



Research Article

Molecular Detection of Methicillin-Resistant *Staphylococcus aureus* from Wound Infections and Its Susceptibility to Aqueous and Methanolic Extracts of *Hymenocardia acida* and *Khaya grandifoliola*

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a leading cause of antimicrobial-resistant wound infection worldwide, and the search for alternative antibacterial agents has drawn renewed attention to medicinal plants. This study evaluated the antibacterial activity of aqueous and methanolic extracts of *Hymenocardia acida* stem bark and *Khaya grandifoliola* leaf against PCR-confirmed MRSA isolated from wound infections among patients in Ilorin, Nigeria. *Staphylococcus aureus* was isolated and identified by standard microbiological methods from wound swabs (n=198) of patients attending selected hospitals in Ilorin. Methicillin resistance was screened phenotypically by the cefoxitin disc diffusion method and confirmed genotypically by PCR amplification of species-specific *nuc* gene and the resistance-associated *mec* gene. Antibacterial activity of the plant extracts was assessed against the MRSA isolates using the agar well diffusion method, with vancomycin (30 µg) as positive control. Of 31 *Staphylococcus aureus* isolates recovered, 23 (74.2%) were phenotypically methicillin-resistant, of which 10 (43.5%) were confirmed genotypically as MRSA (*nuc*+/*mec*+). The aqueous extract of *Khaya grandifoliola* produced the widest mean zone of inhibition (18.6±1.5 mm), exceeding the vancomycin control (16 mm), while its methanolic extract produced a mean zone of 15.9±1.7 mm. The aqueous extract of *Hymenocardia acida* showed no inhibitory activity against any isolate, whereas its methanolic extract produced a mean zone of 11.6±1.1 mm. These findings demonstrate that PCR-confirmed MRSA wound isolates were more susceptible to extracts of *Khaya grandifoliola* than to *Hymenocardia acida*, and support further investigation of *Khaya grandifoliola* as a potential source of plant-derived antibacterial agents against MRSA.

Keywords: *Hymenocardia acida*; *Khaya grandifoliola*; *mec* gene; Methicillin-Resistant *Staphylococcus aureus*; *nuc* gene; Wound infection

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INTRODUCTION

Wound infections remain a major cause of morbidity, prolonged hospitalisation, and healthcare cost worldwide, and are frequently complicated by bacterial colonisation with organisms such as

Staphylococcus aureus (Zewdu *et al.*, 2025). *Staphylococcus aureus* is among the most common pathogens recovered from wound infections, and strains that have acquired resistance to methicillin and other β-lactam antibiotics, collectively termed

methicillin-resistant *Staphylococcus aureus* (MRSA). They are associated with worse clinical outcomes, longer hospital stay, and higher treatment cost than infections caused by methicillin-susceptible strains (Turner *et al.*, 2019). Methicillin resistance in *Staphylococcus aureus* arises principally from acquisition of the *mecA* gene, carried on a mobile genetic element known as the staphylococcal cassette chromosome *mec* (SCC*mec*), which encodes an altered penicillin-binding protein with reduced affinity for β -lactam antibiotics (Lade & Kim, 2023).

The burden of MRSA is particularly pronounced in low- and middle-income countries, including Nigeria, where a systematic review reported MRSA prevalence rising from 18.3% to 42.3% among *Staphylococcus aureus* isolates across hospital-based studies conducted between 2007 and 2017 (Abubakar & Sulaiman, 2018). Locally, an early molecular epidemiological study in Ilorin reported a substantial proportion of *Staphylococcus aureus* isolates from the city to be methicillin-resistant (Taiwo *et al.*, 2005), while more recent Nigerian studies have continued to apply polymerase chain reaction (PCR)-based methods to confirm methicillin resistance genotypically rather than relying on phenotypic testing alone: Bala *et al.* (2026), for example, used PCR-based SCC*mec* typing to characterise MRSA isolates from poultry and poultry farm workers in Kano, while Isa *et al.* (2025) applied PCR to detect antibiotic-resistance genes in *Staphylococcus aureus* isolates from patients with diabetic foot infections in Katsina State, reporting discordance between phenotypic susceptibility results and genotypic resistance-gene carriage. Because phenotypic methods such as the cefoxitin disc diffusion test can both over- and under-classify methicillin resistance relative to the underlying resistance genotype, PCR amplification of the species-confirmatory *nuc* gene and the resistance-associated *mec* gene, first described by Brakstad *et al.* (1992) and Murakami *et al.* (1991) respectively, remains the reference standard for definitive MRSA confirmation (Muawiyya *et al.*, 2025).

The global rise of antimicrobial resistance, including MRSA, has renewed interest in medicinal plants as potential sources of alternative or complementary antibacterial agents, particularly in settings where access to newer antibiotics is limited (Naghavi *et al.*, 2024). Plant-derived phytochemicals such as tannins, flavonoids, alkaloids, and saponins have been associated with antibacterial activity against a range of pathogens, including MRSA, through mechanisms that include disruption of bacterial cell membranes

and inhibition of efflux pumps (Okwu *et al.*, 2019; Abdallah *et al.*, 2023). Similarly, Ewansiha *et al.* (2024) demonstrated antibacterial activity of a plant leaf extract against *Staphylococcus aureus* and other isolates, supporting continued local evaluation of plant extracts against clinically important bacteria. Locally in Ilorin, Baba *et al.* (2025) reported that aqueous and methanolic extracts of *Senna alata* and *Euphorbia hirta* produced substantial zones of inhibition against clinical isolates of *Staphylococcus aureus*, with synergistic effects observed when the extracts were combined with conventional antibiotics. *Hymenocardia acida* (Euphorbiaceae), locally known as “Erupa” among the Yorubas, and *Khaya grandifoliola* (Meliaceae), the African mahogany known locally as “Ewe Oda”, are both used in Nigerian traditional medicine. Stem bark extract of *Khaya grandifoliola* has previously been shown to possess antibacterial activity against *Staphylococcus aureus* and other pathogens, attributed to its tannin, alkaloid, saponin, and flavonoid content (Quartey *et al.*, 2020).

Despite this evidence, the antibacterial activity of *Hymenocardia acida* stem bark and *Khaya grandifoliola* leaf extracts specifically against PCR-confirmed MRSA isolated from wound infections in Ilorin has not been previously reported, and many local antibacterial-activity studies continue to rely on phenotypically-characterised MRSA without genotypic confirmation. This study therefore isolated and phenotypically screened *Staphylococcus aureus* from wound swabs in Ilorin, confirmed methicillin resistance genotypically by PCR amplification of the *nuc* and *mec* genes, and evaluated the antibacterial activity of methanol and aqueous extracts of *Hymenocardia acida* stem bark and *Khaya grandifoliola* leaf powder against the PCR-confirmed MRSA isolates.

MATERIALS AND METHODS

Study Design and Area

This was a hospital-based, laboratory cross-sectional study conducted in Ilorin, Kwara State, Nigeria, at three purposively-selected hospitals representing the three metropolitan Local Government Areas of the city: Ajikobi Cottage Hospital (Ilorin West), Primary Health Centre (PHC) Okelele (Ilorin East), and Sobi Specialist Hospital (Ilorin South).

Collection of Samples and Isolation of *Staphylococcus aureus*

Wound swab samples were collected from 198 patients with clinically-evident wound infections attending the three selected hospitals, using sterile

cotton swabs after the wound surface had been cleaned with sterile normal saline to remove surface debris. Each swab was inoculated onto Mannitol Salt agar and Blood agar, and incubated aerobically at 37°C for 24 hours. Presumptive *Staphylococcus aureus* colonies were identified by colony morphology, mannitol fermentation, Gram staining, catalase testing, and coagulase testing, and confirmed isolates were preserved on Nutrient agar slants for further analysis.

Phenotypic Detection of Methicillin Resistance

Methicillin resistance was screened phenotypically using the cefoxitin disc diffusion method in accordance with Clinical and Laboratory Standards Institute guidelines (CLSI, 2023). A standardised bacterial suspension of each isolate, adjusted to 0.5 McFarland turbidity, was inoculated onto Mueller-Hinton agar, and a 30 µg cefoxitin disc was placed on the inoculated plate. Following incubation at 37°C for 24 hours, isolates with a zone of inhibition below the CLSI breakpoint (≤ 21 mm) were classified as phenotypically methicillin-resistant.

Molecular Detection of Methicillin-Resistant *Staphylococcus aureus*

Genomic DNA was extracted from phenotypic methicillin-resistant isolates using a crude boiling method. Bacterial cells were harvested from agar plates, or by centrifuging 1 mL of broth culture at 6,000-8,000 rpm for 5 minutes, and the resulting pellet was resuspended in 500 µL of sterile distilled water and washed three times by centrifugation at 10,000 rpm. The washed suspension was subjected to heat shock at 95-100°C for 7 minutes in a dry bath to lyse the bacterial cells and release DNA, followed immediately by cold shock in an ice bath for 2 minutes. Cell debris was pelleted by centrifugation at 10,000 rpm for 1-2 minutes, and the supernatant containing the crude DNA template was carefully transferred to a new sterile Eppendorf tube and used immediately for PCR or stored at -20°C if there was any delay.

The PCR was performed to molecularly confirm the presence of the species-specific *nuc* gene (Brakstad *et al.*, 1992). Each 20 µL reaction mixture contained 4 µL of PCR master mix, 0.5 µL each of forward and reverse primers, 5 µL of template DNA, and nuclease-free water to a final volume of 20 µL. Amplification was carried out with an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 1 minute, annealing at 55°C for 1 minute, and extension at 72°C for 2 minutes, with a final extension at 72°C for 10 minutes.

The resistance-associated *mec* gene was detected by PCR under similar reaction conditions (Murakami *et al.*, 1991). Each 20 µL reaction contained 4 µL of PCR master mix, 0.5 µL each of forward and reverse primers, 5 µL of template DNA, and nuclease-free water to the final volume. Amplification consisted of an initial denaturation at 95°C for 5 minutes, followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 55.5°C for 1 minute, and extension at 72°C for 2 minutes, with a final extension at 72°C for 5 minutes. Amplified products were subjected to electrophoresis on agarose gel and visualised under ultraviolet trans-illumination alongside a molecular weight marker; isolates positive for both the *nuc* and *mec* genes were classified as genotypically confirmed MRSA.

Collection and Identification of Plant Materials

Stem bark of *Hymenocardia acida* and immature leaves of *Khaya grandifoliola* were collected within Ilorin, Kwara State, air-dried, and reduced to powder.

Preparation of Plant Extracts

Plant extracts were prepared using a crude solvent extraction method. For each plant material, 200 g of powder was macerated separately in 1 litre of distilled water and 1 litre of methanol. The aqueous and methanolic mixtures were left to stand, filtered, and concentrated to obtain the respective extracts, and the percentage yield of each extract was calculated as the weight of extract obtained divided by the weight of plant powder used, multiplied by 100.

Antibacterial Susceptibility Testing

The antibacterial activity of the aqueous and methanolic extracts of *Hymenocardia acida* and *Khaya grandifoliola* against the 10 PCR-confirmed MRSA isolates was evaluated using the agar well diffusion method. Mueller-Hinton agar plates were inoculated with standardised suspensions of each MRSA isolate, wells were bored into the inoculated agar, and a fixed volume of 10 µL of each extract was introduced into the corresponding well. Plates were allowed to stand for diffusion before incubation at 37°C for 24 hours, after which zones of inhibition were measured in millimetres using a transparent ruler. Vancomycin (30 µg) served as the positive control antibiotic.

Ethical Considerations

Ethical approval for this study was obtained from the Kwara State Ministry of Health Ethical Research Committee (ERC/MOH/2026/06/780). Informed consent was obtained from each patient prior to collection of wound swab, and participant

confidentiality was maintained throughout using coded identifiers.

Statistical Analysis

Data were summarised using descriptive statistics, including frequencies and percentages for isolation and confirmation outcomes, and percentages and mean $\pm$ standard deviation for extraction yield and zone-of-inhibition data, respectively.

RESULTS

Of the 198 wound swabs screened, 31 (15.7%) yielded *Staphylococcus aureus* on culture (Table 1). These isolates were confirmed as *Staphylococcus aureus* based on colony morphology, mannitol fermentation, Gram staining, and positive catalase and coagulase reactions, consistent with the identification criteria described above. Of these 31 isolates, 23 (74.2%) were phenotypically methicillin-resistant by the ceftioxin disc diffusion method. Following PCR amplification of the *nuc* and *mec* genes, only 10 (43.5%) of the 23 phenotypically resistant isolates were positive for both genes and were therefore classified as genotypically-confirmed MRSA (*nuc*+/*mec*+), corresponding to 5.1% of the total wound swabs screened; these 10 PCR-confirmed isolates (designated RMRSA1 to RMRSA10) were used for the subsequent antibacterial susceptibility testing.

The percentage yields obtained from aqueous and methanolic extraction of the two plant materials are shown in Table 2. For *Hymenocardia acida* stem bark, aqueous extraction produced a lower yield (1.86%) than methanolic extraction (4.20%). Similarly, for *Khaya grandifoliola* leaves, aqueous extraction produced a lower yield (7.00%) than methanolic extraction (8.40%), and *Khaya grandifoliola* produced a higher extraction yield than *Hymenocardia acida* with both solvents.

The antibacterial activity of the four plant extracts against the 10 PCR-confirmed MRSA isolates is presented in Table 3. The aqueous extract of *Hymenocardia acida* did not produce a measurable zone of inhibition against any of the 10 isolates and was therefore considered inactive. The methanol extract of *Hymenocardia acida* produced a mean zone of inhibition of 11.6 ± 1.1 mm (range 10-13 mm). Both extracts of *Khaya grandifoliola* produced larger inhibition zones: the aqueous extract produced the largest mean zone of inhibition overall, at 18.6 ± 1.5 mm (range 17-22 mm), exceeding the vancomycin control against every isolate tested, while the methanol extract produced a mean zone of 15.9 ± 1.7 mm (range 13-18 mm), comparable to the vancomycin positive control (16 mm).

Table 1: Occurrence and Molecular Confirmation of Methicillin-Resistant *Staphylococcus aureus* from Wound Swabs

Parameter	Frequency, n (%)
Wound swabs screened	198 (100.0)
<i>Staphylococcus aureus</i> isolated	31 (15.7)
Phenotypic MRSA (ceftioxin-resistant)	23 (74.2)
PCR-confirmed MRSA (<i>nuc</i> +/ <i>mec</i> +))	10 (43.5)

Note. MRSA = methicillin-resistant *Staphylococcus aureus*. Percentages are of the immediately preceding stage: *Staphylococcus aureus* isolated (of 198 wound swabs screened), phenotypic MRSA (of 31 *Staphylococcus aureus* isolates), and PCR-confirmed MRSA (of 23 phenotypic MRSA isolates). PCR-confirmed MRSA also corresponds to 32.3% of all *Staphylococcus aureus* isolates and 5.1% of all wound swabs screened.

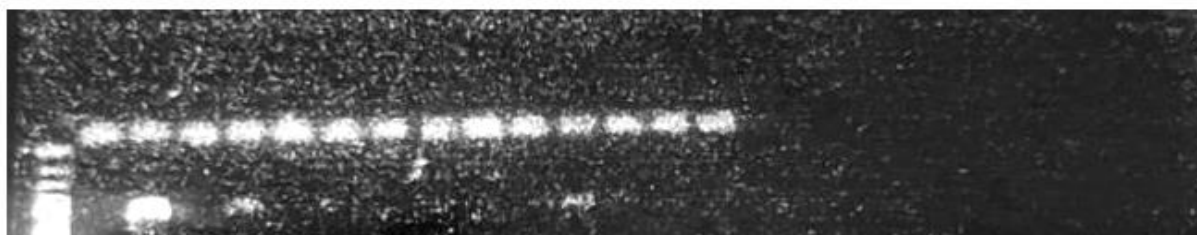


Figure 1: Agarose gel electrophoresis image of the PCR products obtained during molecular confirmation of the methicillin-resistant *Staphylococcus aureus* (MRSA) wound isolates

Table 2: Percentage Yield of Aqueous and Methanol Extracts of *Hymenocardia acida* Stem Bark and *Khaya grandifoliola* Leaf

Plant Material	Solvent	Weight of Extract (g)	Yield (%)
<i>Hymenocardia acida</i> stem bark	Aqueous	3.72	1.86
<i>Hymenocardia acida</i> stem bark	Methanol	8.40	4.20
<i>Khaya grandifoliola</i> immature leaves	Aqueous	14.00	7.00
<i>Khaya grandifoliola</i> immature leaves	Methanol	16.80	8.40

Table 3: Zone of Inhibition (mm) produced by *Hymenocardia acida* and *Khaya grandifoliola* Extracts Against PCR-Confirmed MRSA Wound Isolates

Isolate	<i>H. acida</i> Methanol	<i>H. acida</i> Aqueous	<i>K. grandifoliola</i> Methanol	<i>K. grandifoliola</i> Aqueous
RMRSA1	12	R	13	22
RMRSA2	10	R	14	17
RMRSA3	10	R	16	18
RMRSA4	11	R	17	18
RMRSA5	12	R	18	19
RMRSA6	13	R	17	18
RMRSA7	12	R	15	17
RMRSA8	13	R	18	20
RMRSA9	12	R	16	18
RMRSA10	11	R	15	19
Mean $\pm$ SD	11.6 $\pm$ 1.1	R	15.9 $\pm$ 1.7	18.6 $\pm$ 1.5
Vancomycin (30 μ g) control	16	16	16	16

Key: R = resistant / no measurable zone of inhibition. Vancomycin (30 μ g) positive control produced a 16 mm zone against every isolate tested

DISCUSSION

The recovery of *Staphylococcus aureus* from 15.7% of wound swabs, of which 74.2% were phenotypically methicillin-resistant, is consistent with reports that MRSA constitutes a substantial proportion of *Staphylococcus aureus* isolated from wound infections in Nigeria (Abubakar & Sulaiman, 2018), and extends earlier local evidence of methicillin resistance among *Staphylococcus aureus* isolates in Ilorin (Taiwo *et al.*, 2005) using contemporary genotypic confirmation.

Only 10 of the 23 phenotypic methicillin-resistant isolates (43.5%) were confirmed genotypically positive for both the *nuc* and *mec* genes by PCR. This discordance between phenotypic and genotypic methicillin-resistance testing has been similarly reported in other Nigerian settings: Isa *et al.* (2025) reported discordance between phenotypic susceptibility results and genotypic resistance-gene carriage among *Staphylococcus aureus* isolates from patients with diabetic foot infections in Katsina State, while Bala *et al.* (2026) likewise relied on PCR-based typing rather than phenotypic testing alone to characterise MRSA isolates in Kano. Cefoxitin disc diffusion can be influenced by inoculum size,

incubation temperature, and the expression of borderline or heterogeneous resistance phenotypes, which may explain why not all phenotypically resistant isolates in this study carried a detectable *mec* gene; this finding reinforces the value of PCR-based confirmation, such as that applied in the present study and in related investigations of *Staphylococcus aureus* virulence and resistance genes (Muawiyya *et al.*, 2025), for accurate MRSA classification rather than reliance on phenotypic testing alone.

Both extracts of *Khaya grandifoliola* showed greater antibacterial activity against the PCR-confirmed MRSA isolates than either extract of *Hymenocardia acida*, with the aqueous extract of *Khaya grandifoliola* exceeding the vancomycin control across all 10 isolates tested. This finding is consistent with Quartey *et al.* (2020), who reported dose-dependent antibacterial activity of a *Khaya grandifoliola* stem bark extract against *Staphylococcus aureus* and other pathogens, attributed to its tannin, alkaloid, saponin, and flavonoid content; the present findings extend this evidence to the leaf extract of the same species and to PCR-confirmed MRSA specifically. The broader

antibacterial potential of medicinal plants against MRSA, including through the phytochemical classes identified in *Khaya grandifoliola*, has been reviewed by Okwu *et al.* (2019) and Abdallah *et al.* (2023), and is consistent with related evidence where Ewansiha *et al.* (2024) similarly demonstrated antibacterial activity of a plant leaf extract against *Staphylococcus aureus*. Comparable activity against clinical *Staphylococcus aureus* has also been reported locally in Ilorin, where a methanol extract of *Sennaalata* produced a mean inhibition zone of similar magnitude to that observed for *Khaya grandifoliola* in the present study (Baba *et al.*, 2025). In contrast, the comparatively weaker activity of *Hymenocardia acida*, and the complete lack of activity of its aqueous extract, suggests that the antibacterial phytochemicals present in this plant's stem bark may be more effectively recovered by non-polar or semi-polar solvents such as methanol than by water alone, an observation that warrants further phytochemical and solvent-optimisation studies.

These findings are relevant against the backdrop of the substantial and rising global burden of bacterial antimicrobial resistance, including MRSA (Naghavi *et al.*, 2024), which continues to drive interest in plant-derived antibacterial alternatives, particularly in resource-limited settings such as Ilorin. Several limitations should be noted. This study used a convenience sample of wound swabs from three hospitals in a single city, which may not be representative of the broader MRSA burden in Ilorin or Kwara State. Antibacterial activity was assessed by zone of inhibition alone; minimum inhibitory and minimum bactericidal concentrations were not determined in this analysis and would help to further quantify the relative potency of the extracts. Phytochemical screening of the extracts was also not performed as part of this analysis, so the specific compounds responsible for the observed antibacterial activity could not be identified. Finally, gel images obtained during PCR confirmation were not of sufficient quality for inclusion in this manuscript; band presence was instead scored directly from the electrophoresis system at the time of analysis.

CONCLUSION

This study demonstrated that methanol and aqueous extracts of *Khaya grandifoliola* leaves possess appreciable antibacterial activity against PCR-confirmed MRSA wound isolates in Ilorin, with the aqueous extract exceeding the activity of a standard vancomycin control, while extracts of *Hymenocardia*

acida stem bark showed comparatively weaker or no activity. The study also demonstrated that a substantial proportion of phenotypically methicillin-resistant *Staphylococcus aureus* isolates did not carry both the *nuc* and *mec* genes on PCR confirmation, which points to the importance of molecular confirmation for accurate MRSA classification in this setting. These findings support further investigation of *Khaya grandifoliola* as a potential source of plant-derived antibacterial agents against MRSA, including phytochemical characterisation and determination of minimum inhibitory and bactericidal concentrations.

Author Contributions: Salaudeen Rasaq Adeniyi conceived the study, collected wound swab samples, performed the microbiological and molecular laboratory analyses. AbdulrazaqNuhu supervised the study and its design. Baba Rasheedat Toyosi, Abdullahi Jamui, and Adeyemo Toheeb Adeleke contributed to the laboratory analysis. SeidatOmolabake Hassan drafted, reviewed and edited the manuscript. All authors read and approved the final manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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Data Availability: The dataset analysed during the current study is available from the corresponding author on reasonable request.

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