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Assessment of Antibacterial Activity of Crude and Partially Purified Stem-Bark Extracts of *Alstonia boonei* Against Multidrug-Resistant Bacterial Pathogens

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ABSTRACT

Introduction: Medicinal plants remain important sources of bioactive compounds for antibacterial drug discovery. Although *Alstonia boonei* is widely used in traditional medicine, systematic data on the antibacterial activity of its stem bark fractions against clinically relevant multidrug-resistant (MDR) bacterial pathogens remain limited. This study therefore evaluated the antibacterial activity of crude and solvent-fractionated stem bark extracts of *A. boonei* against selected MDR bacterial strains.

Methods: The stem bark was extracted using ethanolic maceration and subsequently fractionated through liquid–liquid partitioning to obtain n-hexane, ethyl acetate, and aqueous fractions. Antibacterial activity against *Escherichia coli*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Klebsiella aerogenes*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* was assessed using the agar well diffusion method. Zones of inhibition were measured and subjected to statistical analysis.

Results: The crude extract exhibited moderate antibacterial activity against most tested organisms, with inhibition zones ranging from 12.33 ± 0.33 mm to 13.33 ± 0.89 mm. The highest activity was observed against *P. aeruginosa* and *A. baumannii*. Solvent fractionation enhanced antibacterial efficacy and revealed organism-specific activity patterns. The n-hexane fraction showed the strongest inhibition against *S. aureus* (16.67 ± 0.67 mm), *K. aerogenes* (15.66 ± 0.33 mm), and *A. baumannii* (14.67 ± 0.89 mm), while the ethyl acetate and aqueous fractions exhibited the highest activity against *K. aerogenes* (16.67 ± 0.67 mm) and *P. aeruginosa* (16.33 ± 0.33 mm), respectively.

Conclusion: This study addresses an important knowledge gap by systematically evaluating the antibacterial activity of crude and polarity-based fractions of *A. boonei* stem bark against clinically relevant MDR bacterial pathogens. The findings demonstrate the influence of solvent polarity on antibacterial potency and support *A. boonei* as a promising source of lead compounds for future antibacterial drug development.

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Introduction

Medicinal plants have long served as vital sources of therapeutic agents and remain central to modern drug discovery owing to their chemical diversity and abundance of bioactive secondary metabolites. Plant-derived compounds, including alkaloids, flavonoids, phenolics, tannins, terpenoids, and glycosides, exhibit a broad range of pharmacological properties most notably antimicrobial and immunomodulatory activities—which continue to support global healthcare systems and pharmaceutical research (Oluyele et al., 2022; Dasgupta, 2023; Zandavar and Afshari, 2023; Owoyemi and Oladunmoye, 2025).

Renewed interest in medicinal plants is driven largely by the escalating global burden of antimicrobial resistance (AMR). AMR refers to the ability of microorganisms to withstand the effects of antimicrobial agents that were previously effective, resulting in treatment failure and increased morbidity and mortality. Multidrug resistance (MDR), defined as resistance to at least one agent in three or more antimicrobial classes, is particularly prevalent among Gram-negative bacteria such as *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Escherichia coli* (Magiorakos et al., 2012; WHO, 2021; Ahmed et al., 2024). These pathogens employ multiple resistance mechanisms including efflux pump overexpression, biofilm formation, and enzymatic antibiotic degradation and are therefore prioritized in the search for alternative antimicrobial strategies (Kumawat et al., 2023; Belay et al., 2024; Elshobary et al., 2025).

African ethnomedicine represents a valuable reservoir of bioactive plants with significant therapeutic potential. *Alstonia boonei* De Wild. (Apocynaceae) is a widely distributed tropical African tree traditionally used in the management of infectious and inflammatory diseases (Adotey et al., 2012; Okoye et al., 2021). In Nigeria, it is commonly employed in the treatment of malaria, gastrointestinal disorders, fever, and pain. Phytochemical investigations have shown that its stem bark contains diverse secondary metabolites, including flavonoids, alkaloids, phenolics, saponins, and triterpenoids, which collectively contribute to its reported biological activities (Fonkoua et al., 2021; Okoye et al., 2021; Anyanwu et al., 2023).

Previous studies have reported antibacterial activity of *A. boonei* extracts against selected bacterial species, predominantly using crude preparations (Ogueke et al., 2014; Opoku and Akoto, 2015). However, crude extracts may obscure the activity of potent bioactive compounds due to low constituent concentrations or antagonistic interactions among phytochemicals. Solvent fractionation based on polarity provides a rational approach to concentrating active constituents and enhancing antibacterial efficacy. Despite this, systematic comparisons of polar and non-polar fractions of *A. boonei* stem bark against clinically relevant MDR bacterial strains remain scarce.

Furthermore, many earlier investigations relied on reference or non-resistant strains, limiting insight into the potential of *A. boonei* as a source of agents effective against MDR pathogens. The bacterial isolates selected in the present study were therefore chosen based on their clinical relevance, resistance to multiple antibiotic classes, and inclusion among World Health Organization–listed priority pathogens. This study addresses this knowledge gap by systematically evaluating the antibacterial activity of crude and polarity-based fractions of *A. boonei* stem-bark extracts against clinically relevant multidrug-resistant bacterial pathogens, with particular emphasis on the influence of solvent polarity on antibacterial potency.

Materials and Methods

Test Organisms and Inoculum Preparation

Multidrug-resistant (MDR) bacterial isolates used in this study were obtained from the Microbiology Laboratory of our institution. Multidrug resistance was defined as resistance to at least one antimicrobial agent in three or more antibiotic classes. The test panel comprised *E. coli, A. baumannii, K. pneumoniae, K. aerogenes, P. aeruginosa,* and *S. aureus*. These organisms were selected based on their clinical relevance, documented resistance to multiple antibiotics, and inclusion among World Health Organization (WHO) priority bacterial pathogens. Bacterial inocula were standardized to a 0.5 McFarland turbidity standard, which was prepared by mixing 0.05 mL of 1% barium chloride with 9.95 mL of 1% sulfuric acid. Fresh bacterial colonies were emulsified in sterile normal saline and visually adjusted to match the McFarland standard, corresponding to approximately 1×10^6 CFU/mL (Oluyele et al., 2023).

Collection, Authentication, and Extraction of Plant Material

Fresh stem bark of *A. boonei* was collected from Akungba-Akoko, Ondo State, Nigeria. Botanical authentication was carried out at the Herbarium Unit, Department of Plant Science and

Biotechnology, Adekunle Ajasin University, Akungba-Akoko, and a voucher specimen (AAUA/PSB/24/109) was deposited. The plant material was air-dried at room temperature, pulverized into a fine powder, and 530 g of the powdered sample was macerated in 4.35 L of absolute ethanol for 96 h with intermittent agitation to ensure efficient extraction of bioactive constituents. The mixture was filtered sequentially through muslin cloth and Whatman No. 1 filter paper. The resulting filtrate was concentrated under reduced pressure using a rotary evaporator and stored at −20 °C until further analysis (Oluyele, 2025).

Fractionation of the Crude Extract

Liquid–liquid fractionation was performed to separate phytochemicals based on polarity. Twenty-five grams of the crude ethanolic extract were reconstituted in 50 mL of distilled water and transferred into a separatory funnel. Sequential partitioning was carried out using n-hexane (2 × 50 mL) to enhance the extraction of non-polar compounds, yielding hexane fractions (F1 and F2). The aqueous phase was subsequently extracted with 100 mL of ethyl acetate to obtain the semi-polar fraction (F3), while the residual aqueous layer constituted the polar fraction (F4). All fractions were air-dried at room temperature under dark, well-ventilated conditions for 72 h to prevent photodegradation and preserve thermolabile constituents. The dried fractions were stored in airtight containers until antibacterial evaluation (Oluyele, 2025).

Antibacterial Activity Assay

The antibacterial activity of the crude extract and solvent fractions was evaluated using the agar well diffusion method (Oluyele et al., 2025a). Mueller–Hinton agar plates were inoculated with 1 mL of each standardized bacterial suspension, which was uniformly spread over the agar surface. Wells with a diameter of 6 mm were aseptically bored into the agar using a sterile cork borer. Each well was filled with 100 µL of the extract or fraction prepared at a concentration of 100 mg/mL in 5% dimethyl sulfoxide (DMSO), corresponding to an applied dose of 10,000 µg (10 mg) per well. Plates were allowed to stand at room temperature for 15 min to facilitate pre-diffusion of the extracts, followed by incubation at 37 °C for 24 h. Amoxicillin or ofloxacin served as positive controls, while 5% DMSO served as the negative control. All solvent residues from n-hexane and ethyl acetate were completely removed during fraction drying; therefore, separate solvent controls were not required. Zones of inhibition were measured in millimetres after incubation.

Data Analysis

All experiments were performed in triplicate, and results are presented as mean values with associated variability. Differences in inhibition zone diameters among crude extracts, solvent fractions, and bacterial species were evaluated using one-way analysis of variance (ANOVA). Tukey's post hoc multiple comparison test was applied to identify statistically significant differences between treatments. Statistical significance was defined at $p < 0.05$. All statistical analyses were conducted using SPSS version 22.0 (IBM Corp., Armonk, NY, USA).

Results

The antibacterial activity of the crude ethanolic extract and solvent fractions of *A. boonei* stem bark was evaluated against six multidrug-resistant clinical isolates *E. coli, A. baumannii, S. aureus, K. aerogenes, P. aeruginosa,* and *K. pneumoniae*. Overall, both the crude extract and solvent fractions exhibited variable antibacterial effects across the test organisms, as reflected by differences in inhibition zone diameters (Table 1). The crude extract demonstrated moderate antibacterial activity, with inhibition zones ranging from 12.33 ± 0.33 mm to 13.33 ± 0.89 mm against most organisms. The highest activity of the crude extract was observed against *P. aeruginosa* (13.33 ± 0.89 mm), followed by *A. baumannii* (13.00 ± 0.58 mm). In contrast, the crude extract showed weak activity against *K. pneumoniae*. Among the solvent fractions, antibacterial activity varied significantly depending on the organism and fraction type. Fraction 1 (F1; n-hexane fraction) exhibited the highest inhibitory activity against *S. aureus* (16.67 ± 0.67 mm) and *K. aerogenes* (15.66 ± 0.33 mm). Fraction 2 (F2) showed notable activity against *A. baumannii* (14.67 ± 0.89 mm). Fraction 3 (F3; ethyl acetate fraction) displayed pronounced activity against *K. aerogenes* (16.67 ± 0.67 mm). Fraction 4 (F4; aqueous fraction) demonstrated strong inhibitory activity against *P. aeruginosa* (16.33 ± 0.33 mm); however, variability in response was observed across replicates. In addition, F4 showed no detectable activity against K. pneumoniae, indicating reduced susceptibility of this strain to polar constituents. Statistical analysis revealed significant differences ($p < 0.05$) in antibacterial activity among the crude extract

and solvent fractions for most test organisms.

Overall, the crude extract and solvent fractions of *A. boonei* exhibited varying antibacterial activities against multidrug-resistant (MDR) strains, with the highest inhibitory effects depending on the type of fraction and the bacterial species tested. Detailed inhibition zone diameters and statistically significant differences are presented in Table 1.

Table 1: Antibacterial activity of *Alstonia boonei* stem-bark extracts

Organisms	Antibacterial activity of extraxts (mm)				
	Crude extract	**Fraction 1**	**Fraction 2**	**Fraction 3**	**Fraction 4**
Escherichia coli	12.67±0.67 a	15.67±0.34 b	13.67±0.67 a	12.67±0.67 a	13.34±0.67 a
Acinetobacter baumanni	13.00±0.58 a	14.67±0.67 a	14.67±0.89 a	12.67±0.67 a	14.67±0.89 a
Staphylococcus aureus	12.33±0.33 a	16.67±0.67 cd	13.33±0.67 ab	14.67±0.67 bc	14.67±0.89 bc
Klebsiella aerogenes	12.67±0.67 ab	15.66±0.33 c	11.33±0.67 a	16.67±0.67 c	13.67±0.67 b
Pseudomonas aeruginosa	13.33±0.89 b	13.33±0.89 b	13.33±0.89 b	16.33±0.33 c	7.33±0.67 a
Klebsiella pneumoniae	0.00±0.00 a	0.00±0.00 a	0.00±0.00 a	2.67±0.67 b	9.33±0.88 c

Fraction 1: n-hexane fraction 1; Fraction 2: n-hexane fraction 2; Fraction 3: Ethyl acetate fraction; Fraction 4: aqueous fraction; Values sharing different superscripts within the same row differ significantly ($p < 0.05$).

Discussion

In this study, the crude extract of *A. boonei* stem bark exhibited moderate antibacterial activity against most of the tested bacterial pathogens but showed no appreciable inhibition against K. pneumoniae. This observation underscores the importance of solvent fractionation in enriching bioactive constituents while minimizing the influence of inactive or antagonistic compounds. Differences in inhibition profiles among extracts and bacterial species, as presented in Table 1, suggest that antibacterial activity is influenced by both extract composition and organism-specific susceptibility (Lee et al., 2024; Zouine et al., 2024). Fractionation of the crude extract resulted in marked variations in antibacterial activity across the tested pathogens, highlighting the role of solvent polarity in modulating phytochemical efficacy. The ethyl acetate fraction demonstrated pronounced activity, particularly against *P. aeruginosa* and *K. aerogenes*, and was the only fraction to exhibit measurable inhibition against K. pneumoniae. This finding suggests that moderately polar constituents enriched in this fraction may contribute to broader antibacterial effects, although their activity remains lower than that of standard antibiotics.

The n-hexane fraction also showed notable antibacterial effects, especially against S. aureus and E. coli, indicating that non-polar phytochemicals such as terpenoids and sterols may play a contributory role in antibacterial activity. In contrast, the aqueous fraction displayed comparatively weaker and more selective inhibition, including limited or no activity against K. pneumoniae. This pattern suggests that water-soluble constituents may exert strain-specific effects rather than broad-spectrum antibacterial activity.

When compared with the positive controls (amoxicillin and ofloxacin), all extracts and fractions produced smaller zones of inhibition. This outcome is expected, given the complex and unrefined nature of plant extracts relative to purified antibiotics. Nonetheless, the observed antibacterial effects support the potential of *A. boonei* stem bark as a source of bioactive compounds that may serve as leads for future antibacterial development rather than as direct substitutes for existing antibiotics.

The antibacterial activity patterns observed in this study are consistent with previous reports on *A. boonei* and related medicinal plants (Ogueke et al., 2014; Zali et al., 2023). Variations in susceptibility among the tested organisms may be attributed to differences in cell wall structure, membrane permeability, and efflux mechanisms. In particular, the intrinsic resistance of Gram-negative bacteria such as K. pneumoniae has been associated with the presence of an outer membrane that restricts the penetration of many antimicrobial agents (Torrens and Cava, 2024; Zheng et al., 2025). Despite this, the moderate inhibition observed against both Gram-positive (*S. aureus*) and Gram-negative (*P. aeruginosa*) organisms suggests that

A. boonei contains constituents capable of interacting with diverse bacterial targets.

Previous phytochemical investigations of *A. boonei* stem bark have identified flavonoids, phenolic acids, and terpenoid compounds associated with antibacterial effects through mechanisms such as membrane disruption, inhibition of nucleic acid synthesis, interference with quorum sensing, and suppression of key metabolic enzymes (Marchese et al., 2017; Fernandes et al., 2023; Veiko et al., 2023). In the present study, these literature-reported mechanisms are discussed to support interpretation of the observed antibacterial activity rather than as experimentally confirmed modes of action.

Despite these findings, certain limitations should be acknowledged. The agar well diffusion assay provides semi-quantitative information and is influenced by the diffusion characteristics of test substances, which may underestimate the activity of poorly diffusing compounds. Additionally, the absence of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) data limits precise assessment of antibacterial potency. Future studies incorporating broth dilution assays, bioassay-guided fractionation, and mechanistic investigations are warranted to further elucidate the antibacterial potential of *A. boonei* stem bark and to explore its efficacy against a broader panel of multidrug-resistant pathogens.

Conclusion

The findings of this study suggest that *A. boonei* stem bark, particularly its ethyl acetate and n-hexane fractions, represents a promising source of antibacterial agents. Moderate to considerable activity was observed against both Gram-positive and Gram-negative bacteria, including selected multidrug-resistant strains, supporting its ethnomedicinal relevance. However, these results are preliminary, and the study has certain limitations, including the limited number of bacterial strains tested and the absence of MIC and MBC determinations. Further investigations are therefore warranted, including evaluation against a broader panel of multidrug-resistant pathogens, isolation and characterization of active phytochemicals, and preclinical pharmacological studies to validate and optimize their therapeutic potential.

Declarations

Conflict of Interest

The authors declare no conflicts of interest.

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Validation: Not applicable
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Writing – original draft: Tosin Ayodele, Emmanuel Akintokun
Writing – review & editing: Olumide Oluyele

Ethical Considerations

All ethical principles and standards for scholarly research—covering plagiarism, data integrity, originality, and proper citation—were diligently upheld throughout the development and submission of this work.

AI Use Disclosure

During the preparation of this review article, generative AI and AI-assisted technologies were used solely as supporting tools under full human oversight and control. The following discloses the use of such tools in accordance with current journal publishing standards.

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