Phenotypic Antibacterial Resistance Profiles of *Azadirachta indica* and *Calotropis procera* Fresh Leaf Extracts Against Multidrug-Resistant Pathogens

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ABSTRACT

Antibiotic resistance remains a global public health crisis, exacerbated by the rise of multidrug-resistant (MDR) and extensively drug-resistant bacteria. This study investigated the antibacterial activity and resistance mechanisms of *Azadirachta indica* (neem) and *Calotropis procera* (apple of Sodom), two plants traditionally used for their medicinal properties. Both plants contain bioactive compounds with broad antimicrobial effects, yet their genotypic profiles related to resistance mechanisms remain underexplored. We assessed the phenotypic resistance profiles of *A. indica* and *C. procera* leaf extracts using a combination of agar disc diffusion, minimal inhibitory concentration assays, and advanced bacterial identification techniques. Fresh leaves were collected from the Kashere community, Gombe State, Nigeria, and extracts were prepared using methanol and n-hexane. The antibacterial activities of the extracts were tested against several MDR bacterial strains, including *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Salmonella enterica*, *Klebsiella pneumoniae*, and *Aeromonas hydrophila*. Biochemical and morphological characterization of the bacterial isolates revealed significant resistance to a wide array of antibiotics, with the highest resistance observed in *P. aeruginosa*. The plant extracts exhibited promising antibacterial properties, particularly against resistant strains, with variable inhibition zones. The results indicated that *A. indica* and *C. procera* bacteria are resistant to beta-lactams, aminoglycosides, and fluoroquinolones, but are more susceptible to plant extracts, suggesting potential natural alternatives.

Keywords: *Azadirachta indica*, *Calotropis procera*, Multidrug-resistant pathogens, Antibacterial activity, Phenotypic resistance profiles

Introduction

Calotropis procera (Apple of Sodom) and *Azadirachta indica* (neem) have traditionally been used across cultures for their medicinal properties.[1,2] These plants contain various bioactive compounds, including alkaloids, flavonoids, saponins, and terpenoids, demonstrating broad-spectrum antimicrobial effects.[3,4] For example, neem contains compounds like *nimbin* and *azadirachtin*, which have been shown to inhibit various pathogens, including Gram-negative and Gram-positive bacteria.[5] Similarly, due to its high concentration of phenolic compounds and alkaloids, *C. procera* has been found to possess potent antibacterial properties, particularly against resistant bacterial strains.[3]

Despite the well-known antimicrobial potential of both plants, there is limited understanding of their phenotypic profiles regarding antibacterial activity and resistance mechanisms. Gaining insight into genetic factors involved in their antimicrobial effects could help isolate novel compounds and provide deeper insights into how these plants may counteract bacterial resistance.[6] This study aimed to evaluate the phenotypic resistance profiles of *C. procera* and *A. indica* and explore their potential as sources for novel antimicrobial agents, especially in combating ABR.

Multidrug-resistant (MDR) infections pose a serious global health threat due to the diminishing efficacy of existing antibiotics driven by widespread misuse and over prescription in human, veterinary, and agricultural contexts. These infections are challenging to treat because of limited therapeutic options, high morbidity and mortality rates, increased healthcare costs, and a stagnant antibiotic development pipeline.[7] Bacteria rapidly evolve resistance mechanisms, such as efflux pumps and enzymatic degradation of antibiotics, making many conventional treatments ineffective and emphasizing the urgent need for novel therapeutic alternatives.[8]

Plant-based treatments have emerged as a promising strategy to combat MDR pathogens. Phytochemicals such as alkaloids, flavonoids, and terpenoids exhibit broad antimicrobial properties through mechanisms like biofilm disruption and membrane targeting, which differ from traditional antibiotics.[9] Furthermore, some plant extracts demonstrate synergistic effects with conventional drugs, potentially restoring antibiotic efficacy and reducing resistance development.[10] Given their chemical diversity and lower likelihood of inducing resistance, medicinal plants represent a valuable resource for antimicrobial drug discovery. Ethnopharmacological knowledge further enhances this potential by providing culturally validated leads for scientific exploration and clinical development.

Antibiotic resistance (ABR) is one of the most pressing public health concerns globally, as it diminishes the efficacy of widely used antimicrobial drugs, resulting in longer illnesses, higher medical costs, and increased death rates.[11] The rise of MDR and extensively drug-resistant (XDR) bacteria has driven the search for alternative treatments, with plant-based remedies drawing attention due to their promising antimicrobial properties.[12]

C. procera (apple of Sodom) and *A. indica* (neem) have been traditionally used across cultures for their

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medicinal properties.[1,2] These plants contain various bioactive compounds, including alkaloids, flavonoids, saponins, and terpenoids, which have demonstrated broad-spectrum antimicrobial effects.[3,4] Neem, for example, contains compounds like *nimbin* and *azadirachtin*, which have been shown to inhibit various pathogens, including Gram-negative and Gram-positive bacteria.[13] Similarly, *C. procera* has been found to possess potent antibacterial properties, particularly against resistant bacterial strains, owing to its high concentration of phenolic compounds and alkaloids.[3]

Despite the established antimicrobial potential of both plants, there is limited understanding of their phenotypic profiles regarding antibacterial activity and resistance mechanisms. Gaining insights into the genetic factors involved in their antimicrobial effects could help isolate novel compounds and offer deeper insights into how these plants may counteract bacterial resistance.[6] This study aims to evaluate the phenotypic resistance profiles of *C. procera* and *A. indica* while exploring their potential as sources of novel antimicrobial agents, particularly in combating ABR.

Methodology

Plant Collection

Garden-fresh leaves of *A. indica* and *C. procera* were harvested in August 2023 from the Kashere community in Gombe State, Nigeria. The plants were acknowledged and authenticated at the Department of Biological Sciences, Federal University of Kashere, Gombe. The specimens were deposited in the university herbarium under accession numbers FUKHER103 (*A. indica*) and FUKHER102 (*C. procera*).

Extraction of Plant Material

The fresh leaves of both *A. indica* and *C. procera* were thoroughly washed with distilled water to remove dirt and foreign particles. Afterwards, they were air-dried for two weeks in the shade at room temperature (37°C) to avoid exposure to direct sunlight, which could cause oxidation of the chemical components. The dried leaves were ground into coarse particles using a pestle and mortar, then pulverized into a fine powder using an electric blender.[13]

The extraction of bioactive compounds from *A. indica* and *C. procera* often employs a sequential solvent extraction method using methanol and n-hexane to effectively isolate both polar and non-polar phytochemicals. Initially, n-hexane is used to extract non-polar compounds like terpenoids and lipids, followed by methanol to isolate polar constituents such as flavonoids and alkaloids, many of which are associated with antimicrobial properties. This solvent combination is favored due to its ability to maximize phytochemical diversity, thereby increasing the chances of identifying compounds with potential synergistic or complementary activity against MDR pathogens.[14]

The extraction method used was the maceration method, as described by Doughari and Saa-Aondo,[15] utilizing methanol and n-hexane separately. A total of 500 grams of pulverized plant material was soaked in 1.5 L of each solvent for 72 hours, with the extraction process performed on an orbital shaker at 120 rpm. Following the extraction, the macerates were filtered twice using Whatman No. 1 filter paper and cotton wool. The filtrate was concentrated using a rotary evaporator, and the final extract was weighed and stored in a refrigerator at 4°C until needed.[13]

Media Sterilization and Bacterial Culture

All glassware, culture media, and distilled water used in the experiments were autoclaved at 121°C for 15 minutes to ensure sterility.[16] The cork borer and bacteriological wire loop were sterilized by flaming, and a laminar flow cabinet was used for all microbiological procedures. The bacterial cultures were incubated in a controlled environment.[17]

McFarland Turbidity Standard Preparation

To assess bacterial growth, the McFarland turbidity standard was set following the method outlined by Oli et al.[18] Exactly 0.5 McFarland standard was produced by mixing 0.5 mL of 0.048 M $BaCl_2$ (1.17% w/v $BaCl_2 \cdot 2H_2O$) with 99.5 mL of 0.18 M H_2SO_4 (1% v/v). The resultant barium sulfate precipitate was compared visually to a bacterial suspension in sterile saline, regulating the bacterial turbidity to approximately 1×10^6 cells/mL, conforming to a 0.5 McFarland standard.

Preparation of Extract Stock Solutions

Following a modified protocol by Odongo et al,[17] stock solutions of the plant extracts were prepared by dissolving 5 grams of the dried extract in 5 mL of 1% dimethyl sulfoxide (DMSO). DMSO is known for its low vapor pressure, non-toxicity, and enhanced chemical stability, making it suitable for long-term storage and testing.[19]

Agar Disc Diffusion Method

The antibacterial activity of the plant extracts was estimated using the agar disc diffusion method. Mueller-Hinton agar (MHA) was set according to the manufacturer's instructions (38 grams of MHA in 1 L of distilled water, mixed and autoclaved). Bacterial inoculum was made by suspending bacterial colonies in sterile normal saline and regulating the turbidity against a black line to the 0.5 McFarland standard. The bacterial suspension was then evenly spread across the surface of the MHA plate using a sterile wire loop.[20] Sterile 6-mm paper discs were positioned on the inoculated MHA plates, and 50 µL of each plant extract at concentrations of 50, 100, 150, 200, and 250 µg/mL (prepared in 1% DMSO) was applied to the discs. Gentamicin (10 µg/mL) was used as a positive control, and 50 µL of 1% DMSO was the negative control. The plates were incubated at 37°C for 24 hours after a 2-hour diffusion period at room temperature. The zones of inhibition were measured

in millimeters and recorded. Each test was repeated three times, and the average zone of inhibition was calculated.

Antibacterial Activity

The plant extracts' antibacterial potential was evaluated using the standard agar well diffusion method and minimal inhibitory concentration (MIC) tests, following the guidelines set by the Clinical and Laboratory Standards Institute[21] against various bacterial strains.

Morphological Identification of Bacteria

Bacterial isolates were cultured on nutrient agar, mannitol salt agar, and blood agar, and their morphological characteristics, such as colony color, shape, elevation, and edge, were assessed.

Gram Staining of Bacteria

Gram staining was used to classify bacteria based on their cell wall structure, with gram-negative bacteria appearing red and gram-positive bacteria appearing purple. A bacterial smear was prepared on a glass slide, stained with crystal violet, treated with iodine, decolorized with acetone, and counterstained with safranin. The slide was examined under a light microscope.[22]

Biochemical Identification Using the Vitek 2 Compact System

The Vitek 2 Compact system was employed for the automated biochemical identification of bacterial isolates. Gram-negative bacteria were identified using the VITEK 2 GN kit. The bacteria were suspended in normal saline to a turbidity of 0.5–0.62 on the McFarland scale, and the suspensions were inoculated into VITEK 2 identification cards. The results of the identification were accessible within 24 hours.

Determination of Bacterial Resistance Phenotypic Profile

Antibiotic susceptibility testing (AST) was done using the bioMérieux VITEK 2 Compact system. The resistance of Gram-negative bacterial isolates to various antibiotics, including Piperacillin-Tazobactam, Cefotaxime, Ceftazidime, Ertapenem, Meropenem, Gentamicin, and others, was evaluated. The results were examined based on the CLSI break-point standards.[21]

Table 1 | Morphological identification of bacteria

S/N	Bacteria	Colour	Shape	Elevation	Edge	Size	Type
1	*P. mirabilis*	Yellow	Rod	Flat	Entire	Small	GN
2	*P. aeruginosa*	Green	Rod	Flat	Entire	Medium	GN
3	*S. enterica*	White	Rod	Raised	Entire	Small	GN
4	*A. hydrophila*	White	Rod	Flat	Entire	Small	GN
5	*K. pneumonia*	White	Rod	Raised	Entire	Medium	GN

Legend: GN = Gram-negative

Bacteria resistant to three or more antibiotics were categorized as MDR and chosen for testing with plant extracts.[23]

Bacterial Identification and Resistance Phenotyping

Bacterial isolates were identified based on their morphological characteristics and biochemical tests using the Vitek 2 Compact system for automated identification. AST was used to determine ABR, and MDR strains were chosen for further testing with the plant extracts.

Results

The morphological and biochemical features of the bacterial isolates, including *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella enterica*, *Aeromonas hydrophila*, and *Klebsiella pneumoniae*, were confirmed through a series of tests. The resistance profiles of these bacteria revealed resistance to multiple antibiotics, with several strains classified as MDR (Tables 1–6).

The result of the phenotypic resistance profile of the bacteria surveyed is presented in Table 7, with all the bacteria showing resistance to the peak of the antibiotics used in this study. The highest resistance was observed in *P. aeruginosa* (Table 7).

Table 2 | Biochemical identification of *P. aeruginosa*

S/N	Biochemical Details					
1	H_2S	–	ODC	–	PyrA	–
2	APPA	–	BNAG	–	AGLTp	+
3	BGLU	–	dMAL	–	dMAN	+
4	ProA	+	LIP	–	PLE	–
5	GlyA	–	dTAG	–	dTRE	+
6	ILATk	+	AGLU	–	SUCT	+
7	SAC	–	ADO	–	LDC	–
8	O129R	+	GGAA	–	LMLTa	+
9	NAGA	–	dCEL	–	BGAL	–
10	dGLU	+	GGT	+	5KG	–
11	dMNE	+	AGAL	–	BAlap	–
12	TyrA	–	URE	–	dSOR	–
13	CIT	+	MNT	+	OFF	–
14	IARL	–	BXYL	–	PHOS	–
15	LHISa	–	LLATa	+	BGUR	–
16	ELLM	–	CMT	+		

Legend: + = positive, – = negative, PyrA = L-pyrrolydonyl-arylamidase, ADO = adonitol, APPA = ala-phe-pro-arylamidase, H_2S = H_2S production, BGAL = beta-galactosidase, dCEL = D-cellobiose, AGLTP = glutamyl arylamidase PNA, dGLU = D-glucose, GGT = gamma-glutamyl-transferase, OFF = fermentation/glucose, BNAG = beta-N-acetyl-glucosaminidase, BGLU = beta-glucosidase, BXYL = beta-xylosidase, BAlap = beta-alanine arylamidase PNA, ProA = L-proline arylamidase, LIP = lipase, PLE = palatinose, TyrA = tyrosine arylamidase, URE = urease, dMAL = D-maltose, dMAN = D-mannitol, dMNE = D-mannose, dSOR = D-sorbitol, MNT = malonate, CIT = citrate (sodium), SAC = saccharose/sucrose, dTAG = D-tagatose, dTRE = D-trehalose, 5KG = 5-keto-D-gluconate, SUCT = succinate alkalinisation, AGLU = alpha-glucosidase, PHOS = phosphatase, GlyA = glycine arylamidase, ODC = ornithine decarboxylase, LDC = lysine decarboxylase, ODEC = decarboxylase base, LHISa = L-histidine assimilation, CMT = coumarate, BGUR = beta-glucoronidase, o129r = o/129 resistance (comp.vibrio.), GGAA = glu-gly-arg-arylamidase, LMLTa = L-malate assimilation, ELLM = ellman, LLATa = L-lactate assimilation.

Table 3 | Biochemical identification of *S. enterica*

S/N	Biochemical Details					
1	PyrA	–	APPA	–	LLATa	–
2	H_2S	+	AGLTp	–	BNAG	–
3	BGLU	–	dMAN	+	dMAL	+
4	SUCT	+	dTAG	–	LIP	–
5	SAC	–	dTRE	+	PLE	–
6	ILATk	+	ProA	+	AGLU	–
7	GlyA	–	LDC	+	ODC	+
8	CIT	+	5KG	+	GGAA	–
9	IARL	–	BGAL	–	dCEL	–
10	dGLU	+	OFF	+	GGT	+
11	dMNE	+	BAlap	+	BXYL	–
12	TyrA	–	dSOR	+	URE	–
13	O129R	+	LMLTa	+	MNT	+
14	NAGA	–	PHOS	–	AGAL	+
15	LHISa	–	ELLM	+	CMT	+
16	BGUR	+	ADO	–		

Legend: + = positive, – = negative, PyrA = L-pyrrolydonyl-arylamidase, ADO = adonitol, APPA = ala-phe-pro-arylamidase, H_2S = H_2S production, BGAL = beta-galactosidase, dCEL = D-cellobiose, AGLTP = glutamyl arylamidase PNA, dGLU = D-glucose, GGT = gamma-glutamyl-transferase, OFF = fermentation/glucose, BNAG = beta-N-acetyl-glucosaminidase, BGLU = beta-glucosidase, BXYL = beta-xylosidase, BAlap = beta-alanine arylamidase PNA, ProA = L-proline arylamidase, LIP = lipase, PLE = palatinose, TyrA = tyrosine arylamidase, URE = urease, dMAL = D-maltose, dMAN = D-mannitol, dMNE = D-mannose, dSOR = D-sorbitol, MNT = malonate, CIT = citrate (sodium), SAC = saccharose/sucrose, dTAG = D-tagatose, dTRE = D-trehalose, 5KG = 5-keto-D-gluconate, SUCT = succinate alkalinisation, AGLU = alpha-glucosidase, PHOS = phosphatase, GlyA = glycine arylamidase, ODC = ornithine decarboxylase, LDC = lysine decarboxylase, ODEC = decarboxylase base, LHISa = L-histidine assimilation, CMT = coumarate, BGUR = beta-glucoronidase, o129r = o/129 resistance (comp.vibrio.), GGAA = glu-gly-arg-arylamidase, LMLTa = L-malate assimilation, ELLM = ellman, LLATa = L-lactate assimilation.

Table 4 | Biochemical identification of *K. pneumonia*

S/N	Biochemical Details					
1	ODC	–	ADO	+	PyrA	–
2	H_2S	–	BNAG	–	AGLTp	–
3	BGLU	+	dMAL	+	dMAN	+
4	ProA	–	LIP	–	PLE	+
5	SAC	+	dTAG	+	dTRE	+
6	ILATk	+	AGLU	+	LLATa	–
7	GlyA	–	APPA	–	LDC	+
8	O129R	–	GGAA	–	LMLTa	–
9	IARL	–	dCEL	+	BGAL	+
10	dGLU	+	BGUR	–	OFF	+
11	dMNE	+	BXYL	+	BAlap	–
12	TyrA	+	URE	+	dSOR	+
13	CIT	+	MNT	+	NAGA	–
14	5KG	–	AGAL	+	PHOS	+
15	LHISa	–	CMT	–	GGT	–
16	ELLM	–	SUCT	–		

Legend: + = positive, – = negative, PyrA = L-pyrrolydonyl-arylamidase, ADO = adonitol, APPA = ala-phe-pro-arylamidase, H_2S = H_2S production, BGAL = beta-galactosidase, dCEL = D-cellobiose, AGLTP = glutamyl arylamidase PNA, dGLU = D-glucose, GGT = gamma-glutamyl-transferase, OFF = fermentation/glucose, BNAG = beta-N-acetyl-glucosaminidase, BGLU = beta-glucosidase, BXYL = beta-xylosidase, BAlap = beta-alanine arylamidase PNA, ProA = L-proline arylamidase, LIP = lipase, PLE = palatinose, TyrA = tyrosine arylamidase, URE = urease, dMAL = D-maltose, dMAN = D-mannitol, dMNE = D-mannose, dSOR = D-sorbitol, MNT = malonate, CIT = citrate (sodium), SAC = saccharose/sucrose, dTAG = D-tagatose, dTRE = D-trehalose, 5KG = 5-keto-D-gluconate, SUCT = succinate alkalinisation, AGLU = alpha-glucosidase, PHOS = phosphatase, GlyA = glycine arylamidase, ODC = ornithine decarboxylase, LDC = lysine decarboxylase, ODEC = decarboxylase base, LHISa = L-histidine assimilation, CMT = coumarate, BGUR = beta-glucoronidase, o129r = o/129 resistance (comp.vibrio.), GGAA = glu-gly-arg-arylamidase, LMLTa = L-malate assimilation, ELLM = ellman, LLATa = L-lactate assimilation.

Table 5 | Biochemical identification of *A. hydrophila*

S/N	Biochemical Details					
1	ADO	–	APPA	–	LLATa	–
2	BNAG	+	GGAA	+	AGLTp	–
3	dMAL	+	BGLU	–	dMAN	+
4	LIP	–	ProA	–	PLE	+
5	dTAG	–	SAC	+	dTRE	+
6	NAGA	–	OFF	+	SUCT	+
7	ODC	–	GlyA	–	LDC	–
8	H_2S	+	O129R	+	LMLTa	–
9	dCEL	–	IARL	–	BGAL	+
10	GGT	–	dGLU	+	ILATk	+
11	BXYL	–	dMNE	+	BAlap	–
12	URE	+	TyrA	–	dSOR	–
13	MNT	–	CIT	–	5KG	–
14	AGAL	–	AGLU	–	PHOS	–
15	CMT	+	LHISa	–	BGUR	–
16	PyrA	–	ELLM	+		

Legend: + = positive, – = negative, PyrA = L-pyrrolydonyl-arylamidase, ADO = adonitol, APPA = ala-phe-pro-arylamidase, H_2S = H_2S production, BGAL = beta-galactosidase, dCEL = D-cellobiose, AGLTP = glutamyl arylamidase PNA, dGLU = D-glucose, GGT = gamma-glutamyl-transferase, OFF = fermentation/glucose, BNAG = beta-N-acetyl-glucosaminidase, BGLU = beta-glucosidase, BXYL = beta-xylosidase, BAlap = beta-alanine arylamidase PNA, ProA = L-proline arylamidase, LIP = lipase, PLE = palatinose, TyrA = tyrosine arylamidase, URE = urease, dMAL = D-maltose, dMAN = D-mannitol, dMNE = D-mannose, dSOR = D-sorbitol, MNT = malonate, CIT = citrate (sodium), SAC = saccharose/sucrose, dTAG = D-tagatose, dTRE = D-trehalose, 5KG = 5-keto-D-gluconate, SUCT = succinate alkalinisation, AGLU = alpha-glucosidase, PHOS = phosphatase, GlyA = glycine arylamidase, ODC = ornithine decarboxylase, LDC = lysine decarboxylase, ODEC = decarboxylase base, LHISa = L-histidine assimilation, CMT = coumarate, BGUR = beta-glucoronidase, o129r = o/129 resistance (comp.vibrio.), GGAA = glu-gly-arg-arylamidase, LMLTa = L-malate assimilation, ELLM = ellman, LLATa = L-lactate assimilation.

Table 6 | Biochemical identification of *P. mirabilis*

S/N	Biochemical Details					
1	ADO	–	ODC	–	PyrA	+
2	BNAG	–	H2S	–	AGLTp	+
3	dMAL	–	BGLU	+	dMAN	+
4	LIP	+	ProA	+	ILATk	–
5	dTAG	+	SAC	–	dTRE	–
6	AGLU	+	PLE	–	URE	+
7	APPA	–	GlyA	+	LDC	+
8	GGAA	+	O129R	+	LMLTa	–
9	dCEL	–	IARL	+	BGAL	+
10	GGT	–	dGLU	–	TyrA	+
11	BXYL	–	dMNE	–	BAlap	+
12	SUCT	+	OFF	+	dSOR	–
13	MNT	–	CIT	+	5KG	–
14	AGAL	+	NAGA	+	PHOS	+
15	CMT	+	LHISa	+	BGUR	+
16	LLATa	–	ELLM	–		

Legend: + = positive, – = negative, PyrA = L-pyrrolydonyl-arylamidase, ADO = adonitol, APPA = ala-phe-pro-arylamidase, H_2S = H_2S production, BGAL = beta-galactosidase, dCEL = D-cellobiose, AGLTP = glutamyl arylamidase PNA, dGLU = D-glucose, GGT = gamma-glutamyl-transferase, OFF = fermentation/glucose, BNAG = beta-N-acetyl-glucosaminidase, BGLU = beta-glucosidase, BXYL = beta-xylosidase, BAlap = beta-alanine arylamidase PNA, ProA = L-proline arylamidase, LIP = lipase, PLE = palatinose, TyrA = tyrosine arylamidase, URE = urease, dMAL = D-maltose, dMAN = D-mannitol, dMNE = D-mannose, dSOR = D-sorbitol, MNT = malonate, CIT = citrate (sodium), SAC = saccharose/sucrose, dTAG = D-tagatose, dTRE = D-trehalose, 5KG = 5-keto-D-gluconate, SUCT = succinate alkalinisation, AGLU = alpha-glucosidase, PHOS = phosphatase, GlyA = glycine arylamidase, ODC = ornithine decarboxylase, LDC = lysine decarboxylase, ODEC = decarboxylase base, LHISa = L-histidine assimilation, CMT = coumarate, BGUR = beta-glucoronidase, o129r = o/129 resistance (comp.vibrio.), GGAA = glu-gly-arg-arylamidase, LMLTa = L-malate assimilation, ELLM = ellman, LLATa = L-lactate assimilation.

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Table 7 | Phenotypic resistance profile of bacteria tested

Bacteria	Resistance Profile	MDR
P. mirabilis	AMC-TZP-CIP-SXT-CTX-CAZ	Yes
P. aeruginosa	ETP-AMK-CIP-SXT, AMC-TZP-CTX-CAZ-	Yes
S. enterica	AMC-TZP-CTX-CAZ-CIP-SXT	Yes
K. pneumonia	AMC-TZP-SXT-CAZ-ETP-CIP-CTX	Yes
A. hydrophila	CAZ-TZP-CTX-AMC-SXT	Yes

Legend: TZP = piperacillin-tazobactam, CTX = cefotaxime, CAZ = ceftazime, ETP = ertapenem, MEM = meropenem, AMK = amikacin, CIP = cekazime, TGC = tigecycline, FOF = fosfomycin, CST = colistin, SXT = trimethoprim-sulfamethoxazole.

Discussion

This study highlights the genotypic resistance of clinical bacterial isolates and the potential of *C. procera* and *A. indica* as natural sources of antimicrobial agents. The existence of carbapenemase DNAs in the bacterial isolates underlines the importance of developing new therapeutic strategies to combat ABR. Further investigation into the active compounds within these houseplants could lead to the discovery of novel antibiotics or adjunct therapies to enhance the efficacy of existing treatments. Significant activity was noted in the zones of inhibition caused by *C. procera* extracts in methanol and N-hexane against all five tested bacteria. At the highest dose of 250 µg/mL, the *P. mirabilis* strain showed the largest zone of inhibition, measuring 25 mm. *C. procera's* n-hexane extract is ineffective against *P. aeruginosa*.

The antibacterial activity of *A. indica* and *C. procera* observed in this study is consistent with prior research. However, differences in extraction methods, bacterial strains, and geographical variation may account for some variability in results. For *A. indica*, the inhibition zones recorded here, particularly against *P. aeruginosa* and *P. mirabilis*, align well with those reported by Bhowmik et al.[24] and Biswas et al.,[25] who observed zones ranging from 15–25 mm and 16–22 mm, respectively, using ethanol-based extracts. Similarly, the MIC values found in this study are comparable to those documented by Kausik et al. (2002), who reported MICs in the range of 62.5–250 µg/mL against common pathogens like *S. aureus* and *E. coli*, reinforcing neem's potential against MDR strains.

In the case of *C. procera*, this study found moderate-to-strong inhibition zones across several MDR bacteria, slightly lower in magnitude compared to *A. indica* but still noteworthy. These results are consistent with those reported by Qbal et al.[26] and Chandrappa et al.,[27] who documented inhibition zones ranging from 12–20 mm against various pathogens. The MIC values observed here also fall within the range reported by Kumar et al,[28] who found MICs of 125–500 µg/mL, suggesting the efficacy of *C. procera* is maintained across different experimental setups. These comparisons support the continued exploration of both plants as potential sources of alternative antibacterial agents, especially against resistant strains where conventional antibiotics may fail.

The occurrence of flavonoid and tannin compounds may cause antibacterial activity. Flavonoid compounds work as antibacterial agents by forming complex compounds in extracellular proteins that cause the proteins in bacteria to denature and damage the cell membrane. Tannin chemicals also work against bacteria by causing cell walls or membranes to wrinkle, impairing cell permeability, and even killing cells. The present study is in agreement with the findings of Abegunde et al.,[29] Mainasara et al.,[30] Oladimeji et al.,[31] Muthuvelan and Balaji Raja,[32] Filgona et al.[33] and Adamu et al,[34] that *C. procera* demonstrated good antibacterial activity. This work supports the discovery made by Sabzal et al.[35] that *C. procera* methanol extract has antibacterial properties against some bacterial pathogens. *A. indica* methanol extract at 250 µg/mL was reported to have a strong zone of inhibition against *P. mirabilis, P. aeruginosa, S. enterica, K. pneumoniae,* and *A. hydrophila*, measuring 26, 17, 22, 23, and 20 mm, respectively. Regarding *K. pneumoniae*, the N-hexane extract of *A. indica* exhibited good action, with the maximum zone of inhibition measuring 23 mm. The result of this present study agrees with the findings of Edet,[36] Benisheikh et al,[37] Fatima[38] and Bappah et al.[39] that *A. indica* has good antibacterial activity.

Significant activity was seen against five tested bacteria by the MIC and minimum bactericidal concentration of methanol and N-hexane extracts of *C. procera* and *A. indica* leaves. The study's findings demonstrated that every extract tested had bacteriostatic action only against *P. aeruginosa*. The extract showed higher antibacterial efficacy when comparing the plant extract to the commonly used antibiotic (Gentamicin) against *P. mirabilis*. Significant activity of gentamicin against *P. aeruginosa, S. enterica*, and *K. pneumoniae* was recorded; these results are consistent with those of Samsudin et al.[40] and Ayehubizu et al.[41] The result of the negative control of the microorganisms studied displays no bacterial activity when 1% DMSO is reconstituted in distilled water.[42]

Previous research has shown that certain plant-derived compounds can restore antibiotic sensitivity in resistant bacterial strains by disrupting resistance mechanisms or enhancing membrane permeability. Therefore, future investigations should include combination therapy studies, such as checkerboard assays or time-kill kinetics, to evaluate possible synergistic interactions between these plant extracts and widely used antibiotics. Such findings could pave the way for novel, integrated treatment regimens capable of overcoming current therapeutic limitations in managing MDR and XDR bacterial infections.

Conclusion

This study has demonstrated the significant antibacterial potential of *A. indica* (neem) and *C. procera* (apple of Sodom) leaf extracts against a range of MDR bacterial strains, including *P. aeruginosa*, *P. mirabilis*, *S. enterica*, *K. pneumoniae*, and *A. hydrophila*. The plant extracts unveiled variable but promising antibacterial activities, especially against bacteria resistant to common antibiotics such as beta-lactams, aminoglycosides, and fluoroquinolones. The resistance profiles

of the bacterial strains have long established their MDR nature, underlining the growing threat of ABR. Compared to synthetic antibiotics, the plant extracts displayed heightened effectiveness against several resistant strains, stressing their potential as alternative therapeutic agents. The study also sheds light on the need for further investigation into the genotypic resistance mechanisms of these plants, which could lead to the identification of novel bioactive compounds for developing new antimicrobial agents.

Recommendations

While this study provides valuable insights into the antibacterial activity of *A. indica* and *C. procera* against MDR pathogens, several limitations must be acknowledged. Primarily, the research was conducted using *in vitro* methods such as agar disc diffusion and MIC assays, which, although informative, do not fully replicate the complexity of living organisms. The lack of *in vivo* validation means that the efficacy, bioavailability, and potential toxicity of the plant extracts remain uncertain when administered systemically. Furthermore, the study did not explore the pharmacokinetic or pharmacodynamic properties of the bioactive compounds, which are critical for evaluating therapeutic viability. Therefore, future research should focus on *in vivo* studies using appropriate animal models to assess the safety, efficacy, and metabolic behavior of these plant-derived extracts. Such investigations would help bridge the gap between laboratory findings and clinical applications, supporting the development of plant-based alternatives to conventional antibiotics.

Given these promising results, it is recommended that future studies focus on the isolation, characterization, and mechanistic evaluation of specific phytochemicals within these plants. In particular, compounds such as azadirachtin, nimbin, and quercetin from *A. indica*, and cardenolides, calotropin, and uscharin from *C. procera*, have been previously identified as having strong antimicrobial properties and warrant further investigation. Furthermore, *in vivo* studies using appropriate animal models are necessary to evaluate the safety, efficacy, pharmacokinetics, and toxicity profiles of these compounds. Finally, exploring potential synergistic interactions between plant extracts and conventional antibiotics could lead to the development of more effective, lower-dose therapies that help mitigate resistance development.

Future reports should focus on isolating and characterizing the specific bioactive compounds responsible for the perceived antimicrobial activities in *A. indica* and *C. procera*. This will ease the development of targeted therapies and provide a clearer understanding of their mechanisms of action. The phenotypic resistance profiling conducted in this study should be complemented with molecular studies to explore the genetic basis of the antibacterial properties of these plants. This can provide insights into the underlying resistance mechanisms and the potential for resistance development. Investigating the synergistic effects of plant extracts in combination with existing antibiotics could offer promising strategies to overcome ABR. This method may help reduce the effective dosage of antibiotics, thereby lessening side effects and slowing down the development of resistance. *In vivo* studies are recommended to further evaluate the therapeutic potential of *A. indica* and *C. procera*. Animal models could provide more information on the efficacy, safety, and pharmacokinetics of the extracts in treating infections caused by MDR bacteria. To guarantee the safety and consistency of plant-based treatments, there is a need for standardized extraction methods and quality control measures. The efficacy of these plant extracts can be optimized by establishing proper dosages and formulations for clinical use. Given the growing global distress over antimicrobial resistance, awareness campaigns should be launched to educate the public and healthcare providers about the potential benefits of plant-based antimicrobials. This could boost the adoption of sustainable and alternative approaches to managing resistant infections. By advancing these research directions, *A. indica* and *C. procera* could become essential sources of new antimicrobial agents, offering a promising solution to the global challenge of MDR infections.

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