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Original Research Article

GC-MS AND *IN VITRO* ANTIBACTERIAL EVALUATION OF *Indigofera arrecta* ROOT EXTRACTS AGAINST MULTIDRUG-RESISTANT (MDR) *Staphylococcus aureus* CLINICAL ISOLATES

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ABSTRACT

Indigofera arrecta has a long history in traditional medicine within the Zaria metropolis for managing bacterial infections. This study sought to identify the antibacterial efficacy and bioactive constituents responsible for combating multidrug-resistant *Staphylococcus aureus* clinical isolates. The roots of *I. arrecta* were extracted via cold maceration and subsequent solvent fractionation techniques. The crude extract and its fractions were evaluated against MDR *S. aureus* clinical isolates from blood, urine, and wounds utilising agar well diffusion, broth dilution, and agar dilution techniques. Phytochemical analyses were conducted. Bioactive substances were isolated via column chromatography and direct bioautography TLC, and their identification was accomplished through GC-MS analysis. An acute toxicity assessment was performed per the recommendations of the Organization for Economic Co-operation and Development (OECD). The mechanism of action was assessed using in vitro microbiological methods. The root extract and its fractions revealed the presence of cardiac glycosides, saponins, flavonoids, terpenoids, phenols, tannins, and alkaloids. All exhibited activity at different concentrations, with ethyl acetate the most active at a minimum inhibitory dosage of 25.00±0.58 mg/mL. The most activity was noted in Fractions 12 and 60. The bioautography plate indicated R_f 0.13 as the active region in Fraction 12. GC-MS analysis identified 14 chemicals, predominantly fatty aldehydes. Majority of compounds were recognised for their antibacterial activities. Included are 13-octadecenal, 8-hexadecenal, 2-tridecenal, Quinuclidine alkaloid, (E)-1,2-Benzenedicarboxylic acid, Behenic alcohol, Tetradecyl Oxirane, and Oleic Acid. The active portion enhanced cell membrane permeability and induce the leaking of cell contents. Acute toxicity experiments demonstrated that the active extract and its fractions did not cause mortality in Wistar rats. The root extract and fractions of *I. arrecta* showed substantial efficacy against multidrug-resistant *S. aureus* clinical isolates.

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INTRODUCTION

Antimicrobial resistance (AMR) represents one of the most urgent global health challenges, carrying profound consequences for both mortality and morbidity. In Nigeria alone, AMR was estimated to account for approximately 64,500 deaths in 2019, while overall AMR-associated mortality reached 263,400 [1]. Among resistant pathogens, *Staphylococcus aureus* is particularly significant, with nationwide prevalence rates reported to range between 13% and 82% [2,3]. More than half of the disease burden linked to multidrug-resistant (MDR) *S. aureus* has been attributed to this organism [4]. The situation is exacerbated in low-income settings such as Nigeria, where treatment costs and mortality are often underreported, making effective management increasingly difficult [1].

Given these challenges, exploring alternative therapeutic strategies is essential. Plant extracts and plant-derived compounds (PDCs) have emerged as promising candidates, functioning either as direct antimicrobials or as agents capable of modulating resistance [5]. Within Nigeria and other tropical regions, *Indigofera arrecta* has long been employed in traditional medicine to treat diverse conditions, including gastrointestinal, inflammatory, dermatological, respiratory, and infectious diseases, as well as parasitic disorders of the skin, blood, and vasculature. The plant is known internationally as Java indigo, Bengal indigo, or Natal indigo, and locally as *Baaban-rini* and *Aniyaa makoomiyaa* in Hausa, *Uri* in Igbo, and *Elu-aja* in Yoruba [6].

The species is widely distributed across tropical and subtropical regions of Africa, Asia, and the Americas, with documented presence in northern, southwestern, and eastern Nigeria [7]. Previous investigations have demonstrated that *Indigofera arrecta* contains bioactive compounds with notable antibacterial properties; however, evidence of its activity against antimicrobial-resistant pathogens remains limited. Ongoing exploration of therapeutic plants with antibacterial properties may yield promising leads for drug discovery and development. In line with this, the present study was undertaken to evaluate the antibacterial activity of *I. arrecta* root extracts and to characterize their bioactive constituents against multidrug-resistant *S. aureus*

MATERIAL AND METHODS

Materials

Chemicals and Reagents: Sterile deionized water, hydrochloric acid, ethanol (95%), ethyl acetate, sucrose, normal saline, methanol, *n*-butanol, and *n*-hexane were purchased from Loba Chemie Pvt. Ltd. (Colaba, Mumbai, India).

Laboratory Equipment and Instruments: The study employed silica gel 60 (60–120 µm), TLC plates, capillary tubes, a fraction collector, preparative plates, and reagents for phytochemical screening. Analytical devices included an atomic absorption spectrophotometer (AAS), a UV–Visible spectrophotometer, and centrifuges. The laboratory setup incorporated a Model-630 system (Aheinicke Company, Portland, Oregon, USA), equipped with an incubator, microtiter plate reader, thermal cycler (PCR), gel electrophoresis apparatus, and a thermal plate reader.

Culture Media: Mueller–Hinton agar and broth, Congo red agar, mannitol salt agar, DNase agar, brain heart infusion agar, nutrient agar and broth, and Luria–Bertani (LB) broth were procured from Oxoid Ltd. (Basingstoke, Hampshire, England).

Methods

Assessment of Isolates: Multidrug-resistant (*MDR*) *Staphylococcus aureus* isolates were obtained from a previous clinical study conducted in two hospitals within Zaria Metropolis, Kaduna State [3]. Identification was carried out using standard microbiological approaches, including both phenotypic and genotypic methods [3]. The isolates were preserved under refrigeration until required for experimentation.

Ethical Approval: Institutional clearance was obtained from the Health Research Ethics Committee of Ahmadu Bello University Teaching Hospital, Shika (ABUTHZ/HREC/F58/2022), as well as from the Committee on the Use of Human Subjects for Research, ABU Zaria (ABUCUHSR/2022/001). Authorization for animal experimentation was further granted by the ABU Animal Use and Care Committee under approval number ABUCAUC/2023/171.

Acquisition and Authentication of Plant Material: Roots of *Indigofera arrecta* were collected in December 2022 from Sauyoyi, Biye, Giwa Local Government Area, Kaduna State. Taxonomic identification was confirmed by Dr. Namadi Sanusi at the Herbarium Unit, Department of Botany, Ahmadu Bello University, Zaria. A voucher specimen was deposited under accession number ABU0677.

Preparation of Extract: The collected roots were rinsed thoroughly with sterile water, air-dried at ambient temperature for two weeks, and finely ground into powder. A 500 g portion of the powdered material was macerated in 2.5 L of methanol for 72 h. The mixture was then filtered, and the resulting extract was concentrated to dryness [8], [9].

Fractionation of Methanol Extract: Fractionation of the methanol extract was carried out by suspending 48 g of the extract in 400 mL of distilled water, followed by filtration through Whatman No. 1 paper. The resulting filtrate was sequentially partitioned with *n*-hexane, ethyl acetate, and *n*-butanol (3 × 300 mL each), yielding four fractions: *n*-hexane (HF), ethyl acetate (EF), *n*-butanol (BF), and aqueous (AF). Each fraction was concentrated using a water bath and stored for subsequent analysis [8].

Standardization of Inoculum: For inoculum standardization, MDR *Staphylococcus aureus* isolates were subcultured on mannitol salt agar and incubated for 24 h. A loopful of the resulting culture was suspended in 2 mL of 0.85% saline and adjusted to a 0.5 McFarland standard, corresponding to approximately 10^7–10^8 CFU/mL [10].

Antibacterial Susceptibility Testing: Antibacterial susceptibility testing was carried out using the agar well diffusion method. Mueller–Hinton agar plates were inoculated with standardized bacterial suspensions and allowed to dry prior to application of the test samples. Aseptic wells (6 mm) were bored in the agar. The extracts and fractions were solubilized in 50% DMSO and serially diluted from 200 mg/mL to 6.25 mg/mL concentrations. After incubation, the diameters of the inhibition zones were measured in millimeteres [12] and the results were expressed as mean ± SEM.

Determination of MIC and MBC: The minimum inhibitory concentration (MIC) was determined by broth dilution in the range 200–3.125 mg/mL. Tubes with Mueller–Hinton broth, test extract or fraction and standardized inoculum were incubated at 37°C for 24 h. MIC was defined as the minimum concentration that resulted in no observable turbidity. For the minimum bactericidal concentration (MBC), aliquots from MIC tubes showing no growth were sub-cultured onto Mueller–Hinton agar and incubated for 18–24 h. The lowest concentration at which no colony growth was observed was recorded as the MBC [13].

Column Chromatography: Column chromatography was performed using silica gel 60 (60–120 µm) as the stationary phase. A slurry prepared with *n*-hexane was packed into a glass column (3.0 × 60 cm). Seven grams of the ethyl acetate portion were loaded onto the column and eluted with gradients of hexane–ethyl acetate. Fractions of 50 mL each were collected and monitored by thin layer chromatography (TLC) on silica gel 60 F254 plates. Active fractions were pooled and subjected to further purification by preparative TLC. The purified compounds were then centrifuged, filtered, and analyzed using GC–MS.

Thin Layer Chromatography (TLC): For thin layer chromatography (TLC), fractions were dissolved in ethyl acetate, and 2 µL of each sample was applied onto silica gel 60 F254 plates. The plates were developed in a solvent system of *n*-hexane and ethyl acetate (8:2, v/v). Spots were visualized under UV light at 254 and 366 nm, followed by derivatization with anisaldehyde–sulfuric acid reagent. Retention factor (Rf) values were then calculated.

Direct Bioautography: In direct bioautography, developed TLC plates were briefly immersed in a bacterial suspension for 10 seconds, dried, and incubated at 25 °C for 48 hours. Following incubation, the plates were treated with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution and maintained at 37 °C for 4 h. The appearance of yellow zones against a blue background signified antibacterial activity. Active regions were subsequently scraped, extracted with ethyl acetate, centrifuged, and subjected to GC–MS analysis [13].

GC–MS Analysis: The analysis was carried out using a PerkinElmer Clarus 680 gas chromatograph coupled with a Clarus SQ 8T mass spectrometer. Isolation was performed with an Elite 5 MS column (30 m × 0.25 mm × 0.25 µm). Helium was the carrier

gas at a flow rate of 1 mL/min. The volume of injection was 1 µL in split mode. The content of the oven temperature was set from 60°C to 250°C. Mass spectra were obtained at 70 eV in the range of 45–700 m/z and the compounds were identified by matching them with NIST library.

Acute Toxicity Study: Toxicity was assessed according to Organisation for Economic Cooperation and Development (OECD) guideline 425 [14]. Five Wistar rats received a single oral dose of 5000 mg/kg of the most active fraction after fasting. The animals were watched for 14 days for changes in behaviour, central nervous system, autonomic, and somatic signs.

Mechanism of Action Studies: *Ion Leakage Assay:* Active fractions at concentrations related to their MIC were incubated with washed bacterial cells [15]. After incubation, samples were centrifuged, and supernatants analysed for Na^+ and K^+ ions using atomic absorption spectroscopy. *Nucleotide Leakage Assay:* Treated bacterial suspensions were centrifuged, and supernatants were measured at 260 nm using a UV spectrophotometer to detect nucleotide leakage [15].

Statistical Analysis

Data are presented as mean ± standard deviation (SD). Statistical evaluation was carried out using SPSS version 20, and the outcomes are displayed in tables and figures.

RESULTS

Fresh roots of *Indigofera arrecta* displayed characteristic macroscopic features, including a dark coloration, hard fibrous texture, distinctly bitter taste, and a typical odour (Table 1). Extraction yields are summarized in Table 2: the crude methanol extract produced 11.59%, the *n*-hexane fraction yielded the highest at 17.77%, while the *n*-butanol fraction gave the lowest at 8.78%.

Phytochemical screening (Table 3) confirmed the presence of carbohydrates, cardiac glycosides, alkaloids, phenols, saponins, terpenoids, and flavonoids across both the extract and its fractions.

Antibacterial susceptibility assays showed that all MDR *Staphylococcus aureus* isolates were inhibited by the methanol extract and solvent fractions at concentrations of 25 mg/mL or higher. Zones of inhibition increased proportionally with concentration (Figure 1). Based on MIC values, the ethyl acetate fraction demonstrated the strongest activity (25 mg/mL), whereas the *n*-hexane fraction was least effective (100 mg/mL). Moderate activity was noted for the *n*-butanol fraction (37.50 mg/mL), the methanol extract (50 mg/mL), and the aqueous fraction (50 mg/mL).

No minimum bactericidal concentration (MBC) was observed for any extract or fraction, as regrowth appeared after 24 hours without treatment. This suggests the extracts acted in a bacteriostatic manner rather than being bactericidal (Figure 2).

Further separation of the ethyl acetate portion by column chromatography produced 82 fractions, of which 20 showed clear antibacterial activity with inhibition zones ≥15 mm (Table 4). Bioautography outcomes for the active compound zones are summarized in Table 5 and illustrated in Plate 1.

Thin-layer chromatography of the active fraction revealed five distinct spots under UV light at 254 nm, including one with an Rf value of 0.13 corresponding to the antibacterial activity zone. Under visible light, a single spot was observed, while derivatization with anisaldehyde–sulfuric acid revealed eight distinct spots.

GC–MS analysis of bioactive regions identified 14 compounds (Table 6), comprising five fatty aldehydes, one fatty acid, four esters, two alcohols, one pheromone, and one alkaloid.

Mechanistic assays showed that the active fraction increased nucleotide leakage and disrupted K^+/Na^+ balance, suggesting enhanced membrane permeability and probable cell membrane damage (Figure 3).

Acute toxicity evaluation in Wistar rats showed that both the crude extracts and solvent fractions of Indigofera arrecta root were non-toxic at doses above 5000 mg/kg (Table 7), suggesting the extract is relatively safe for oral use.

Table 1: Macroscopic characteristics of fresh and powdered *Indigofera arrecta* root.

`	***Indigofera arrecta***	
Features	Fresh Root	Powdered Root
Texture/surface	Smooth	Rough
Fracture	Hard and fibrous	-
Colour	Light brown	Brown
Odour	Characteristics	Characteristics
Taste	Mask bitter	Mask bitter

Table 2: Extraction yields of crude methanol extract and solvent partitions.

S/NO	Extraction/Fractions	Initial weight (g)	Weight (g)	% Yield
1	Methanolic	500	57.95	11.59
2	n-Hexane	48	8.52	17.77
3	Ethyl acetate	48	11.97	24.97
4	n-Butanol	48	4.21	8.78
5	Residual Aqueous	48	19.53	40.74

Table 3: Qualitative phytochemical constituents detected in root extract and fractions of *I. arrecta*

		Indigofera arrecta				
Phytochemicals	Test	MeOH	AQF	NBF	EAAF	NHF
Carbohydrate	Molisch	+	+	+	-	-
	Fehling	+	+	+	-	-
Cardiac glycoside	Keller Killiani	+	+	+	+	-
Flavonoids	Shinoda	+	+	+	+	-
	Sodium Hydroxide	+	+	+	+	-
Saponins	Frothing	+	+	+	-	-
Steroids/	Salkwoski	+	+	+	+	-
Terpenoids	Libermann-buchard	+	+	+	+	+
Tannins	Ferric chloride	+	+	+	+	+
	Lead subacetate	-	-	-	+	+
Alkaloids	Wagner	+	+	+	-	-
	Dragendorff	+	+	+	+	+
	Mayer	+	+	+	+	+
Anthraquinones	Bontragers	-	-	-	-	-
	Modified Borntrager	-	-	-	-	-

Key: + (positive/present), - (absent)

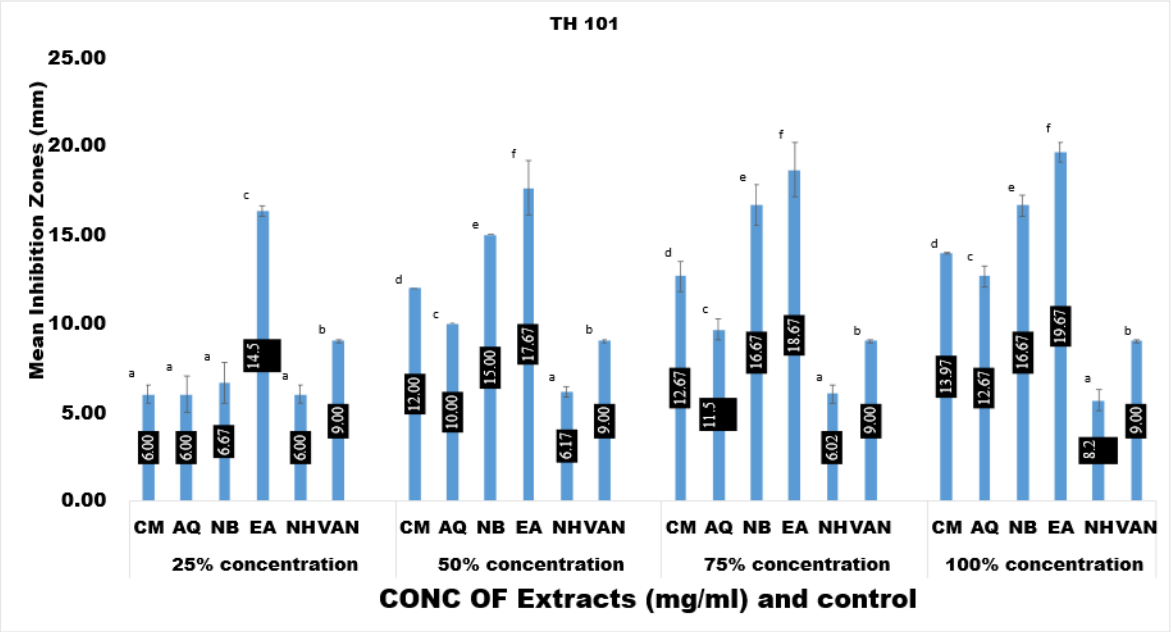


Figure 1: Zones of inhibition produced by crude extract and solvent fractions of *Indigofera arrecta* against MDR *Staphylococcus aureus*.
Key: CM: methanol: AQ: Aqueous fraction: NB: n-Butanol fraction: EA: ethyl acetate fraction: NH: n-Hexane fraction: VAN-Vancomycin

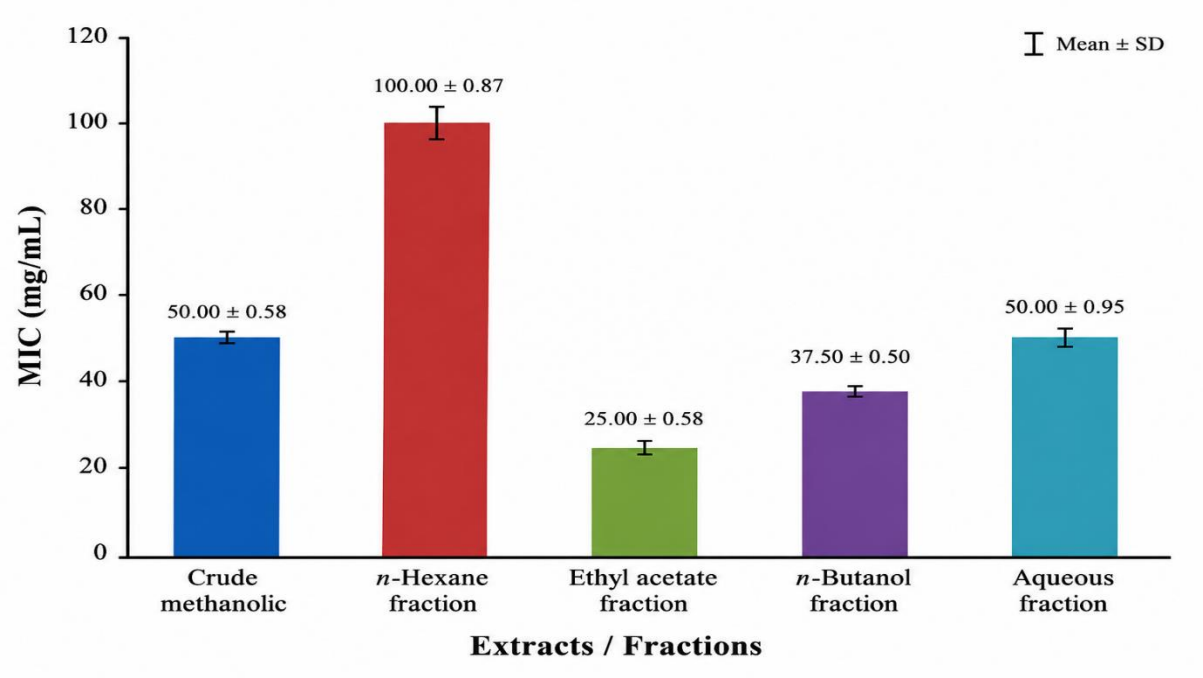


Figure 2: Bacteriostatic effect of extracts and fractions showing regrowth after 24 h in MBC assay.

Table 4: Antibacterial activity of column chromatography fractions (zones ≥15 mm).

S/NO	Solvent system	F/NO	Yield/g	Concentration (mg/ml)	ZI/mm
1	H: E 8:2	F 12	1.23	20	30.0
2	H: E 6:4	F 22	0.31	20	20.0
3	H: E 5:5	F 28	0.31	20	16.0
4	H: E 4:6	F 31	0.23	20	15.0
5	H: E 3:7	F 38	2.46	20	20.0
6	H: E 2:8	F 39	1.92	20	20.0
7	H: E 2:8	F 40	2.77	20	20.0
8	H: E 2:8	F 41	3.46	20	20.0
9	H: E 1:9	F 45	3.31	20	18.0
10	H: E 1:9	F 46	2.77	20	18.0
11	E 100%	F 49	3.08	20	15.0
12	E:M 9:1	F 53	0.23	20	15.0
13	E:M 8:2	F 57	1.15	20	15.0
14	E:M 8:2	F 60	0.69	20	30.0
15	E:M 7:3	F 61	0.54	20	25.0
16	E:M 7:3	F 62	2.31	20	15.0
17	E:M 6:4	F 66	0.15	20	15.0
18	E:M 6:4	F 67	0.38	20	18.0
19	E:M 6:4	F 68	0.38	20	15.0
20	M 100%	F 76	0.23	20	24.0
21		CIP		5	0.0
22		DO		5	0.0

Key: S/NO-Serial number, F/NO-Fraction number, F-Fraction, CIP-Ciprofloxacin, DO-Doxycycline, H-Hexane, E-Ethyl acetate, M-Methanol, ZI-Zone of inhibition

Table 5: Bioautography results indicating active compound zones of ethyl acetate column fraction of *I. arrecta* against MDR *S. aureus*.

No. of spots	Retention Factor	Bioactive Spots
1	0.77	NA
2	0.60	NA
3	0.40	NA
4	0.27	NA
5	0.13	Active

NA- Not Active.

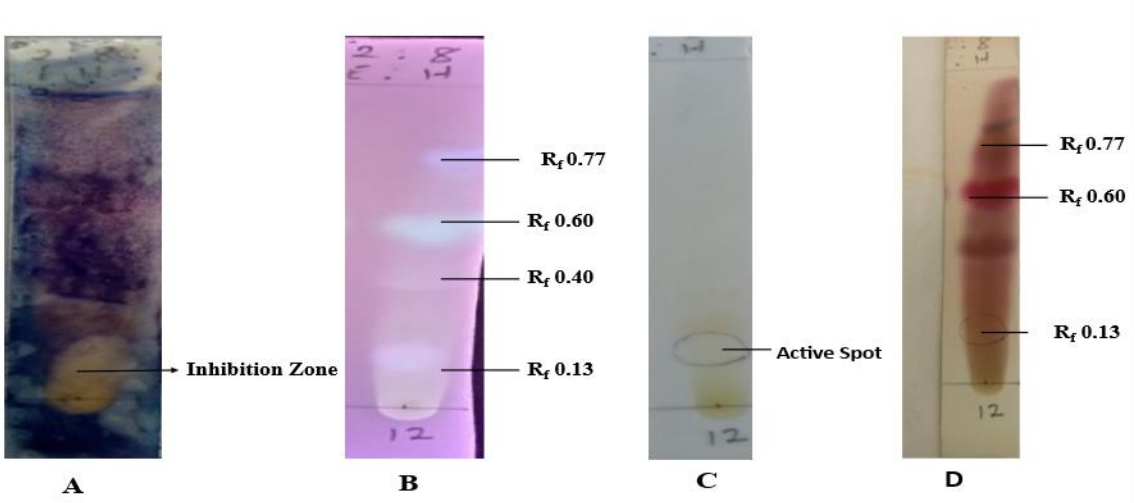


Plate 1: Bioautogram/TLC profile of the most active ethyl acetate column fraction of *I. arrecta* showing inhibitory band at Rf = 0.13
A-Thin Layer Chromatogram (Bioautogram)
B- TLC of the most active ethyl acetate column fraction *I. arrecta* viewed at 254 nm.
C- Strip tested with *I. arrecta* viewed with the naked eye
D- Anisaldehyde sulfuric acid reagent-stained strip

Table 6: Compounds detected by GC–MS within the bioactive zone of the most active ethyl acetate portion of *Indigofera arrecta* root extract.

S/no	RT (min)	Area Peaks	Qty	Compound	Formular/ MW	PubChem ID
1.	38.3786	0.4697	58	Oxirane, tetradecyl-	$C_{16}H_{32}O$ 240.42g/mol	23741
2.	32.3249	0.9317	58	12-Methyl-E, E-2,13-octadecadien-1-ol	$C_{19}H_{36}O$ 280.5g/mol	90107969
3.	31.6248	1.0237	46	Bromoacetic acid, pentadecyl ester	$C_{17}H_{33}BrO_2$ 349.3g/mol	537044
4.	38.051	1.0302	49	Undec-10-ynoic acid, undecyl ester	$C_{22}H_{40}O_2$ 336.6g/mol	91692431
5.	38.1419	1.0334	58	9-Methyl-Z, Z-10,12-hexadecadien-1-ol acetate	$C_{19}H_{34}O_2$ 294.5g/mol	5363214
6.	31.5408	1.4639	64	13-Octadecenal, (Z)-	$C_{18}H_{34}O$ 266.5g/mol	5364497
7.	6.3419	1.7896	50	Pentafluoropropionic acid, 10-undecenyl ester	$C_{14}H_{21}F_5O_2$ 316.31g/ mol	543371
8.	31.4342	2.5811	64	Behenic alcohol	$C_{22}H_{46}O$ 326.6g/mol	12620
9.	32.2394	2.5998	72	8-Hexadecenal, 14-methyl-, (Z)-	$C_{17}H_{32}O$ 252.4g/mol	5364688
10.	21.5061	3.3874	64	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	$C_{20}H_{30}O_4$ 334.4g/mol	6818
11.	6.2714	7.609	46	1-Azabicyclo [2.2.2] octane, 2,6-dimethyl-, (1.alpha.,2.alpha.,4.alpha.,6.alpha .)-	$C_9H_{17}N$ 139.24g/mol	544800
12.	5.1395	11.2234	90	9,12-Octadecadienal	$C_{18}H_{32}O$ 264.4g/mol	5283383
13.	31.8369	20.6987	58	Oleic Acid	$C_{18}H_{34}O_2$ 282.5g/mol	445639
14.	24.2896	47.7893	47	2-Tridecenal, (E)-	$C_{13}H_{24}O$ 334.4g/mol	5283363

Key: RT-retention time, Qty-quality, MW-molecular weight, ID-identification

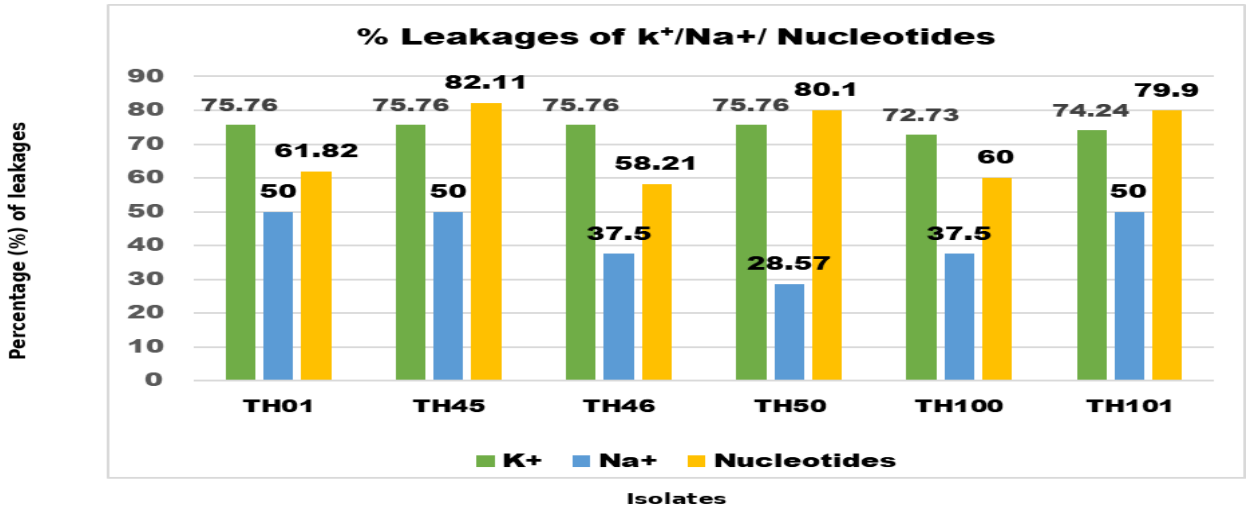


Figure 3: Membrane disruption indicated by nucleotide leakage and ion imbalance in MDR *S. aureus* treated with the active fraction

Key: TH- teaching hospital isolate code (TH01, TH45, TH46, TH50, TH101)

Table 7: Acute toxicity results in Wistar rats after oral dosing of the active fraction of *Indigofera arrecta.*

S/No	Extract/ Fractions	Route of Administration	Dosage Limits
1	Crude methanolic	Oral	>5000mg/kg
2	n-Hexane fraction	Oral	>5000mg/kg
3	Ethyl acetate fraction	Oral	>5000mg/kg
4	n-Butanol fraction	Oral	>5000mg/kg
5	Aqueous fraction	Oral	>5000mg/kg

DISCUSSION

The crude methanol extract and solvent-derived fractions of *Indigofera arrecta* root were tested against multidrug-resistant *Staphylococcus aureus* at different concentration levels. All fractions demonstrated inhibitory activity, with the ethyl acetate fraction showing the strongest effect at the lowest concentration tested. In contrast, the *n*-hexane fraction exhibited the weakest activity, requiring higher concentrations to achieve inhibition (Figure 2). The progressive increase in inhibition with rising concentration indicates a clear dose-dependent response.

These results are in line with earlier studies showing that the ethyl acetate portion of *Indigofera arrecta* leaves displayed strong antibacterial activity [16]. In contrast, some reports have found greater effects in the *n*-hexane fractions of fresh leaves [17]. Such discrepancies may be attributed to differences in plant part utilized, extraction solvent, harvest season, and geographical origin, all of which influence phytochemical composition [18]. Variations in extraction methodology may also contribute to differences observed across studies.

Reports from diverse regions further highlight the variability in plant-derived antibacterial activity. For instance, stem bark extracts collected in Southwestern Nigeria [17], [19], [20] inhibited multiple pathogens, including MRSA, MDR *Salmonella*, *S. pyogenes*, and *S. faecalis*. Conversely, leaf extracts from Ethiopia showed no activity against *Xanthomonas campestris* [21]. Similarly, studies in South Africa reported inhibitory effects of both root and leaf extracts of *I. arrecta* against *S. aureus*, *M. aurum*, and *K. pneumoniae* [22]. These observations suggest that the antibacterial potential of *I. arrecta* is influenced by plant part, geographic origin, and phytochemical diversity.

Column chromatography applied to the ethyl acetate portion produced 82 fractions, of which 20 showed inhibition zones of 15 mm or greater (Table 5). Fraction 12 exhibited the strongest activity and was

selected for direct bioautography. The bioautogram revealed an inhibitory band at Rf = 0.13 (Plate 1), indicating the presence of bioactive constituents in that region.

Phytochemical screening confirmed the presence of carbohydrates, cardiac glycosides, alkaloids, phenols, flavonoids, saponins, and terpenoids (Table 3). GC–MS analysis of the active zone identified 14 compounds (Table 6), with fatty aldehydes being the most abundant. Other compounds included esters, quinuclidine alkaloids, fatty acids, fatty alcohols, and pheromone-related molecules.

Several of these compounds are known antimicrobials. Fatty aldehydes and acids have been reported to possess antibacterial, antiviral, antiparasitic, and antifungal properties [23–25]. Oleic acid, along with other related fatty acids, can disrupt bacterial resistance mechanisms by affecting efflux pumps, quorum sensing, and horizontal gene transfer. Alkaloids are also recognized for disrupting bacterial respiration, DNA topoisomerase activity, and protease function [27, 28].

Mechanistic assays revealed leakage of potassium and sodium ions, along with nucleotide release, indicating that the active fraction compromised membrane integrity and increased permeability (Figure 3). This disruption likely led to loss of essential cellular contents, impaired energy metabolism, and reduced bacterial survival. These outcomes align with mechanisms often described for plant-based antimicrobials [15]. Such compounds can disrupt bacterial processes by interfering with cell wall construction, DNA replication, protein synthesis, and other essential pathways in Gram-positive organisms [27, 28].

Acute toxicity was assessed following OECD guideline 425. Oral dosing of *Indigofera arrecta* root extract in Wistar rats produced no significant adverse effects. These observations indicate the extract is safe at the tested level and point to its promise for future pharmacological exploration [14].

CONCLUSION

The root extract and solvent fractions of *Indigofera arrecta* showed clear antibacterial effects against MDR *Staphylococcus aureus* clinical samples. Among the tested fractions, the ethyl acetate portion was the most effective, likely because of its richer content of active phytochemicals. These findings point to *I. arrecta* root as a valuable source of compounds that could be developed into therapeutic agents for managing MDR *S. aureus* infections.

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AUTHORS' CONTRIBUTION

GS and IYKE designed the study, carried out the experiments, and prepared the initial draft. IYKE and DUH reviewed the first draft. OAF and DMT contributed to data curation. FMM and OJS assisted with rewriting, reviewing, editing, and software-related tasks. Each author confirmed the accuracy of the work and approved the manuscript for publication.

CONFLICT OF INTEREST

This research was conducted independently, without any commercial or personal conflict affecting the results.

FUNDING

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