

Original Research Article

***Terminalia avicennioides* root bark extract: A promising phytotherapeutic agent against methicillin-resistant *Staphylococcus aureus* hospital strains**

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Abstract

Purpose: To investigate the antibacterial activity of a partially purified ethyl acetate fraction obtained from methanol extract of the root bark of *Terminalia avicennioides* (Family: Combretaceae) against a hospital strain of methicillin-resistant *Staphylococcus aureus* (MRSA).

Methods: Gas chromatography-mass spectrometry (GC-MS) was used to identify and characterize compounds in the bioactive fraction of *T. avicennioides* root bark, while Fourier Transform Infrared (FTIR) spectroscopy was used to elucidate the functional groups. The MRSA hospital strain was isolated from a clinical sample and the isolate biochemically characterized by colonial morphology, Gram staining, and strain reaction. Resistance to methicillin/oxacillin was confirmed by repeat susceptibility testing using oxacillin. The antibacterial efficacy of the fraction was compared with those of standard antibiotics including meropenem, amoxiclav, nitrofurantoin, ceftriaxone, nidof, and ciprofloxacin. Antimicrobial activity was evaluated using agar well diffusion method, while the minimum inhibitory concentration (MIC) was determined using a 10-fold serial broth dilution assay.

Results: Mass spectrometry analysis revealed that the ethyl acetate fraction with anti-MRSA activity comprised a mixture of seven compounds in varying proportions, viz: *n*-hexadecanoic acid, 11-octadecenoic acid, oleic acid, octadecanoic acid, 3,11-tetradecadien-1-ol, 9-octadecenal, and (9Z)-9-tetradecenal. The FTIR spectra indicated the presence of alkyl halides. At 100 µg/mL, the ethyl acetate fraction exhibited a bacteriostatic effect, with a minimum bactericidal concentration (MBC) of 9.48 ± 0.90 µg/mL. Based on Kirby-Bauer standard, it demonstrated bactericidal activity (MBC) at 150 µg/mL, with mean inhibition zone diameter of 14 mm.

Conclusion: The synergistic action of the seven identified compounds in the bioactive fraction may offer a promising foundation for developing novel drug candidates with potent bactericidal activity against hospital-acquired MRSA strains.

Keywords: *Terminalia avicennioides*, Methicillin-resistant *Staphylococcus aureus*, Bioactive fraction, Antibacterial activity

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INTRODUCTION

Staphylococcus aureus (*S. aureus*) is a human-associated pathogen that causes toxin-mediated illnesses such as toxic shock syndrome, cellulitis, impetigo and boils. According to the World Health Organization (WHO), in parts of Africa, up to 80 % of *S. aureus* infections are methicillin-resistant, while in some regions of America, this figure reaches as high as 90 %, posing a serious public health threat [1]. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a high-priority pathogen responsible for both hospital and community-associated infections, with significant clinical and public health implications [2]. In Nigeria, it accounts for a substantial proportion of urinary tract infections [2,3], with 69 % of *S. aureus* isolates from urine samples identified as methicillin-resistant [4]. Notably, MRSA is resistant to methicillin and β -lactamase antibiotics, including dicloxacillin, cloxacillin, nafcillin, penicillin, and oxacillin [5]. In Sub-Saharan Africa, the burden of infections caused by *S. aureus*, *S. pneumoniae*, and β -lactamase-producing *Enterobacteriaceae* is particularly high [6]. Community-associated MRSA, which emerged in the 1990s, now causes skin and soft tissue infections in healthy individuals [7]. Methicillin, a β -lactam antibiotic, functions by inhibiting penicillin-binding proteins (PBPs), which are necessary for the synthesis of peptidoglycan, a vital macromolecule that forms the bacterial cell wall. Resistance to β -lactam antibiotics arises from the production of penicillin-binding protein 2a (PBP2a), making conventional treatments ineffective. This highlights the need for alternative antimicrobial agents. *Terminalia avicennioides* Guill. & Perr. (Combretaceae) has been traditionally applied to treat soft tissue infections. Specifically, ethyl acetate root bark extract has demonstrated bacteriostatic and bactericidal effects against MRSA standard strains (ATCC 33591) [8] while the leaf extract also exhibited susceptibility against MRSA isolates [9]. Given the limitations of synthetic antibiotics, this study evaluated the efficacy of a bioactive fraction of *T. avicennioides* root bark against hospital strain MRSA. The findings of this study may validate the fractionated compounds as promising bactericidal candidates against hospital strain MRSA.

EXPERIMENTAL

Reagents

Methicillin-resistant *Staphylococcus aureus* (MRSA) was isolated from a clinical sample obtained at the Department of Medical Microbiology, Ahmadu Bello University Teaching

Hospital (ABUTH), Zaria, Nigeria. The reagents used in the study were of analytical grades and sourced from Sigma-Aldrich (St. Louis, MO, USA) and Thermo Fisher Scientific (Waltham, MA, USA).

Plant material and extract

All experimental procedures complied with institutional guidelines and the revised ARRIVE 2.0 framework. The root bark of *T. avicennioides* was collected during the rainy season in 2018, with the informed consent of the district head, Samaru, Sabon-Gari Local Government Area, Kaduna State, Nigeria. The plant material was authenticated by a taxonomist and verified through <http://www.theplantlist.org>. A voucher specimen no. 901452 was assigned and the sample was deposited in the institutional herbarium for future reference. The root bark was thoroughly washed with clean water, air-dried at room temperature, pulverized, sieved, and stored in a sterile container. A total of 50 g of the pulverized root bark was soaked in 500 mL 80 % methanol and subjected to cool maceration for 48 h. To inhibit fungal growth, few drops of chloroform were added. The resulting crude extract was then filtered and freeze-dried for 4 h at 4 °C using a bench-top freeze dryer (Labconco™).

Partial purification of *T. avicennioides* crude extract

The methanol extract was partitioned into separate fractions using ethyl acetate, n-butanol, and chloroform. Preliminary bioassay-guided analysis revealed that the ethyl acetate fraction exhibited activity against MRSA. To partially purify this fraction, preparative thin-layer chromatography (TLC) was performed on a Sorbtech® USA TLC plate coated with a 0.5 mm layer of silica gel (60 F254). A sterile capillary tube was used to spot 10 μ L of the methanol extract (100 mg/mL stock solution) at the base of the plate, forming a horizontally spread band approximately 1 mm thick. The plate was then air-dried for 30 min. Another 10 μ L of extract was similarly spotted, forming a second band of 1 mm thick, and air-dried for 30 min. The TLC plate was vertically placed in a chromatographic tank containing a solvent system of n-butanol, acetic acid, and water (BAW) in a 10:2:1 ratio. Chromatography was stopped when the solvent was approximately 1 cm from the top of TLC plate. The chromatogram was carefully removed and examined under ultraviolet (UV) light to identify compounds represented by the bands. Each band was scraped off the plate and

suspended in 2 mL distilled water, followed by centrifugation at $3000 \times g$ for 20 min.

This process was repeated three times to ensure the complete removal of any residual solvent system from the partially purified extract. The resulting supernatant was reconstituted with 2 mL distilled water. Each recovered and reconstituted band (referred to as the partially purified fraction) was tested for antibacterial activity against MRSA. The band showing antibacterial activity was selected for further use and labeled as the partially purified fraction of the extract. To minimize the risk of chemical inhalation, this process was conducted in a fume hood. The active purified fraction was then analyzed using gas chromatography-mass spectrometry (GC-MS) for compound identification and Fourier transform infrared spectroscopy (FTIR) to determine functional groups.

Identification and confirmation of bacterial isolate

The MRSA sample was inoculated in triplicate onto mannitol salt agar plates and incubated at 37°C for 24 h. After incubation, MRSA was aseptically isolated and subjected to biochemical analysis, focusing on characteristics such as colonial morphology, Gram stain, and strain reaction. To assess the resistance of MRSA strain to oxacillin (Oxoid, UK), its sensitivity to the antibiotic was evaluated and compared to standards established by the Institute for Clinical and Laboratory Standards (CLSI) [10].

Antibacterial assay

The antibacterial assay was conducted in triplicate using the agar well diffusion method [11], with minor modifications. A sterile cotton swab was used to inoculate 0.1 mL of a standard inoculum (1.5×10^8 cfu/mL; 0.5 McFarland standard) cultured for 24 h, onto the surface of freshly prepared sterile Mueller-Hinton agar. The inoculated plates were allowed to stand for 2 h to enable proper diffusion. Wells measuring 5 mm in diameter were created in the agar, and 0.5 mL of extract suspended in 1 % dimethyl sulfoxide (DMSO) was introduced into each well at varying concentrations (50, 100, 150, and 200 $\mu\text{g/mL}$). The plates were incubated at 37°C for 24 h. Inhibition zone diameter was measured and interpreted using the standard Kirby-Bauer chart. The test sample was ethyl acetate fraction of the methanol extract of *Terminalia avicennioides*, which was partially purified via preparative thin-layer chromatography (TLC). Among the bands recovered, only band 2, which exhibited clear

antibacterial activity against MRSA, was selected for further analysis. For comparative evaluation, standard antibiotics were used in accordance with CLSI guidelines. These included Meropenem (10 μg), Amoxiclav (30 μg), Nitrofurantoin (300 μg), Ceftriaxone (30 μg), and Ciprofloxacin (5 μg). An additional disk, Nidof (10 μg), was also included based on its relevance in the local clinical setting, although it is not listed under CLSI standardized disks. Inhibition zones for each of the antibiotics were measured and compared to those of the bioactive fraction (band 2).

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC was determined using the agar-well diffusion method. Wells were created aseptically on Muller-Hinton agar to evaluate the antibacterial activity [12]. The ethyl acetate bioactive fraction (band 2) of the partially purified extract was added to the wells at concentrations ranging from 50 to 200 $\mu\text{g/mL}$. Bacterial cultures were inoculated and incubated at 30°C for 24 h. Two controls were included: control A, consisting of broth and the test organism, and control B, consisting of broth and distilled water. Inhibition zone diameter defined as the clear area around the well, was measured. The MIC of the ethyl acetate fraction was determined as the concentration that completely inhibited visible growth. To determine the MBC, a broth microdilution assay was performed. The 96-well microtiter plate was serially diluted with the bioactive fraction, inoculated with test strains, and incubated at 30°C for 24 h. Bacterial growth was quantified by measuring the absorbance at 660 nm using a microtiter plate reader. The MBC was defined as the lowest concentration of the test fraction that inhibited bacterial growth. Known antibiotics, including Meropenem (10 μg), Amoxiclav (30 μg), Nitrofurantoin (300 μg), Ceftriaxone (30 μg), and Ciprofloxacin (5 μg), along with an additional control disk, Nidof (10 μg), were used as a control.

Analysis of the purified fraction by GC-MS and FTIR spectroscopy

The GCMS-QP 2010 Plus instrument (Shimadzu, Kyoto, Japan) was used to analyze samples of the purified bioactive fraction. Fatty acid methyl esters (FAMES) were prepared using a high-temperature acid-catalyzed method [13]. In this process, a portion of the bioactive fraction was transferred to a 10 mL centrifuge tube, and 2 mL of 1 % H_2SO_4 -MeOH solution was added.

Table 1: Inhibition zone diameter of different concentrations of TLC fractions of *T. avicennioides* root bark extract against MRSA (Mean $\pm$ SD)

Bands recovered after purification	50 μ g/mL	100 μ g/mL	150 μ g/mL	200 μ g/mL
Band (fraction) 1	15.00 $\pm$ 2.00 ^b	15.33 $\pm$ 1.53 ^b	17.00 $\pm$ 1.41 ^{bc}	15.00 $\pm$ 2.00 ^c
Band (fraction) 2	12.67 $\pm$ 0.58 ^b	16.50 $\pm$ 0.71 ^b	19.00 $\pm$ 1.41 ^a	19.00 $\pm$ 1.41 ^b
Band (fraction) 3	13.67 $\pm$ 2.08 ^b	15.00 $\pm$ 2.00 ^b	14.33 $\pm$ 0.58 ^c	14.50 $\pm$ 0.71 ^c
Band (fraction) 4	13.67 $\pm$ 2.08 ^b	15.00 $\pm$ 0.00 ^b	17.00 $\pm$ 2.83 ^{bc}	14.00 $\pm$ 0.00 ^c

Values bearing the same superscript down the column are not significantly different at the same concentration of the recovered bands against methicillin-resistant *S. aureus*. 'a' represents values with a significant difference from b. 'b' represents values significantly different from c. Superscript 'bc' represents values with no significant difference from 'bc'

The mixture was heated at 85 °C for 2 h. After cooling to room temperature, 3 mL n-hexane and 2 mL distilled water was added and mixed thoroughly. The resulting supernatant was subjected to GC analysis under specific chromatographic conditions (flow rate was set to 1.8 mL/min, pressure ratio of 1:0, injection syringe was programmed to 280 °C, with pressures of 100 and a 60 % column oven, helium was used as the carrier gas at a flow rate of 1.8 mL/min, transfer line and source were both set to 280 °C, oven temperature was initially set to 80 °C for 2 min, then increased to 200 °C at a rate of 9 °C per min until it reaches 280 °C, where it was maintained for 5 min with a flow rate of 10 °C, full scan mode (40 – 800 m/z) was used to capture the chromatograms). The compounds were identified using the NIST/EPA/NIH Mass Spectral Library (NIST/EPA/NIH 11, version 2.0). To determine the functional groups of the compounds, a sample disk was created by encapsulating 10 mg of the sample in a 100 mg KBr pellet. This translucent disk was then loaded into a Shimadzu FTIR spectrometer (IR Affinity1, Japan) with a scan range of 400 to 4000 cm⁻¹ and a resolution of 4 cm⁻¹.

Statistical analysis

Data was analyzed using Statistical Package for Social Sciences (SPSS version 22.0, IBM, Armonk, NY, USA). Results were presented in mean $\pm$ standard deviation (SD) and compared using one-way analysis of variance (ANOVA) and multiple post hoc tests. $P < 0.05$ was considered statistically significant.

RESULTS

Inhibition zone diameter of the bioactive fraction at various concentrations

The results identified *S. aureus* as gram-positive cocci, exhibiting positive catalase and coagulase reactions. At 100 μ g/mL, the fraction exhibited bacteriostatic effect on MRSA, with a minimum

bactericidal concentration (MBC) of 9.48 $\pm$ 0.90 (Table 1). At 150 μ g/mL, the fraction demonstrated bactericidal activity against MRSA (Figure 1). Furthermore, at 150 μ g/mL, the bioactive fraction showed significant antibacterial activity ($p < 0.05$) compared to other fractions.

Preliminary phytochemical profiling of the bioactive fraction

Qualitative phytochemical analysis of the bioactive fraction revealed the presence of flavonoids, tannins, and alkaloids (Table 2).

Table 2: Phytochemical determination of the partially purified fraction (band 2) with anti-MRSA activity

Phytochemical	Present/Absent
Alkaloids	Present
Flavonoids	Present
Tannins	Present
Cardiac glycosides	Absent
Triterpenes	Present
Terpenoids	Present
Anthraquinones	Present

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The TLC fraction (band 2), demonstrated an MBC of 9.48 $\pm$ 0.90. The inhibition zone is shown in Figure 1. The bioactive fraction (band 2) of the extract exhibited inhibition zone of ≥ 14 mm, indicating the sensitivity of MRSA, as confirmed by the standard Kirby-Bauer test for antibiotic susceptibility. Furthermore, the compounds within the fraction showed bacteriostatic activity against MRSA at 100 μ g/mL and bactericidal activity at 150 μ g/mL (Table 3).

Chemical profiling of bioactive fractions

The bioactive fraction (band 2) comprises seven compounds which include n-hexadecanoic acid (3.95 %), 11-octadecenoic acid (2.90 %), oleic acid (22.10 %), octadecanoic acid (4.07 %), 3,11-tetradecadien-1-ol (3.08 %), 9-octadecenal (4.39 %), and (9Z)-9-teradecenal (1.47 %).

These compounds have molecular weights ranging from 210 to 295 kDa (Table 4 and Table 5).

Table 3: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC; mean $\pm$ SD)

Extract/fraction	MIC (100 μ g/mL)	MBC (150 μ g/mL)
Methanol	11.42 $\pm$ 0.20	15 $\pm$ 0.40
Chloroform	21.54 $\pm$ 1.03	34 $\pm$ 1.20
Ethyl acetate	16.06 $\pm$ 0.62	21 $\pm$ 2.01
n-Butanol	9.87 $\pm$ 1.46	12 $\pm$ 1.11
TLC band 2	4.00 $\pm$ 1.00	9.48 $\pm$ 0.90

Fourier transform infrared (FTIR) spectroscopy

Fourier transform infrared (FTIR) analysis revealed several functional groups present in the fraction, including alkyl halides (R-Br, R-F), methyne (CH), nitro compounds (R-NO₂),

amides (R-C(O)-NH₂, R-C(O)-NH-R), isothiocyanates (-NCS), nitriles (CN), carboxylic acids, saturated alkyl methylene C-H, and alkanes (Figure 2).

DISCUSSION

The emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) presents a significant challenge to healthcare systems, necessitating the search for effective antimicrobial agents. In this study, a bioactive fraction partially purified via preparative thin-layer chromatography (TLC) demonstrated both bacteriostatic and bactericidal activity against MRSA. The ability of the compounds within this fraction to inhibit and kill MRSA highlights their potential as promising candidates for novel drug development. These findings support ongoing efforts to discover and develop new therapeutic molecules capable of combating MRSA resistance.

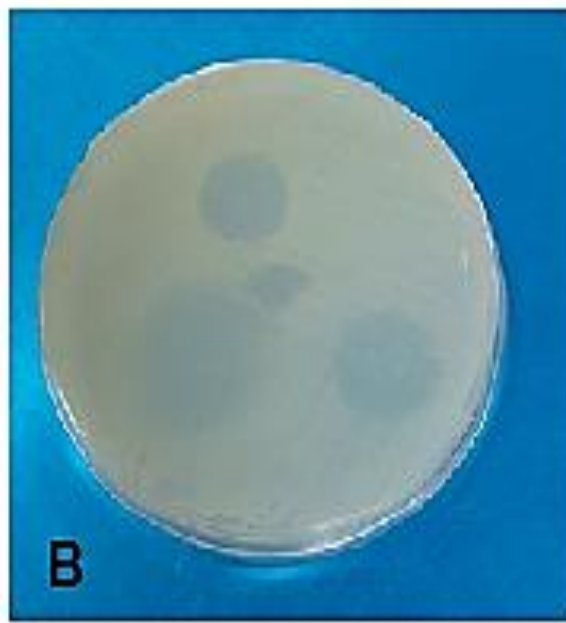
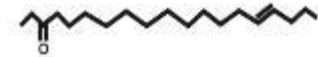
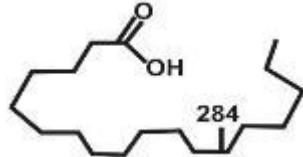
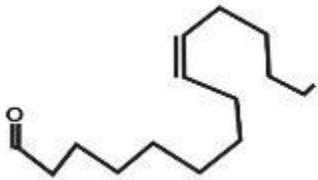


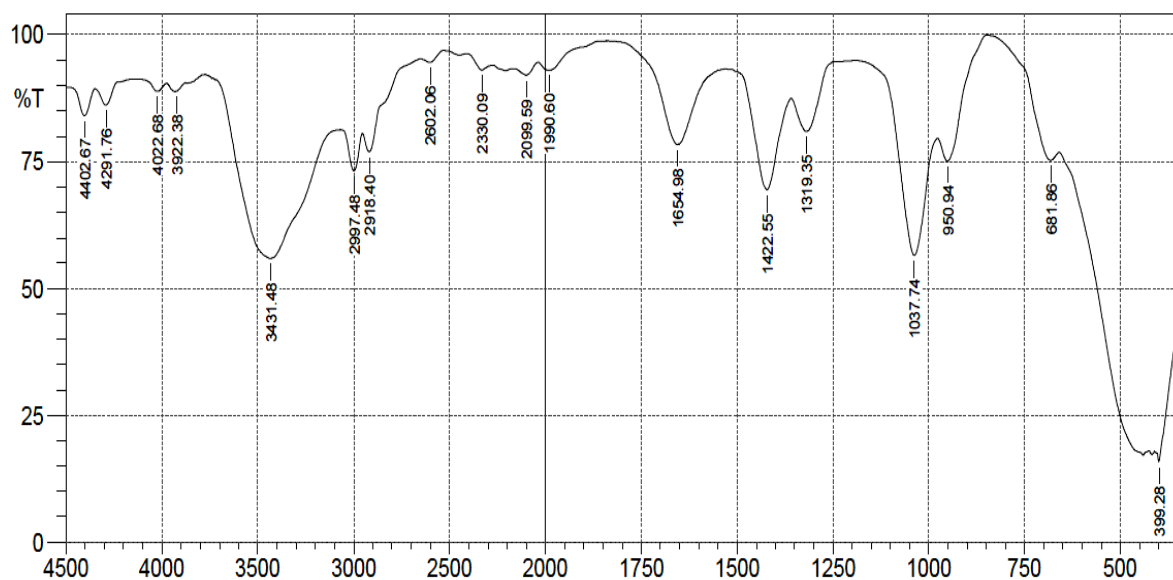
Figure 1: Test demonstrating the antibacterial effect of the bioactive fraction and multidrug resistance in MRSA hospital strains. A: MRSA hospital strains show multidrug resistance to Meropenem, Amoxiclav, Nitrofurantoin, Ceftriazone, Nidof, and Ciprofloxacin. B: Inhibition zone diameter of the bioactive fraction at 150 μ g/mL

Table 4: Gas chromatography-mass spectroscopy characteristics of seven identified compounds

Peak no.	Stable ion	Compound name	Mol. weight (kDa)	Mol. formula	Retention time (min)	Peak area	Abundance (%)
1	93	n-Hexadecanoic acid	256	C ₁₆ H ₃₂ O ₂	18.546	9986682	3.95
2	92	11-Octadecenoic acid	295	C ₁₉ H ₃₆ O ₂	20.205	7339337	2.90
3	94	Oleic Acid	282	C ₁₈ H ₃₄ O ₂	21.350	55865451	22.10
4	90	Octadecanoic acid	284	C ₁₈ H ₃₆ O ₂	21.537	10294647	4.07
5	91	3,11-Tetradecadien-1-ol	210	C ₁₄ H ₂₆ O	24.153	7787536	3.08
6	91	9-Octadecenal	266	C ₁₈ H ₃₄ O	24.589	11094234	4.39
7	90	(9Z)-9-Teradecenal	210	C ₁₄ H ₂₆ O	26.550	3722523	1.47

Table 5: Structure of the seven compounds in the bioactive fraction of the root bark extract of *T. avicennioides*

Name	Structure
n-Hexadecanoic acid	
11-Octadecenoic acid	
Oleic acid	
Octadecanoic acid	
3,11-Tetradecadien-1-ol	
9-Octadecenal	
(9Z)-9-Tetradecenal	

**Figure 2:** Fourier transform infrared spectroscopy spectra showing the profile of seven compounds in the bioactive fraction

Phytochemicals identified in the bioactive fraction may act synergistically to modulate bacterial resistance mechanisms, supporting their candidacy as scaffolds for anti-MRSA therapies. The lipophilic nature of several compounds in the fraction suggests that they may disrupt bacterial lipid membranes, resulting in bactericidal activity.

One proposed mechanism involves epicatechin gallate, a flavonoid known to integrate into the cytoplasmic membrane and interfere with the localization of penicillin-binding protein 2a (PBP2a), thereby reversing methicillin resistance [14]. Alternatively, the observed bacteriostatic effect may arise from inhibition of DNA synthesis

or tannin-mediated disruption of bacterial cell walls.

Previous study has shown that the secondary metabolites from various phytochemical classes exert bactericidal activity against *S. aureus* by compromising cell wall integrity and broadly inhibiting both hospital and community-associated MRSA strains [15]. Such phytochemicals, which impair membrane function and energy production, hold considerable promise as therapeutic agents. Findings from this study align with an earlier report that identified palmitic acid, stearic acid, and (9Z)-9-tetradecenol (Table 4) as effective anti-MRSA compounds [16]. Additionally, oleic acid, detected in this study, has been shown to exert antimicrobial activity by disrupting the bacterial cell wall and inhibiting the growth of Gram-positive organisms, including MRSA [17].

Anti-MRSA activity observed in the ethyl acetate fraction of *Terminalia avicennioides* root bark may be attributed, in part, to the presence of oleic acid. Furthermore, this study identified 3,11-tetradecadien-1-ol as a potential novel compound with anti-MRSA activity. These effects are likely due to the collective action of compounds belonging to different chemical classes. Fatty acids, in particular, are known to disrupt bacterial membranes, impair energy metabolism, interfere with the electron transport chain, and inhibit oxidative phosphorylation. They may also block essential enzymes, inhibit nutrient uptake, generate toxic intermediates, or directly lyse bacterial cells [13].

Antimicrobial efficacy of the bioactive fraction may be attributed to the functional groups within its constituent compounds. Functional groups serve as reactive centers of organic molecules and critically influence biological activity. In this study, the presence of alkyl halides may contribute to the bactericidal activity observed. Nitrogen-containing heterocycles and amides, also identified through FTIR analysis, are known to inhibit dihydrofolate reductase—an essential enzyme in bacterial DNA synthesis, thereby exerting anti-MRSA effects [18]. Some other active substances may cause bacteriolysis due to cytoplasmic and cell wall distortion [19].

Plant-derived compounds have long been instrumental in treating infectious diseases, offering a vast array of bioactive molecules for therapeutic use. In parallel, snake venom has emerged as a potent source of antimicrobial agents. The growing interest in venom-based research within the pharmaceutical industry underscores its potential to develop novel

antibacterial and antimicrobial treatments. Together, plant-based and venom-derived compounds represent valuable leads in global efforts to address antibiotic resistance and discover next-generation therapeutics.

Limitations of the study

Lack of comprehensive characterization of the extract using high-performance liquid chromatography (HPLC) fingerprinting would have provided deeper insights into its pharmacologically active constituents. Also, the antibacterial activity was assessed against only a single clinical MRSA isolate, thereby limiting the generalizability of the results to broader MRSA populations.

CONCLUSION

Ethylacetate fraction of *T. avicennioides* demonstrates dose-dependent antibacterial effect, with bactericidal activity occurring at higher concentrations. The fraction furnishes an array of seven compounds which may offer a promising foundation for developing novel drug candidates with potent bactericidal activity against hospital-acquired MRSA strain.

DECLARATIONS

Acknowledgement/Funding

None.

Ethical approval

This study did not involve human or animal participants. All experimental procedures complied with institutional guidelines and the revised ARRIVE 2.0 framework.

Use of Artificial intelligence/Large language models

We also declare that we did not use Generative artificial intelligence (AI) and AI-assisted technologies in writing the manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

Emeka John Dingwoke and Fatima Adis Adamude conceptualized and designed the study. Emeka John Dingwoke drafted the initial manuscript. All authors contributed to the experimental work, data analysis, critical review, and approval of the final version.

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