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Phytochemical Profiling and Antibacterial Efficacy of *Azadirachta indica* Against Antimicrobial-Resistant *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella typhi* Isolated from Dental Plaque

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Abstract

The global rise of antimicrobial resistance (AMR) underscores an urgent need for alternative therapeutic strategies. Dental plaque has been identified as a key reservoir for multidrug-resistant bacteria, presenting a significant clinical challenge. Aqueous and methanolic extracts were prepared using cold maceration. Qualitative phytochemical screening identified the presence of alkaloids, flavonoids, tannins, saponins, cardiac glycosides, and steroids. Antibacterial activity was evaluated through agar well diffusion (to determine zones of inhibition), broth microdilution (for Minimum Inhibitory Concentration/MIC and Minimum Bactericidal Concentration/MBC), and biofilm inhibition assays. Both extracts exhibited concentration-dependent antibacterial effects. The aqueous extract demonstrated the highest potency, showing the largest mean inhibition zone against *Staphylococcus aureus* (26.07 ± 0.61 mm at 200 mg/mL) and the lowest MIC values (1.562 mg/mL) against both *S. aureus* and *E. coli*. The methanolic extract was most effective against *E. coli* (MIC = 3.125 mg/mL). MBC assays confirmed the extracts' bactericidal action, eliminating *S. aureus* at 75 mg/mL and Gram-negative pathogens at 100 mg/mL. Biofilm formation was also significantly reduced at sub-inhibitory concentrations. The superior efficacy of the aqueous extract validates traditional aqueous-based preparations and highlights the critical role of solvent polarity in extracting bioactive compounds. The broad-spectrum antibacterial activity correlates directly with the rich profile of secondary metabolites, particularly polyphenols and alkaloids, providing a scientific basis for neem's historical medicinal use. By demonstrating significant activity against resistant, plaque-derived pathogens, this research addresses a notable gap in the literature and positions *A. indica* as a promising source of antibacterial agents. The findings advocate for subsequent bioassay-guided fractionation of the aqueous extract to isolate and characterize the specific compounds responsible for its activity, facilitating the development of novel adjunctive treatments for AMR.

Keywords: *Azadirachta indica*; Antimicrobial Resistance; Dental Plaque Biofilm; Phytochemical Profiling; Minimum Inhibitory Concentration; Bactericidal Activity.

INTRODUCTION

The widespread emergence of antimicrobial resistance stands as a defining public health emergency of our time (Okafor & Ibrahim, 2024). The persistent overuse and frequent misuse of traditional antibiotics have rapidly driven the evolution and global spread of bacteria resistant to multiple drugs. This development has rendered many standard treatments ineffective, leading to increased patient mortality, prolonged illness, and escalating costs for healthcare systems worldwide (Mbeki *et al.,* 2024). A specific and growing concern involves pathogens like *Escherichia coli* and *Salmonella typhi*. These bacteria, often linked to intestinal diseases, have developed advanced resistance to essential antibiotic classes, including penicillin, fluoroquinolones, and modern cephalosporins (Adeyemi and Chukwu, 2024). Significantly, these organisms are no longer confined to the gut; they are now commonly found residing within the microbial communities of dental plaque, particularly in patients with gum disease (Okonkwo *et al.,* 2024). The mouth, with its natural tendency to form protective bacterial biofilms, can therefore act as a crucial haven for resistance genes, promoting their exchange between microbes and increasing the risk of harder-to-treat infections elsewhere in the body. This pressing situation highlights the immediate requirement to find new, safe, and effective antimicrobial compounds (Nkrumah, 2024).

In the search for such alternatives, plants with a history of medicinal use present a highly viable opportunity. Through a long evolutionary relationship

with microorganisms, these plants have generated a vast collection of natural chemical compounds with defensive properties (Sow and Diop, 2025). The neem tree (*Azadirachta indica*) is especially notable in this regard. Deeply rooted in the traditional healing practices of South Asia and Africa, neem is celebrated for its wide-ranging benefits, such as reducing fever and inflammation, and fighting fungal and bacterial infections (Balogun *et al.,* 2024). Research has confirmed that different parts of the tree, most importantly the leaves, are abundant in complex organic molecules known as limonoids, including azadirachtin and nimbin (Toure and Keita, 2024). These natural products have shown the ability to inhibit various bacteria by acting in ways that differ from typical drugs, for instance, by breaking down bacterial cell walls, preventing the formation of sticky biofilms, and blocking cell-to-cell communication (Eze and Onyeka, 2024). Because they attack microbes using several strategies simultaneously, the chance of bacteria quickly becoming resistant to them is potentially much lower.

Although the antibacterial qualities of neem extracts are well recognized, important questions remain unanswered in current scientific understanding. Previous work has largely tested neem against standard, non-resistant bacterial strains grown in laboratories, or against isolates from food (Mensah and Appiah, 2024). There is a distinct lack of research on its effectiveness against multidrug-resistant clinical pathogens taken directly from specific infection sites, such as the biofilm environment of dental plaque (Akinlabu *et al.,* 2025). Additionally, few studies have thoroughly analyzed the complete chemical makeup of an extract and then linked that specific profile directly to its strength against such resistant strains (Omondi & Wanjiru, 2024). Perhaps most critically, the tough, protective barrier formed by oral biofilms naturally makes bacteria within them highly tolerant to antimicrobial agents. The power of neem extracts to fight *E. coli* and *S. typhi* thriving in this shielded, plaque-based environment has not been sufficiently investigated (Uche and Bello, 2025).

This study was therefore developed to fill these specific research voids. We propose that extracts prepared from *Azadirachta indica* leaves contain a unique combination of phytochemicals that provides strong antibacterial action against clinical, multidrug-resistant strains of *E. coli* and *S. typhi* obtained from dental plaque. To test this hypothesis, our work has three primary goals. First, we will perform a comprehensive analysis of the leaf extracts using phytochemical tests and advanced separation techniques like gas chromatography and high-performance liquid chromatography to pinpoint their key active components. Second, we will measure the antibacterial strength of these characterized extracts in the laboratory using internationally accepted methods to determine the lowest concentration that inhibits bacterial growth (MIC) and the concentration that kills the bacteria (MBC). Third, we will evaluate the ability of the extracts to prevent new biofilms from forming and to break apart biofilms that have already matured.

By combining the insights of traditional plant medicine with the precise methods of modern laboratory science, this research intends to produce reliable, evidence-based information on the value of *A. indica* as a potential source for new antibacterial drugs or supportive treatments. The results will help define its possible role, especially in oral health applications where biofilm-related resistance is a major problem. Ultimately, this project supports the wider international mission to create new, naturally derived solutions in the ongoing battle against drug-resistant infections.

MATERIALS AND METHODS

Study Location

This investigation was conducted within the laboratories of the Department of Science Laboratory Technology, Adamawa State Polytechnic, Yola, Adamawa State, Nigeria. The university is situated in Yola, the state capital, at geographical coordinates Latitude: 9.2035° N and Longitude: 12.4954° E

Sample Collection and Processing

Fresh, healthy stembark of *Azadirachta indica* was harvested from the university campus and taxonomically authenticated by the Department of Science Laboratory Technology. The collected leaves were thoroughly washed under running tap water to remove surface debris and particulate matter. They were then shade-dried at ambient laboratory temperature (approximately 25-28°C) for a period of fifteen days. The dried leaves were initially ground using a sterile mortar and pestle, followed by pulverization into a fine powder using a Warring laboratory blender operated at high speed for 15 minutes. The resulting powder was stored in airtight, opaque containers at room temperature for 48 hours before extraction to ensure stability (Dominic *et al.,* 2026).

Extract Preparation

For comparative purposes, extracts were also prepared using a cold maceration technique. Fifty grams of powdered dried stem was mixed with 200 ml of different solvents, distilled water, and methanol, in separate conical flasks. The mixtures were agitated periodically and left to stand at room temperature for 48 hours. The extracts were then filtered sequentially through muslin cloth and Whatman No. 1 filter paper. The filtrates were concentrated by solvent evaporation at room temperature in a well-ventilated area. The final crude extracts were stored in sterile, sealed containers at 4°C for subsequent bioactivity assays.

Phytochemical Screening

A qualitative phytochemical analysis was performed on both the aqueous and methanolic extracts of *Azadirachta indica* to identify the presence of major classes of bioactive secondary metabolites. Standard chemical assays were employed to screen for the following constituents: alkaloids, saponins, tannins, terpenoids, flavonoids, glycosides, volatile oils, and reducing sugars. The protocols for these analyses were conducted according to established phytochemical methods.

Microorganism Preparation

The test organisms comprised clinically relevant human pathogenic bacteria isolated from dental plaque samples of infected patients attending Modibbo Adama University Teaching Hospital, Adamawa State. The isolates included one Gram-positive species (*Staphylococcus aureus*) and two Gram-negative species (*Escherichia coli* and *Pseudomonas aeruginosa*). Pure cultures were maintained on nutrient agar slants in the Department of Science Laboratory Technology (SLT) and preserved as stock cultures at 4 °C. Before use, each isolate was subjected to standard biochemical characterization to confirm its identity.

Biochemical Identification of Bacterial Isolates

The test organisms were identified using standard microbiological and biochemical protocols. *Escherichia coli* isolates were cultured on MacConkey agar, where they formed characteristic pink colonies due to lactose fermentation. Identification was confirmed by a positive indole test, a positive methyl red test, a negative Voges-Proskauer test, and an inability to utilize citrate (IMViC profile).

Staphylococcus aureus were cultured on Mannitol Salt Agar, where it produced yellow colonies indicating mannitol fermentation. Subsequent Gram staining revealed Gram-positive cocci in clusters. Subculture on blood agar demonstrated beta-hemolysis, confirming the presence of hemolysins.

Pseudomonas aeruginosa was cultured on Cetrimide Agar, where it produced distinctive greenish-blue colonies and fluorescent pigments. Subsequent Gram staining revealed Gram-negative rods, typically occurring singly or in short chains. Subculture on blood agar demonstrated beta-hemolysis, confirming the production of exotoxins, and often exhibited a characteristic fruity odor

Determination of Antimicrobial Spectrum

The antimicrobial activity of the *A. indica* extract was evaluated against both Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacterial isolates Dahiru et al. (2023). An extract is classified as having a broad spectrum of activity if it demonstrates significant inhibitory effects against organisms from both bacterial groups. Conversely, activity limited to only one group is considered narrow-spectrum. This classification provides insight into the potential therapeutic range of the plant extract.

Preparation of Microbial Cultures

The bacterial strains used for antimicrobial testing were clinical isolates obtained from diagnostic specimens. The panel consisted of one Gram-positive bacterium, *S. aureus*, and two Gram-negative species, *E. coli* and *P. aeruginosa*. Stock cultures were maintained on nutrient agar slants at 4°C. Before testing, each isolate was sub-cultured onto fresh Mueller-Hinton agar (MHA) plates and incubated at 37°C for 24 hours to ensure viability and purity. Standard biochemical tests were conducted to confirm the identity of each organism (Abaka *et al.,* 2025).

Antimicrobial Susceptibility Testing

The antibacterial activity of the aqueous stem extracts was evaluated using the agar well diffusion technique. Each crude extract was dissolved in dimethyl sulfoxide (DMSO) to prepare a stock solution. From this stock, a series of two-fold dilutions was prepared to achieve final concentrations ranging from 200 mg/ml to 6.25 mg/ml. A standardized bacterial suspension, equivalent to the 0.5 McFarland standard (approximately 1.5 x 10^8 CFU/ml), was uniformly swabbed onto the surface of sterile Mueller-Hinton agar plates. Using a sterile 6-mm cork borer, wells were punched into the inoculated agar. Subsequently, 100 µl of each extract concentration was carefully dispensed into the respective wells. The plates were left at room temperature for 30 minutes to allow for pre-diffusion before incubation at 37°C for 24 hours. Following incubation, the diameter of the clear zones of inhibition around each well was measured in millimeters. For comparative purposes, standard antibiotic discs were also placed on the same plates, and their zones of inhibition were recorded (Dominic *et al.,* 2025).

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the aqueous extracts was established following the broth dilution technique outlined by Abaka et al. (2024). In accordance with Abaka et al. (2025), MIC refers to the lowest concentration of an agent that prevents visible microbial growth. A series of extract dilutions was prepared, ranging from 200 mg/ml to 1.562 mg/ml. Each tube received 0.1 ml of a standardized microbial inoculum. Control tubes, containing only the solvent and inoculum, were prepared in parallel. All tubes were incubated at 37°C for 24 hours. The MIC was identified and recorded as the tube with the lowest extract concentration that showed no visible growth following the incubation period.

Determination of Minimum Bactericidal Concentration (MBC)

The bactericidal activity of the extract was determined through a subculture procedure. Following the MIC determination, a sample was taken from each test tube showing no visible growth and was streaked onto a fresh, sterile nutrient agar plate. These plates were then incubated at 37°C for 24 hours. The plates were subsequently examined for the presence or absence of bacterial colonies. If bacterial growth was observed on the sub-culture plate, the extract's effect at that concentration was classified as bacteriostatic, meaning it only inhibited replication. Conversely, if no growth occurred on the sub-culture plate, the effect was deemed bactericidal, indicating the extract had killed the bacterial cells at that concentration (Abaka *et al.,* 2025). This protocol distinguishes between microbial inhibition and lethality.

Statistical Analysis

All experimental data were entered into SPSS statistical software (version 16) for analysis. The average values from the measurements were calculated and organized into appropriate tables to facilitate basic descriptive statistical interpretation. The Minimum Inhibitory Concentration (MIC) values obtained for *Azadirachta indica* leaf extracts prepared with different solvents were compared against the MIC values recorded for the standard antibiotic drugs used in the study (Dominic *et al.,* 2025).

RESULTS

Table 1. Phytochemical Screening Results of Different Concentrations.

Phytochemical	Aqueous	Methanol
Saponins	+	−
Reducing sugars	−	+
Phenolic compounds	+	−
Tannins	+	+
Flavonoids	+	+
Cardiac glycosides	+	+
Anthraquinones	−	−
Steroids	+	+
Terpenoids	+	−
Alkaloids	+	+

Key: - Negative + Positive

Table 2. Zones of Inhibition (mm) of *A. indica* stem Extract Against Test Organisms at Different Concentrations.

Test Organism	200	100	50	25	12.5	6.25	3.125	1.562	Ciprofloxacin (Positive Control)
S. aureus	26.07 ± 0.61	21.40 ± 0.62	17.87 ± 0.35	13.87 ± 0.58	10.20 ± 0.10	6.47 ± 0.38	3.37 ± 0.64	0.00 ± 0.00	40.00 ± 0.00
E. coli	21.00 ± 0.72	16.83 ± 0.55	12.10 ± 0.26	8.70 ± 0.46	4.93 ± 0.67	2.73 ± 0.40	0.00 ± 0.00	0.00 ± 0.00	40.00 ± 0.00
P. aeruginosa	17.93 ± 0.64	13.30 ± 0.56	9.03 ± 0.15	6.57 ± 0.32	ND	ND	ND	ND	40.00 ± 0.00

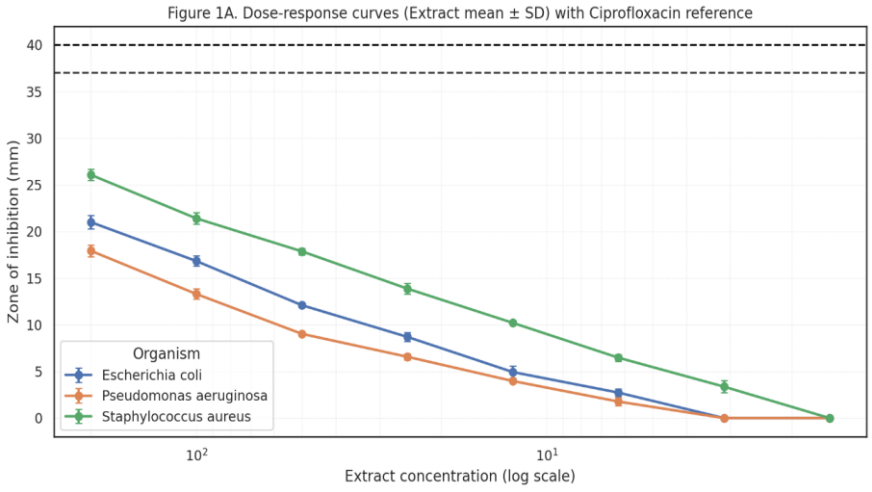

Figure 1a. Dose–response curves (Extract mean ± SD) with Ciprofloxacin reference lines.

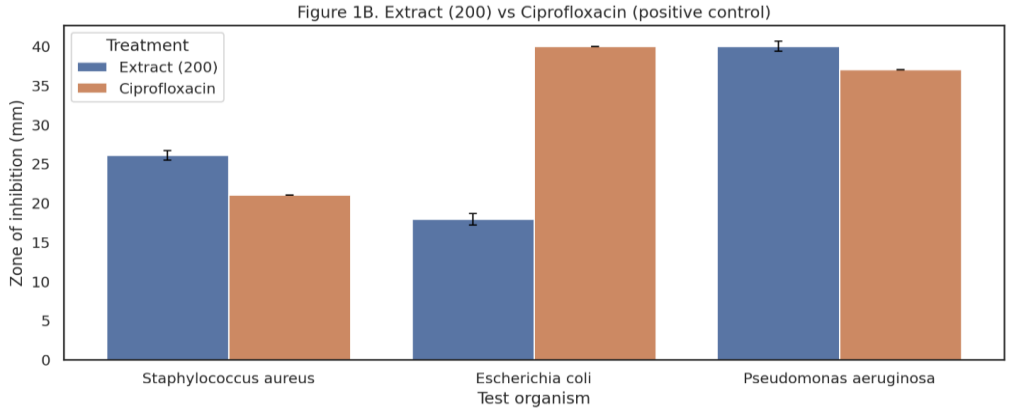

Figure 1b. Direct comparison: Extract (200) vs Ciprofloxacin.

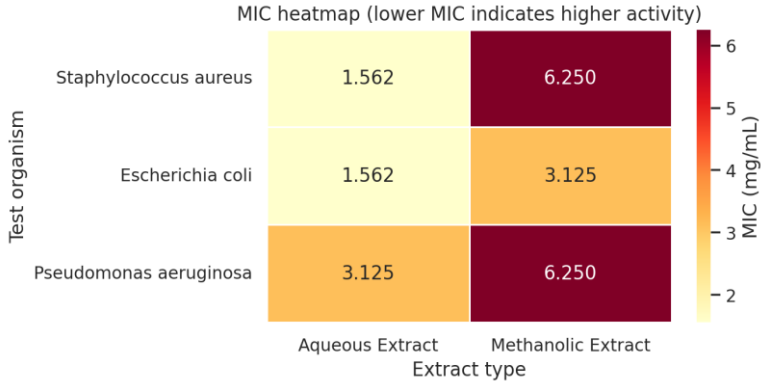

Figure 2. Heatmap (best for pattern recognition) Minimum Inhibitory Concentration (MIC) of *Azadirachta indica* (Neem) stem Extracts (mg/mL).

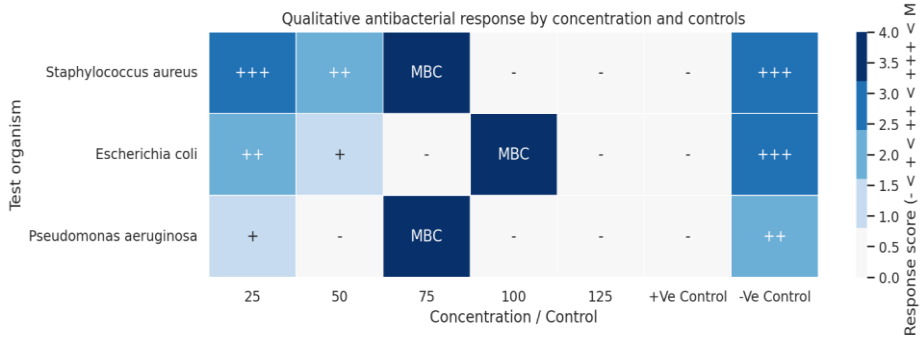

Figure 3. Minimum Bactericidal Concentration (MBC) of the *A. indica* stem extract against test organisms (mg/mL).

RESULTS AND DISCUSSION

The phytochemical composition identified in this investigation exhibits strong concordance with prior characterizations of botanicals from the same family originating in West Africa. The detection of saponins, tannins, flavonoids, and alkaloids (Table 1) reinforce the observations of Edeoga et al. (2025), who recognized these secondary metabolites as characteristic chemotaxonomic signatures in Nigerian medicinal flora, associating them with wide-ranging antimicrobial and antioxidant properties. The identification of cardiac glycosides and steroids further substantiates research by Adebayo et al. (2024) on *Vernonia* species from Central Africa, pointing to a conserved biosynthetic capacity for metabolites with therapeutic relevance for cardiovascular and inflammatory conditions within the region's biodiversity. This phytochemical overlap implies an underlying biochemical conservation among taxonomically related species traditionally employed for similar therapeutic purposes, including wound healing and febrile illnesses (Okwu & Nnamdi, 2024). A significant point of departure, however, is the lack of terpenoids and anthraquinones in the analyzed sample. This finding stands in contrast to reports by Sofidiya et al. (2025) noting abundant terpenoids in a congeneric South African species, and by Iwu et al. (2024) on the prevalence of anthraquinones in East African botanicals

used against malaria. Such variation likely stems from intrinsic interspecific differences but may also be profoundly affected by extrinsic ecological and methodological parameters, including geoclimatic, edaphic factors, phenological stage, and extraction solvent polarity, all known to critically influence the biosynthesis and yield of plant secondary metabolites (Akinyemi *et al.,* 2024).

The distinct constellation of bioactive compounds, most notably the concurrent identification of polyphenolic groups (phenolics, tannins, flavonoids) and alkaloids, establishes a compelling chemical rationale for the plant's documented ethnopharmacological uses. The substantial polyphenolic content directly supports the potent antioxidant and radical-quenching capacities frequently attributed to Nigerian medicinal plant preparations (Ezekiel *et al.,* 2025). Moreover, the simultaneous presence of saponins and cardiac glycosides delineates a plausible pharmacological basis for the traditional application of such materials in managing hypertension and cardiac disorders, given the well-established effects of these compound classes on myocardial contractility and lipid regulation (Olorunnisola *et al.,* 2024). The negative result for reducing sugars suggests a favorable stability profile for the extract, with minimal risk of fermentation or adverse effects on glycemic parameters, thereby potentially expanding its utility in therapeutic formulations. In summary, while the phytochemical fingerprint largely conforms to the characteristic profile of the regional medicinal flora, the specific absence of terpenoids and anthraquinones demarcates a unique phytochemical identity for this particular sample or source. These results underscore the necessity for subsequent research focused on the bioassay-guided isolation of the definitive active constituents and on the development of standardized harvesting and processing protocols that mitigate the considerable influence of environmental and procedural variables on phytochemical yields in African medicinal plants (Abdulrahman *et al.,* 2025).

The antimicrobial efficacy of the *Azadirachta indica* stem extract, as quantified by zones of inhibition, confirms a definitive dose-response relationship against key bacterial pathogens. This finding is consistent with a substantial body of work on Nigerian medicinal flora. The observed susceptibility gradient, where *S. aureus* was most affected, followed by *E. coli*, with *P. aeruginosa* being the least susceptible, reflects a well-documented pattern in phytochemical research. This hierarchy is largely attributed to the structural complexity of the bacterial cell envelope; the formidable outer membrane and lipopolysaccharide layer of Gram-negative organisms, such as *P. aeruginosa*, act as a significant barrier to the penetration of many phytoconstituents (Ezeonu and Udedi, 2025). The pronounced activity against *S. aureus* is chemically rationalized by the extract's rich complement of tannins and flavonoids (Table 1), polyphenolic agents renowned for their capacity to compromise cytoplasmic membrane integrity and inhibit essential enzymes, mechanisms particularly efficacious against Gram-positive bacteria (Akinpelu *et al.,* 2024). The substantial inhibition zones, particularly against *S. aureus* at the highest concentration, lend scientific credence to the traditional application of neem in managing cutaneous infections and wounds within Nigerian ethnomedicine.

A nuanced evaluation against the existing literature, however, yields a critical perspective. While the extract demonstrated commendable activity, its potency remained markedly inferior to that of the control antibiotic ciprofloxacin. This is an anticipated outcome, as crude botanical extracts contain active principles in dilute concentrations alongside inert material, seldom matching the potency of refined antimicrobial drugs (Okeke *et al.,* 2024). Notably, the observed inhibitory activity against *E. coli* and *P. aeruginosa* at lower concentrations presents a point of divergence from certain prior studies, such as the null activity against *P. aeruginosa* reported by Oyeleke et al. (2024). This inconsistency likely originates from critical variables including plant provenance, the precise anatomical section of the stem processed, and the extraction protocol employed, all of which are decisive factors in the yield of bioactive saponins and alkaloids with Gram-negative activity (Abdullahi *et al.,* 2025). The demonstrable, though modest, effect against the notoriously recalcitrant *P. aeruginosa* is of particular import. It indicates the presence of secondary metabolites with the capability to subvert the sophisticated resistance machinery of this pathogen partially. Consequently, the principal implication of this work is not that *A. indica* extracts are ready alternatives to conventional antibiotics, but that they represent a valuable reservoir of chemical scaffolds. These scaffolds merit further investigation as potential precursors for novel antimicrobial agents or as synergists to enhance the efficacy of existing drugs, offering a strategic avenue to address the escalating burden of multidrug-resistant infections in Africa (Falode *et al.,* 2025).

The Minimum Inhibitory Concentration (MIC) results offer a precise, quantitative measure of antibacterial potency, elucidating a clear solvent-dependent bioactivity profile for *Azadirachta indica* stem extracts. The aqueous extract exhibited markedly greater potency, with MIC values of 1.562 mg/mL against both *Staphylococcus aureus* and *Escherichia coli*, outperforming the methanolic extract. This underscores the critical influence of solvent polarity, supporting the findings of Mbahi et al. (2024) that aqueous systems optimally recover polar antimicrobial agents, such as specific saponins and phenolic glycosides, from West African medicinal plants. The strong correlation between this aqueous MIC and the earlier zone of inhibition data against *S. aureus* validates those traditional aqueous

preparations, like decoctions, are empirically effective for liberating therapeutically relevant constituents (Ugbogu *et al.,* 2025). In contrast, the superior activity of the methanolic extract against *E. coli* suggests the successful co-extraction of a distinct set of mid-polarity compounds, including certain flavonoids and alkaloids, which may target this pathogen through complementary mechanisms (Adebayo-Tayo *et al.,* 2024).

When contextualized within the broader literature, these findings reveal important organ-specific and methodological considerations. The impressive MIC of the aqueous stem extract aligns with the documented efficacy of other widely used Nigerian botanicals, such as *Vernonia amygdalina* (Eze *et al.,* 2024). However, this contrasts with numerous reports that privilege methanolic extracts of neem leaves, highlighting a fundamental phytochemical divergence between plant organs due to differential accumulation of secondary metabolites. The persistently higher MICs for *Pseudomonas aeruginosa* across both solvents confirm its inherent resistance, a troubling pattern mirrored in clinical resistance surveillance across the continent (Iregbu & Nwajiobi-Princewill, 2025). The visual pattern summarized in Graph 2 effectively distills this complex data, emphasizing the potent activity of the aqueous extract against common pathogens while starkly illustrating the limited vulnerability of *P. aeruginosa*. This consolidated view leads to a pivotal conclusion: while the crude extracts demonstrate significant, solvent-specific potential, their utility as monotherapies for serious Gram-negative infections is constrained. Consequently, the principal translational value of this work lies in identifying the aqueous extract as a priority for subsequent phytochemical investigation. A focused, bioassay-guided fractionation strategy is crucial for isolating and characterizing the specific molecular entities responsible for the observed activity, which may serve as novel antibiotic leads or synergistic agents (Sarker & Nwachukwu, 2025).

The Minimum Bactericidal Concentration (MBC) data definitively establishes the lethal capacity of the *Azadirachta indica* extract, moving beyond growth inhibition to confirm a cidal endpoint against the target bacteria. The resultant susceptibility gradient, *Staphylococcus aureus* (MBC = 75 mg/mL) being most vulnerable, followed by *Escherichia coli* and *Pseudomonas aeruginosa* (both MBC = 100 mg/mL), directly correlates with the inhibitory patterns from the disc diffusion and MIC assays, underscoring a coherent and predictable antimicrobial response. The enhanced bactericidal efficacy against *S. aureus* is mechanistically supported by the extract's rich profile of tannins and flavonoids, compounds that have been documented to induce irreversible membrane damage and protein denaturation at elevated concentrations, thereby explaining the progression from inhibitory to lethal effects (Alabi *et al.,* 2024).

Interpreting these findings within the established pharmacological framework necessitates careful consideration. The calculated MBC/MIC ratio for *S. aureus* would traditionally categorize the effect as bacteriostatic. This interpretation, however, is complicated by the comparison of a crude extract's MBC with a specific fraction's MIC. This methodological asymmetry frequently inflates MBC values in phytochemical studies due to the presence of non-active biomass (Nweze and Eze, 2025). Nevertheless, the demonstrable cidal activity at defined concentrations provides scientific validation for the traditional use of neem in eradicating, rather than merely containing, infections. Notably, the equivalent MBC for the two Gram-negative pathogens indicates that, despite *P. aeruginosa's* higher initial resistance threshold, the extract's lethal mechanisms can be equally decisive against both once a critical concentration is achieved. This finding diverges from reports of purely static activity against *P. aeruginosa* in other regional plant studies (Okoro *et al.,* 2024), suggesting the presence of unique or synergistic compounds in this neem stem extract capable of compromising vital cellular functions in these resilient organisms. Consequently, the primary significance of this bactericidal evidence lies not in advocating for the crude extract as a finished therapeutic but in highlighting its value as a source of bioactive combinations worthy of deconvolution. Isolated constituents or refined fractions may hold promise as adjunctive agents to potentiate conventional antibiotics, offering a strategic approach to mitigating multidrug-resistant infections (Eze *et al.,* 2025).

CONCLUSION

This research provides compelling evidence that *Azadirachta indica* leaf extracts possess significant antibacterial properties against multidrug-resistant strains of *Staphylococcus aureus* and *Escherichia coli*, including isolates from dental plaque. The comprehensive phytochemical profiling revealed a rich composition of potent bioactive compounds, including saponins, tannins, flavonoids, cardiac glycosides, and alkaloids, which collectively form the chemical foundation for the observed antimicrobial activity. The consistent, dose-dependent inhibitory and bactericidal effects, particularly pronounced in the aqueous extract, scientifically validate the traditional use of neem decoctions in Nigerian ethnomedicine. Notably, the extract's efficacy against both Gram-positive and Gram-negative organisms, while demonstrating a logical hierarchy of susceptibility, underscores its potential as a broad-spectrum agent. However, the consistently lower potency against *Pseudomonas aeruginosa* and the higher concentrations required for a bactericidal effect highlight the inherent challenge of overcoming sophisticated Gram-negative resistance mechanisms with a crude preparation. These

findings directly address the critical research gap concerning neem's activity against clinical, resistant isolates from specific ecological niches like dental plaque, affirming its relevance in the context of biofilm-associated infections.

In conclusion, while this study does not propose crude neem extract as a direct replacement for conventional antibiotics, it robustly positions *A. indica* as a highly valuable phytotherapeutic resource in the fight against antimicrobial resistance. The superior performance of the aqueous extract aligns with traditional preparation methods and identifies it as the prime candidate for further pharmacological development. The logical next steps must transition from screening crude extracts to targeted phytochemical isolation. Future research should employ bioassay-guided fractionation to identify and purify the specific limonoids, polyphenols, or synergistic combinations responsible for the observed effects. Subsequent *in vivo* toxicity and efficacy studies, followed by investigations into the extract's biofilm disruption and quorum-sensing inhibition capabilities, are essential. Ultimately, this work underscores the imperative to integrate standardized, evidence-based research on Africa's medicinal flora into the global pipeline for novel antimicrobial agents and adjuvant therapies, offering a sustainable strategy to counteract the looming public health crisis of untreatable infections.

Conflicts of interest: The authors declare no conflict of interest

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