseminate resistance genes

has rendered many conventional antibiotics ineffective, thereby complicating treatment strategies and increasing the risk of therapeutic failure. In response to the growing ineffectiveness of synthetic antibiotics, attention has increasingly turned toward natural products as potential sources of new antimicrobial compounds. Medicinal plants, in particular, have long been recognized for their therapeutic value, largely due to the diverse array of secondary metabolites they produce (Panda *et al.*, 2025) These bioactive compounds such as alkaloids, flavonoids, tannins, saponins, and terpenoids have demonstrated antimicrobial, antioxidant, anti-inflammatory, and immunomodulatory properties in numerous studies (Roy *et al.*, 2022). Traditional medicine systems across the world have harnessed plant-based remedies for centuries, underscoring the relevance of ethnopharmacological knowledge in contemporary drug discovery.

*Acacia nilotica* (Linn), commonly known as gum arabic tree or Egyptian thorn, is a multipurpose tree of the Fabaceae family distributed widely across Africa, Asia, and the Indian subcontinent. It has been extensively used in traditional medicine for the treatment of various infectious and inflammatory conditions, including gastrointestinal infections, respiratory tract infections, urinary tract infections, and wound healing (Hafez *et al.*, 2024). Several parts of the plant - bark, leaves, pods, and fruits are employed in herbal preparations. Phytochemical investigations have confirmed that *A. nilotica* contains a wide spectrum of secondary metabolites, notably polyphenols and condensed tannins, which have been implicated in its antimicrobial effects (Fraga-Corral *et al.*, 2021).

Despite the increasing interest in the antimicrobial potential of *A. nilotica*, limited comparative studies have investigated the influence of extraction solvents on its efficacy against multidrug-resistant pathogens such as MRSA. The polarity of the extraction solvent plays a significant role in determining the types and yields of phytochemicals extracted, which in turn affects antimicrobial activity (Wakeel *et al.*, 2019). Ethanol, a polar organic solvent, is known to extract a broad range of phenolic compounds, while aqueous extracts are typically limited to highly polar constituents (Kozhantayeva *et al.*, 2024). Comparing these two extraction methods offers insight into the optimal approach for harnessing the plant's therapeutic potential.

Given the urgent need for alternative antimicrobial agents and the underexplored potential of *Acacia nilotica* fruit extracts against drug-resistant bacteria, this study aims to comparatively evaluate the antimicrobial activity of aqueous and ethanolic fruit extracts of *A. nilotica* against clinical and reference strains of MRSA. The investigation employs the agar well diffusion method to assess antimicrobial

efficacy, as well as determination of minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) using standard microbiological techniques. By bridging traditional medicine and modern antimicrobial screening, this research contributes to the search for novel, plant-based interventions in the fight against antimicrobial resistance.

## MATERIALS AND METHODS

### Equipment, Solvents and Culture Media

Sterile Petri Dish (Pyrex England), Pipette (Pyrex England), Conical Flask (Pyrex, England), Beakers (PEC, India), Measuring Cylinder (PEC, India), Weighing Balance (Mettler Toledo, Switzerland), Autoclave (YX-280B3, Yongxin, China), Laboratory Incubator (Surgfriend Medical, England), Hot Air Oven (Ambassador, India), Electric Blender (Panasonic, Japan), Mueller Hinton Agar (MHA), Nutrient Agar (NA), Nutrient Broth (NB). All media used for the research work are of Oxoid brand.

## METHODS

### Test Organism

A total of eight (8) *Staphylococcus aureus* bacterial strains consisting of six (6) clinical strains of methicillin-resistant *staphylococcus aureus* and two (2) typed strains consisting of methicillin-resistant *Staphylococcus aureus* NCTC 12493 and methicillin-susceptible *Staphylococcus aureus* ATCC 29213). They were collected from the Microbiology Laboratory Unit of Medecins Sans Frontieres (MSF) at Gwange Primary Health Care Centre, Maiduguri, Borno state and were confirmed through standard identification techniques for MRSA (coagulase test and cefoxitin/oxacillin disk test).

### Collection and Identification of Plant Material

The fruits of *Acacia nilotica* were collected from an herb seller in Baga Road Market Maiduguri, Borno state, Nigeria (13°7'7.7"N 13°51'23.7"E) in April, 2024. The medicinal fruit was identified and authenticated by Dr Cletus Ukwubule of the Department of Pharmacognosy, Faculty of Pharmacy, University of Maiduguri, Nigeria with the Voucher number UMM/FPH/FAA/003.

### Preparation of Plant Material

The fruits of *Acacia nilotica* were air-dried in shade, away from sunlight, to avoid a change in the nature of plant constituents. After drying, the fruits were ground into fine powder using an electric blender (Panasonic, Japan). The powdered fruit was weighed using a weighing balance and stored in an airtight container for further use.

#### **Ethanol Extraction of Active Constituents from *Acacia nilotica* Fruit**

Two litres of ethanol were used as the extraction solvent. 500g of powdered fruits of *Acacia nilotica* was weighed and macerated in 600 mL of 99% ethanol for 72 hours, after which it was extracted successively with the remaining 1400 mL of the solvent according to the method previously reported (Stephen *et al.*, 2024).

#### **Aqueous Extraction of Active Constituents from *Acacia nilotica* Fruit**

Two litres of distilled water were used as the extraction solvent. 500g of powdered fruits of *Acacia nilotica* was weighed and macerated in 600 mL of distilled water for 72 hours, after which it was extracted successively with the remaining 1400 mL of the solvent as previously reported (Stephen *et al.*, 2024).

#### **Standardization of Inoculum**

The viscosity of culture of test organisms grown on Mueller Hinton agar and inoculated into a test tube containing normal saline was compared with 0.5 McFarland's standard to get a bacterial density equivalent to approximately  $1.0 \times 10^8$  CFU/mL (Hombach *et al.*, 2015).

#### **Antimicrobial Testing of Different Concentrations of Ethanol and Aqueous Plant Extracts**

Four different concentrations, 10 mg/mL, 8 mg/mL, 6 mg/mL, and 4mg/mL. of both ethanol and aqueous extracts were prepared. Antimicrobial susceptibility was determined using the agar-well diffusion method by culturing standardized test isolate on Mueller Hinton agar plates as previously reported (Hossain *et al.*, 2024). Vancomycin and sterile water were used as positive and negative controls, respectively. The plates were kept at 8 °C for 30 minutes before incubation for 24 hours at 37 °C. After incubation, a ruler was used to measure the zones of inhibition to the closest millimetre. The assay for both ethanol and aqueous extracts were performed in duplicates.

#### **Determination of the Minimal Inhibitory Concentration (MIC) and Minimum Bactericidal Concentrations (MBC) of the extracts**

Minimum Inhibitory Concentration (MIC) was determined using the solid dilution method on double-strength Mueller-Hinton agar. Briefly, to make a stock solution of 8 mg/mL, 4 mg/mL, 2 mg/mL, 1 mg/mL, 5 mL was taken from each extract stock solution and aseptically diluted with the same volume of sterile water in a petri dish. Then, 100 µL

of each of the standardized test bacterial isolates was added to the petri dishes. All the petri dish cultures, including the experimental controls, were incubated at 37 °C for 24 hours and their growth was monitored. The lowest concentration at which bacterial growth was inhibited was recorded as the extract's MIC (Kowalska-Krochmal and Dudek-Wicher, 2021).

By inoculating fresh Mueller Hinton agar plates with one loopful of culture from each cultured plate that did not exhibit growth, including the MIC plates, the MBC assay was carried out. The MBC value for each extract was determined using the lowest concentration of extract that killed the bacteria on the solid agar after the plates were incubated for 48 hours at 37 °C (Strompfova *et al.*, 2024).

#### **RESULTS**

The extraction yield for ethanol and aqueous extracts were 10.3% and 12% (Table 1). The antimicrobial susceptibility tests revealed that the six clinical isolates of Methicillin-resistant *Staphylococcus aureus* and the typed strains: methicillin-susceptible *Staphylococcus aureus* (MSSA ATCC 29213) and methicillin-resistant *Staphylococcus aureus* (MRSA NCTC 12493) were susceptible to the ethanol and aqueous extracts (Table 2). The antibacterial activity of the aqueous and ethanol extracts of *Acacia nilotica* on clinical isolates of MRSA and the typed strains demonstrated a concentration-dependent activity, with increasing zones of inhibition in mm at increased concentration. The zones of inhibition for ethanol and aqueous extracts at various concentration ranged from 21-35.5mm and 20-32mm respectively. All the clinical isolates including the typed strains showed appreciable zone of inhibition with vancomycin. However, the results further demonstrated that the ethanol extract has better activity as evidenced by larger zone of inhibition compared to the aqueous extract.

The MIC values against the tested MRSA pathogens range from 1.5-4 mg/mL for aqueous extracts and 1-2 mg/mL for ethanol extracts. The highest MIC was 4 mg/mL, which was observed with aqueous extract against MRSA 1 and 2 clinical isolates while the lowest MIC value was 1mg/mL observed with ethanol extract against MSSA ATCC 29213 (Figure 1). The minimal bactericidal concentration (MBC) against the organisms was computed following the MIC determination. The MBC values obtained varied from 2-6 mg/mL for aqueous extracts and 2-4 mg/mL for ethanol extracts (Figure 2).

Table 1: Percentage yield of ethanolic and aqueous extracts of *Acacia nilotica* fruit

Parameter	Ethanol extract	Aqueous extract
Weight of crude plant material used (g)	500	500
Volume of solvent used (mL)	2000	2000
Weight of extracts obtained (g)	60	52
Percentage yield (%)	10.3	12

Table 2: Antibacterial activity of ethanol extract of *Acacia nilotica* against clinical isolates of MRSA and typed strains.

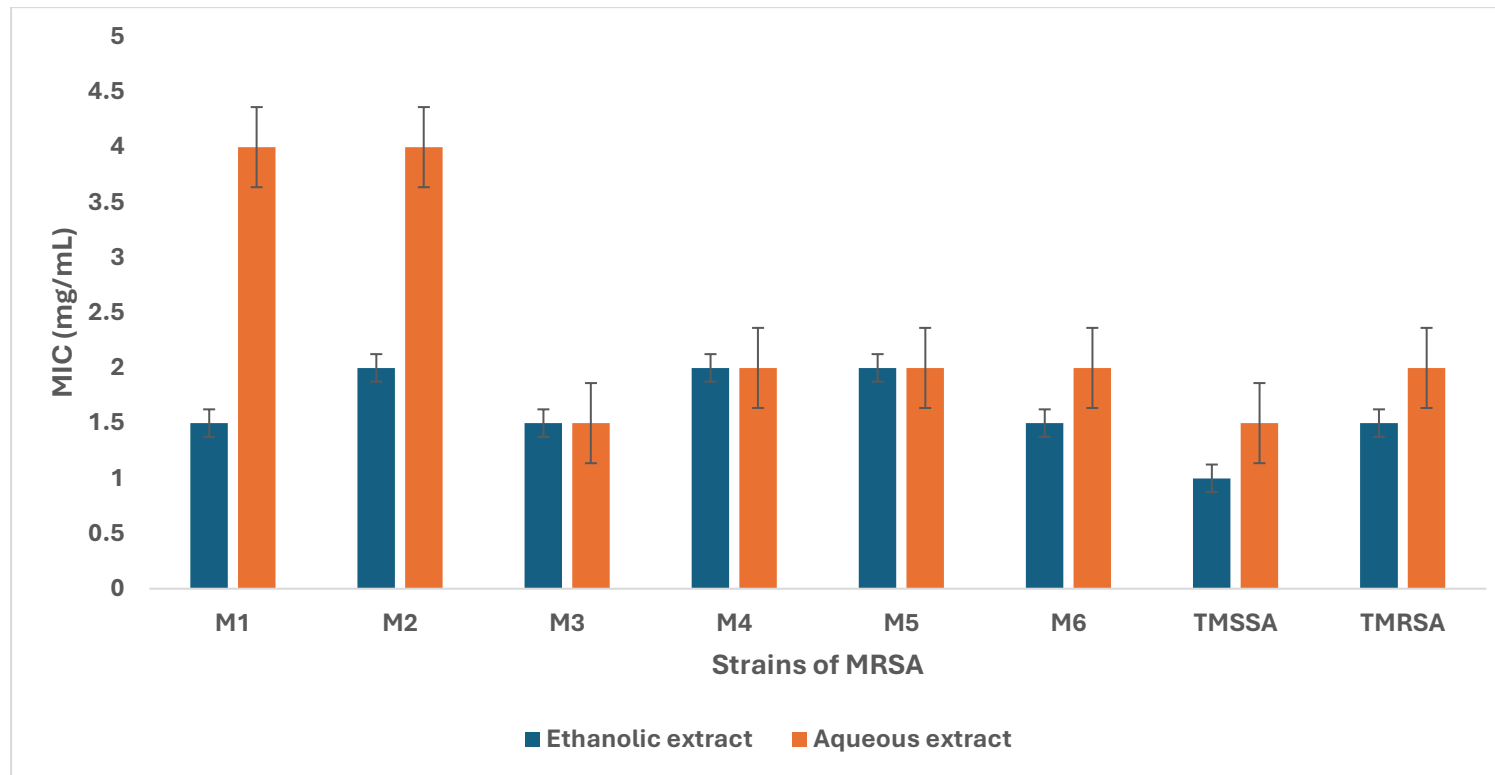
Plant extracts	Concentration (mg/ml)	Zone of inhibition in mm							
		M1	M2	M3	M4	M5	M6	TMS SA	TMR SA
Ethanol extract	10	29	27.5	25.5	25.5	30	27	35.5	32.5
	8	25.5	26	23.5	24.5	27	24.5	33	29.5
	6	25	25	21.5	22	26.5	24	30	27.5
	4	23	24	21	21	24	23	25	24.5
	Sterile water	-	-	-	-	-	-	-	-
Aqueous extract	10	23.5	29	29	25.5	26	26	30	32
	8	23	27.5	27.5	24	25	25	29.5	29
	6	22	25.5	25.5	22.5	24	24	25.5	28
	4	20.5	20	24	20	21	22.5	22	27
	Sterile water	-	-	-	-	-	-	-	-
Vancomycin (30 µg/mL)		26.5	28	30	26.5	32.5	27.5	33	30

Keys: M1-M6 – methicillin-resistant *Staphylococcus aureus* clinical isolate

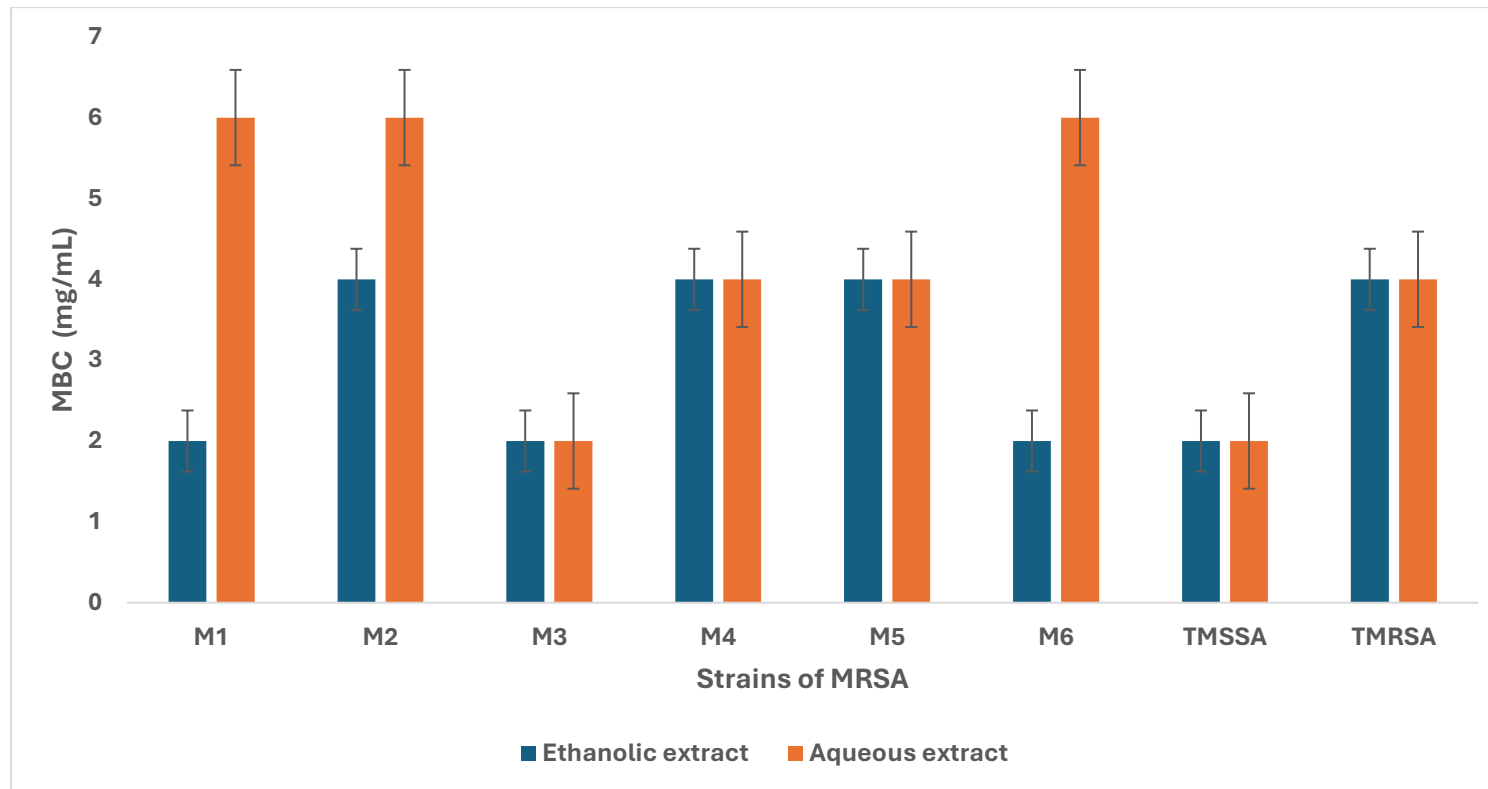
TMSSA- typed methicillin-susceptible *Staphylococcus aureus* ATCC29213

TMRSA- typed methicillin-resistant *Staphylococcus aureus* NCTC12493

(-): No inhibition



**Figure 1: Average MIC values for ethanolic and aqueous extracts of *Acacia nilotica* fruits against clinical and reference strains of MRSA.** M1-M6 represent clinical isolates of MRSA. TMSSA and TMRSA represent typed methicillin-sensitive and methicillin-resistant *Staphylococcus aureus*. MIC stands for minimum inhibitory concentration while MRSA stands for methicillin-resistant *Staphylococcus aureus*



**Figure 2: Average MBC values for ethanolic and aqueous extracts of *Acacia nilotica* fruits against clinical and reference strains of MRSA.** M1-M6 represent clinical isolates of MRSA. TMSSA and TMRSA represent typed methicillin-sensitive and methicillin-resistant *Staphylococcus aureus*. MBC stands for minimal bactericidal concentration, while MRSA stands for methicillin-resistant *Staphylococcus aureus*

## DISCUSSION

This study evaluated and compared the antibacterial efficacy of aqueous and ethanolic extracts of *Acacia nilotica* fruit against clinical and reference strains of methicillin-resistant *Staphylococcus aureus* (MRSA). The findings demonstrate that both extracts possess significant antibacterial activity, with the ethanolic extract exhibiting better activity, as evidenced by larger zones of inhibition, lower minimum inhibitory concentrations (MICs), and lower minimum bactericidal concentrations (MBCs) across all tested strains. These results are consistent with previous reports demonstrating the antibacterial activity of ethanolic fruit extracts of *A. nilotica* against a range of Gram-positive and Gram-negative pathogens (Fasogbon *et al.*, 2022; Sufi and Mustapha, 2020).

The better activity of the ethanolic extract may be attributed to the enhanced solubility and extraction efficiency of ethanol for a broader spectrum of phytochemicals, particularly moderately polar and non-polar bioactive compounds. Although phytochemical profiling was not conducted in this study, *A. nilotica* fruits are widely reported to contain tannins, flavonoids, alkaloids, saponins, and phenolic compounds, many of which possess established antimicrobial properties (Kumar *et al.*, 2022; Hernandez-Fuentes *et al.*, 2024). Qualitative phytochemical screenings from previous studies have consistently identified these compounds as major constituents, with tannins and flavonoids being particularly abundant and strongly associated with antibacterial activity (Hernandez-Fuentes *et al.*, 2024). These phytoconstituents are known to exert antimicrobial effects through protein precipitation, membrane disruption, enzyme inhibition, and metal ion chelation, thereby impairing essential bacterial functions. The higher efficacy observed in the ethanolic extract is therefore plausibly linked to its ability to extract higher concentrations and a wider diversity of these bioactive compounds compared to aqueous extraction (Asfaw *et al.*, 2025; Rehman *et al.*, 2014).

The sensitivity of both clinical and reference strains of MRSA to the extracts highlights the efficacy of *A. nilotica* and supports its potential use as a complementary or alternative therapeutic agent in managing MRSA infections. Notably, the observed activity against MRSA, an organism characterized by resistance mediated primarily through the *mecA* gene encoding altered penicillin-binding protein 2a (PBP2a)—suggests that the bioactive compounds in *A. nilotica* may act via mechanisms distinct from  $\beta$ -lactam antibiotics. Polyphenolic compounds such as flavonoids and tannins have been reported in *A. nilotica* and are known to interfere with bacterial cell wall synthesis, potentially through indirect inhibition or conformational disruption of PBPs, including PBP2a, thereby restoring susceptibility or

bypassing resistance mechanisms (Sadiq *et al.*, 2017; Vaou *et al.*, 2021). Additionally, these compounds may compromise membrane integrity, increase cell permeability, or disrupt quorum sensing and biofilm formation, all of which are critical for MRSA survival and pathogenicity. Such multi-target mechanisms may explain the comparable or, in some cases, superior activity of the extracts relative to conventional antibiotics such as vancomycin observed in this study.

While the precise mechanism of action of *A. nilotica* extracts was not experimentally elucidated in this study, the observed antibacterial effects likely result from a synergistic interplay of multiple phytochemicals acting on different cellular targets. This highlights the importance of further investigations employing bioassay-guided fractionation and molecular studies to isolate, characterize, and validate the specific compounds responsible for anti-MRSA activity, as well as to elucidate their mechanisms of action at the molecular level.

This study, however, has certain limitations. First, it is confined to in vitro assays, which may not fully replicate the complex interactions occurring in vivo. Secondly, the absence of phytochemical characterization limits the direct correlation of specific bioactive compounds with the observed antimicrobial effects. Future studies incorporating qualitative and quantitative phytochemical analyses would significantly strengthen the mechanistic interpretation of the findings. Moreover, potential cytotoxic effects on human cells were not evaluated, which is an essential consideration in drug development. Despite these limitations, our findings provide a strong scientific basis for the traditional use of *A. nilotica* in treating infectious diseases and reinforce its potential role in antimicrobial drug discovery, particularly against drug-resistant pathogens such as MRSA. Future work should focus on in vivo efficacy studies, toxicity profiling, detailed phytochemical characterization, and identification of lead compounds for further therapeutic development.

## CONCLUSION

This study has demonstrated that both aqueous and ethanolic fruit extracts of *Acacia nilotica* possess significant in vitro antimicrobial activity against clinical and reference strains of methicillin-resistant *Staphylococcus aureus* (MRSA). The ethanolic extract consistently exhibited better efficacy across all parameters assessed, indicating the presence of potent phytochemicals with potential therapeutic relevance.

These findings validate the traditional use of *A. nilotica* in managing infectious diseases and suggest its promising role in developing alternative treatments for multidrug-resistant pathogens,

particularly MRSA. However, further investigations are required to isolate and characterize the active compounds, evaluate their mechanisms of action, and assess their safety and efficacy in vivo.

In conclusion, *A. nilotica* fruit extracts represent a valuable resource in the ongoing search for novel antimicrobial agents and may contribute meaningfully to the global effort to combat antimicrobial resistance.

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