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Research Article

Antibacterial Activity of *Azadirachta indica* Leaf Extracts Against Clinical Isolates of *Klebsiella pneumoniae* and *Staphylococcus aureus*

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ABSTRACT
The increasing prevalence of antibiotic-resistant bacterial pathogens has necessitated the search for alternative antimicrobial agents from natural sources. This study investigated the phytochemical constituents and antibacterial activity of crude leaf extracts of *Azadirachta indica* against *Staphylococcus aureus* and *Klebsiella pneumoniae*. Fresh *A. indica* (neem) leaves were collected, authenticated, air-dried, and pulverized before extraction using ethanol and distilled water. Standard qualitative phytochemical screening methods were used to identify the presence of Phytochemical constituents. Antibacterial activity was evaluated using the agar well diffusion method, while Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined using broth dilution techniques. The results revealed the presence of flavonoids, steroids, tannins, alkaloids, saponins, terpenoids, glycosides, and phenolics compounds in both extracts, with higher concentrations observed in the ethanolic extract. Both extracts demonstrated antibacterial activity against the test organisms, with the ethanolic extract showing larger zones of inhibition ranging from 12.5 ± 0.4 -25.1 ± 0.5 and lower MIC and MBC values compared to the aqueous extract. The findings confirm the antibacterial potential of *Azadirachta indica* leaves and support their traditional use in the management of bacterial infections. The study highlights the potential of *A. indica* leaf extracts as alternative antimicrobial agents against *Klebsiella pneumonniae* and *Staphylococcus aureus*.

Keywords: Antibacterial Activity; *Azadirachta indica*; *Klebsiella pneumoniae;* Medicinal plants; Phytochemicals; *Staphylococcus aureus*

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INTRODUCTION

The alarming global rise in antimicrobial resistance (AMR) is one of the most disturbing to modern medicine, public health, and global development. Wound and urinary tract infections remain a major cause of morbidity in healthcare settings, particularly due to the involvement of opportunistic bacterial pathogens such as *Staphylococcus aureus*, a gram-positive bacterium and *Klebsiella pneumoniae*. *Staphylococcus aureus* is commonly isolated from infected wounds and it is known for its ability to cause tissue damage, delayed healing, and resistance to several antimicrobial agents. Similarly, *Klebsiella pneumoniae* a gram-negative bacterium is frequently associated with urinary tract infections, especially those related to the use of urinary catheters (Nordmann *et al*., 2011). The increasing resistance of these pathogens to conventional antibiotics has necessitated the search for alternative antimicrobial agents, including medicinal plants such as *Azadirachta indica* (O'Neill, 2016; WHO, 2023)

These pathogens have evolved multiple resistance mechanisms, including drug-degrading enzymes, altered target sites and biofilm formation which together contribute to their persistence and virulence. The growing failure of synthetic antibiotics has triggered a significant shift toward alternative therapeutic approaches, especially those derived from natural sources (David and Daum, 2017; Centers for Disease Control [CDC] 2022). Medicinal plants, which have been used for centuries in traditional medicine, are now being re-investigated through modern scientific lenses for their potential to combat resistant microbes (Seyedalinghi *et al*., 2025).

MATERIALS AND METHODS

Study Area

The study was carried out within Kaduna metropolis, Kaduna State, Nigeria. All laboratory analyses were carried out in the Department of Microbiology, Kaduna State University (KASU).

Collection and Identification of Plant Material

Azadirachta indica leaves were collected from Kaduna State University, and authenticated at the Department of Biology (Botany) of the University with an assigned voucher number KASU/BSH/3342. The Fresh neem leaves were washed under running tap, air dried, and ground into fine powder using mortar and pestle, the powder was stored in an airtight container until use.

Extraction of Plant Material

Five hundred grams (500 g) of powdered leaves were extracted separately using 2500 mL of ethanol and 2500 mL of distilled water in a Soxhlet apparatus. Aqueous extraction was carried out at 95–100°C, while ethanol extraction was conducted at 60–70°C for 6–8 hours. Extracts were filtered and concentrated using a rotary evaporator at ≤45°C. The dried extracts were weighed and stored at 4°C (Harborne, 1998).

Percentage yield (%) = (W2 − W1) / W0 × 100 (Musa *et al*., 2021).

Phytochemical Screening

Qualitative phytochemical screening was carried out to detect the presence of major secondary metabolites, including alkaloids, flavonoids, tannins, saponins, terpenoids, glycosides, and phenolic compounds, using standard laboratory procedures as described by Cheesbrough (2010)

Collection and Reconfirmation of Bacterial Isolates

Collection of Clinical and Reference Isolates

Clinical isolates of *Klebsiella pneumoniae* and *Staphylococcus aureus* were obtained from General Hospital Kawo, Kaduna State, Nigeria. Reference (typed) strains of *Klebsiella pneumoniae* (ATCC 13883) and *Staphylococcus aureus* (ATCC 25923) were obtained from the National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria, and were used as positive controls.

Bacterial Culture

An inoculum from overnight cultures of *Klebsiella pneumoniae* and *Staphylococcus aureus* was streaked onto freshly prepared MacConkey agar (MA) and Mannitol Salt Agar (MSA), respectively. The plates were incubated at 37°C for 24 hours to obtain pure cultures of the test isolates (Andrew, 2019).

Reconfirmation of Clinical Isolates

Reconfirmation of the isolates was carried out using Gram staining and biochemical tests including Methyl red, voges proskauer catalase, coagulase, oxidase, and citrate tests (Cheesbrough, 2010).

Phytochemical Screening of Extracts

Phytochemical screening of the extracts was carried out to determine the following bioactive compounds; tannins, flavonoids, steroids, glycosides, terpenoids and phenolics, alkaloids (Musa *et al*., 2021).

Preparation of Plant Extract and Antibiotic Concentrations

One gram (1 g) each of the aqueous and ethanolic crude extracts of *Azadirachta indica* leaves was weighed and dissolved in 10mL of 10% dimethyl sulfoxide (DMSO) to obtain a stock concentration of 100mg/mL**.** Using two-fold serial dilution, concentrations of 50 ,25 and 12.5mg/mL were prepared from each stock solution. Similarly, 0.5g of ciprofloxacin was dissolved in 10mL of 10% DMSO to obtain a concentration of 50 mg/mL**,** which served as the positive control (Shrivastava *et al*., 2018).

Standardization of Inoculum

A 0.5 McFarland standard (1.5×10^8 CFU/mL) was used to standardize bacterial suspensions (Cheesbrough, 2010).

Antibacterial Activities of Aqueous and Ethanolic Crude Extracts of *Azadirachta indica* Leaf

The antibacterial activity of the extracts was determined using the agar well diffusion method as described by Bauer *et al*. (2020). Mueller–Hinton agar was prepared according to the manufacturer's instructions and poured into sterile Petri dishes. After

solidification, the surface of the agar was inoculated with a standardized bacterial suspension. A sterile 6 mm cork borer was used to create wells in the agar. Different concentrations of the extracts were introduced into the wells. The plates were allowed to stand at room temperature for 2 hours to allow diffusion of the extracts into the agar. Thereafter, the plates were incubated at 37°C for 24 hours. Zones of inhibition were measured using a transparent ruler and recorded in millimeters (mm). Ciprofloxacin was used as the standard antibiotic control.

Determination of MIC and MBC

The Minimum Inhibitory Concentration (MIC) of the extracts was determined using the broth dilution method. Serial two-fold dilutions (100, 50, 25, and 12.5 mg/mL) were prepared in Mueller–Hinton broth. Each tube was inoculated with a standardized bacterial suspension and incubated at 37°C for 24 hours. The MIC was defined as the lowest concentration of the extract that showed no visible bacterial growth.

The Minimum Bactericidal Concentration (MBC) was determined by sub-culturing aliquots from tubes showing no visible growth onto nutrient agar plates, followed by incubation at 37°C for 24 hours. The MBC was recorded as the lowest concentration that resulted in no bacterial growth on the agar plates.

The negative control consisted of Mueller–Hinton broth only and Mueller–Hinton broth containing the highest concentration of the extract (100 mg/mL) without bacterial inoculum. The positive control consisted of Mueller–Hinton broth inoculated with the test organism and Mueller–Hinton broth containing the standard antibiotic CLSI (2025)

Data Analysis

The agar well diffusion assay was performed in triplicate, and results were expressed as mean ± standard deviation (SD). Statistical analysis was carried out using analysis of variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT) to determine significant differences among the test isolates and the control. A p-value ≤ 0.05 was considered statistically significant.

RESULTS

The physical characteristics and percentage yields of aqueous and ethanolic crude extracts of *A. indica* are represented in Table 1. The extraction process yielded 12.98% and 6.48% respectively.

The phytochemical screening revealed the presence of several Phytochemical constituents in both aqueous and ethanolic extracts of *Azadirachta indica* leaf such as tannins, flavonoids, steroids, glycosides, terpenoids and phenolics, alkaloids (Table 2)

The antibacterial activities of aqueous and ethanolic leaf extracts, ciprofloxacin, and DMSO against clinical and reference isolates of *Klebsiella pneumoniae* and *Staphylococcus aureus* are presented in (Table 3). Aqueous and ethanolic extracts exhibited concentration-dependent antibacterial activities against the test bacterial isolates at concentrations of 100, 50, 25, and 12.5 mg/mL. The ethanolic extract showed higher antibacterial activity across all tested concentrations compared to the aqueous extract. At 100 mg/mL, the ethanolic extract produced mean zones of inhibition ranging from 23.7 ± 1.0 to 25.1 ± 0.5 mm, while the aqueous extract at the same concentration yielded zones ranging from 20.2 ± 0.4 to 23.0 ± 1.0 mm. The standard antibiotic ciprofloxacin at 50 mg/mL demonstrated the highest antibacterial activity, with inhibition zones ranging from 27.8 ± 0.6 to 32.7 ± 0.4 mm, significantly outperforming both plant extracts. DMSO, used as the negative control, showed no antibacterial activity against any of the test organisms (0.0 ± 0.0 mm). Means with same letter(s) in a column are not significantly different.

The MIC and MBC values of the and aqueous and ethanolic extracts against clinical and reference isolates of *Klebsiella pneumoniae* and *Staphylococcus aureus* are presented in (Table 4). For the clinical isolate of *K. pneumoniae*, aqueous extract showed weaker activity, with a MIC of 100 mg/mL and an MBC value not detected. The reference isolate was more susceptible than the clinical isolate. In contrast, the ethanolic extract exhibited a MIC of 50 mg/mL and an MBC of 100 mg/mL, indicating moderate antibacterial activity.

Table 1: Cultural and Biochemical Characteristics of Bacterial Isolates

Bacteria	Colony Appearance	Gram Reaction	Cell Morphology	IN	MR	VP	CI	MO	CA	CO	OX	Number of isolates confirmed
Klebsiella pneumoniae	Large, mucoid, cream colonies	Negative	Rod	−	−	+	+	−	+	−	−	3
Staphylococcus aureus	Smooth, golden yellow colonies	Positive	Cocci (clusters)	−	+	−	−	−	+	+	−	4

KEYS: IN = Indole, MR =Methyl Red, VP =Voges Proskauer, CI = Citrate utilization, MO = Motility, CA = Catalase, CO = Coagulase, OX =Oxidase, + = Positive, -= Negative

Table 2: Physical characteristics and percentage yields of Aqueous and Ethanolic Crude Extract of *Azadirachta indica* Leaf

Solvent	Physical Characteristics	Solubility	Weight of Extract (g)	%yield
Aqueous	Dark green Solid	Water	64.9	13.0
Ethanol	Brownish green Semi-solid	DMSO	32.4	6.8

Table 3: Qualitative Phytochemical Constituents of *Azadirachta indica* Leaf extracts

Phytochemical	Aqueous	Ethanolic
Saponins	+	+
Tannins	+	+
Flavonoids	+	+
Alkaloids	+	+
Steroids	_	+
Glycosides	+	+
Terpenoids	+	+
Phenolics	+	+

KEYS: + (Present), – (absent)

Table 4: Antibacterial Activities of Aqueous and Ethanolic Extracts of *Azadirachta indica* Leaf (MZI±SD) mm

Solvent	Conc. (mg/mL)	*Klebsiella pneumoniae* (CL)	*Klebsiella pneumoniae* (RI)	*Staphylococcus aureus* (CL)	*Staphylococcus aureus* (RI)
Aqueous	100	20.3 ± 0.5^{a}	20.2 ± 0.4^{a}	23.0 ± 1.0^{a}	22.7 ± 0.6^{a}
	50	18.0 ± 0.0^{b}	17.5 ± 0.5^{b}	20.3 ± 0.6^{b}	19.7 ± 0.6^{b}
	25	15.4 ± 0.6^{c}	14.0 ± 0.0^{c}	16.0 ± 1.0^{c}	15.0 ± 0.0^{c}
	12.5	0.0 ± 0.0^{d}	10.7 ± 0.6^{d}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}
Ethanolic	100	24.3 ± 1.0^{a}	23.7 ± 1.0^{a}	24.2 ± 1.0^{a}	25.1 ± 0.5^{a}
	50	21.3 ± 0.5^{b}	21.7 ± 0.6^{b}	22.3 ± 0.6^{b}	22.0 ± 0.0^{b}
	25	17.3 ± 0.6^{c}	17.7 ± 0.5^{c}	18.0 ± 1.0^{c}	18.3 ± 0.6^{c}
	12.5	12.5 ± 0.4^{d}	13.3 ± 0.6^{d}	14.0 ± 1.0^{d}	13.0 ± 0.0^{d}
Ciprofloxacin	50	27.8 ± 0.6^{a}	29.4 ± 0.4^{a}	31.5 ± 0.5^{a}	32.7 ± 0.4^{a}
DMSO	–	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}

KEYS: Means with same letters in a column are not significantly different at P≤ 0.05 level of significance. MZI ± SD= Mean Zones of inhibition ± Standard deviation, CL= Clinical, RI= Reference isolates, DMSO= Dimethyl sulfoxide

Table 5: Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentration (MBCs) of Aqueous and Ethanolic Extracts of *Azadirachta indica* Against Bacterial isolate

Bacteria	Extracts	MIC (mg/mL)	MBC (mg/mL)
*K. pneumoniae (*Cl*)*	Aqueous	100	ND
	Ethanolic	50	100
*K. pneumoniae (*RI*)*	Aqueous	50	100
	Ethanolic	25	50
*S. aureus (*Cl*)*	Aqueous	50	100
	Ethanolic	25	50
*S. aureus (*RI*)*	Aqueous	25	50
	Ethanolic	12.5	25

KEYS: ND= Not detected, CI= Clinical isolate, RI= Reference isolate

DISCUSSION

The extraction of *Azadirachta indica* leaf (neem) using distilled water and ethanol resulted in extracts with different phytochemical constituents and antibacterial activities. Although the aqueous extract yielded a greater quantity of crude extract than the ethanol extract, the ethanol extract demonstrated stronger antibacterial activity. This observation indicates that the biological effectiveness of plant extracts depends more on the quality and concentration of Phytochemical constituents than on the total quantity of extract obtained. This finding aligns with the report of Mudenda *et al*. (2023), who noted that solvent polarity significantly influences the type of phytochemicals extracted and that antimicrobial activity is primarily determined by the richness of active constituents rather than extract yield.

Phytochemical screening of the aqueous and ethanolic extracts revealed the presence of several secondary metabolites, including flavonoids, tannins, alkaloids, terpenoids, steroids, glycosides, saponins, and phenolics. These phytochemicals are widely recognized for their antimicrobial properties. Flavonoids and phenolic compounds are known to disrupt bacterial cell membranes, inhibit nucleic acid synthesis, and interfere with essential metabolic processes, while alkaloids inhibit bacterial enzyme systems. Terpenoids and saponins further enhance antimicrobial effects by increasing membrane permeability and causing leakage of intracellular components. These mechanisms provide a scientific explanation for the antibacterial activities observed in the present study. This observation corresponds with the work of Khanal (2024), who reported that phytochemical constituents such as flavonoids, phenolics, and alkaloids contribute significantly to the antimicrobial activity of medicinal plants.

The antibacterial activities demonstrated that both aqueous and ethanolic extracts of *Azadirachta indica* leaf exhibited inhibitory activity against *Staphylococcus aureus* and *Klebsiella pneumoniae*. However, the ethanol extract consistently produced larger zones of inhibition and lower MIC and MBC values than the aqueous extract across all tested concentrations. This result indicates that ethanol was more effective in extracting antibacterial compounds from neem leaves. The superior performance of the ethanolic extract may be attributed to its ability to dissolve a broader range of phytochemicals, including moderately polar and non-polar compounds, which are often responsible for antimicrobial activity. This finding aligns with the study by Shrestha (2024), Bhatt (2024), who reported that ethanolic extracts of *Azadirachta indica* leaves exhibited stronger antibacterial activity against both Gram-positive and Gram-negative bacteria compared to aqueous extracts.

Overall, the results of this study demonstrate that *Azadirachta indica* leaf extracts possess significant antibacterial activity against *Staphylococcus aureus* and *Klebsiella pneumoniae*, with the ethanolic extract showing greater potency than the aqueous extract. These findings support the continued exploration of plant-based antimicrobials as affordable, accessible, and sustainable alternatives to conventional antibiotics, particularly in regions where antimicrobial resistance poses a major public health challenge.

CONCLUSION

Aqueous leaf extract of A*zadirachta indica* yielded a greater quantity of crude extract than the ethanol extract, but the ethanolic extract demonstrated stronger antibacterial activity, with lower MIC and MBC. These findings confirm that *Azadirachta indica*

leaves possess significant antibacterial potential and support their traditional use in the treatment of bacterial infections.

Further studies should be conducted to isolate and characterize the specific bioactive compounds responsible for the antibacterial activity of *Azadirachta indica* leaf extracts.

In vivo antimicrobial and toxicity studies should be carried out to evaluate the safety and therapeutic effectiveness of the extracts before clinical application.

The potential synergistic effects of *Azadirachta indica* extracts in combination with conventional antibiotics should be investigated to enhance antibacterial efficacy and reduce the development of antibiotic resistance.

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Conflict of interest

The authors declare no conflict of interest

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