Pharmacognosy Communications, 2026; 16(2):85-94.
https://www.phcogcommn.org

Original Article

Antibacterial Efficacy and Cytotoxicity of *Terminalia avicennioides* Guill. and Perr. Stem Bark Extract Against Resistant Pathogens

Temiyefunolu A. Ngwu[1], Oluwaseun. O Olasunkanmi[1], Victor Akpe[2], Ian Edwin Cock[2,*]

[1]Department of Microbiology, Faculty of Science, Lagos State University, Lagos, NIGERIA.
[2]School of Environment and Science, Griffith University, Nathan Campus, Queensland, AUSTRALIA.

ABSTRACT

Background: The global rise of antimicrobial resistance (AMR) has created an urgent need for alternative therapeutic agents, particularly from medicinal plants used in traditional medicine. *Terminalia avicennioides* Guill. and Perr. is widely used in West Africa for treating bacterial infections. **Objectives:** To evaluate the antibacterial efficacy and *in vitro* cytotoxicity of *Terminalia avicennioides* Guill. and Perr. stem bark extract. **Materials and Methods:** Stem bark was extracted using methanol-water (3:2, v/v; 60% methanol). Antibacterial activity was assessed using agar well diffusion assays, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) tests against selected Gram-positive and Gram-negative bacteria. Cytotoxicity was evaluated using the brine shrimp lethality assay (BSLA). **Results:** The extract produced zones of inhibition ranging from 16-22 mm at test concentrations of 30-35 mg/mL. MIC values were as low as 0.05 mg/mL against *Chromobacterium* spp. and 0.25 mg/mL against *Staphylococcus aureus*, with similar MBC values. An LC_{50} of 820 µg/mL was determined for the extract. **Conclusion:** These findings validate the traditional use of *Terminalia avicennioides* Guill. and Perr. for bacterial infections and highlight its potential as a source of novel antimicrobial agents. Despite moderate *in vitro* toxicity, further in vivo studies and dosage standardization are recommended before therapeutic application.

Keywords: Antibacterial activity, Antimicrobial resistance, Brine shrimp lethality assay (BSLA), Cytotoxicity, Medicinal plants, Minimum inhibitory concentration (MIC), Phytochemical constituents.

Correspondence:
Dr. Ian Edwin Cock
School of Environment and Science, Griffith University, Nathan Campus, Queensland, AUSTRALIA.
Email: i.cock@griffith.edu.au
ORCID: 0000-0002-8732-8513

Received: 24-12-2025;
Revised: 09-02-2026;
Accepted: 16-04-2026.

INTRODUCTION

Antimicrobial resistance (AMR) has emerged as one of the most critical challenges to global public health in the 21st century. The misuse and overuse of antibiotics in human medicine, agriculture, and aquaculture have accelerated the development of multidrug-resistant (MDR) bacterial strains, rendering many conventional antibiotics ineffective.[1] This has led to prolonged illnesses, increased healthcare costs, and higher mortality rates. The World Health Organization (WHO) estimates that AMR is responsible for at least 1.27 million deaths annually, with projections suggesting that by 2050, AMR could cause 10 million deaths per year.[2] The need for alternative therapeutic agents to combat AMR is therefore urgent.

OPEN ACCESS

Manuscript TECHNOMEDIA

DOI: 10.5530/pc.2026.2.9

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Medicinal plants have historically played a crucial role in treating infections, particularly in traditional medicine systems. They are rich in antimicrobial compounds, including alkaloids, flavonoids, tannins, and phenolic compounds.[3] These compounds often act synergistically, targeting multiple bacterial pathways, thereby reducing the likelihood of resistance development.[4] The exploration of medicinal plants as sources of novel antimicrobial agents has gained momentum in recent years, driven by the structural diversity and unique mechanisms of action of phytochemicals.[5]

Terminalia avicennioides Guill. and Perr. (family Combretaceae) is widely distributed across sub-Saharan Africa and is extensively used in African traditional medicine. The stem bark, leaves, roots, and fruits of this plant are employed in treating bacterial infections, gastrointestinal disorders, and inflammation.[6] Ethnobotanical surveys have revealed its use in managing wounds, diarrhea, and respiratory tract infections, suggesting the presence of potent antimicrobial agents.[7] Despite its widespread use, scientific validation of its pharmacological properties and safety is limited.

Phytochemical investigations have identified a diverse array of bioactive compounds in *T. avicennioides*, including tannins, flavonoids, saponins, alkaloids, phenolics and glycosides.[8] These compounds are known for their antimicrobial, antioxidant, and anti-inflammatory properties. Tannins inhibit bacterial adhesion and disrupt bacterial enzymes, whilst flavonoids act as free radical scavengers and enhance bacterial susceptibility to antibiotics.[9] Additionally, synergistic action between these compounds may contribute to the plant's therapeutic efficacy.

Previous studies have demonstrated the antimicrobial activity of *T. avicennioides* extracts against *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa*, although this species remain to be tested against many pathogens.[10] Additionally, critical gaps remain in understanding its toxicological profile. Cytotoxicity assessments, particularly using human cell lines or animal models, are limited, and data on acute or chronic toxicity are scarce.[11] This study aims to address these gaps by evaluating both the antibacterial activity and cytotoxicity of *T. avicennioides* stem bark extract.

Experimental

Study Area and Plant Material Collection

Fresh stem bark of *Terminalia avicennioides* was collected in September 2024 from Iseyin, Oyo State, Nigeria, located at longitude 8°1'50"N and latitude 3°23'55"E. This site was selected due to its rich vegetation and history of traditional medicinal plant usage, which enhances the likelihood of obtaining bioactive specimens. The collection was conducted at approximately 2:00 p.m., a time chosen based on evidence suggesting that the biosynthesis and concentration of certain phytochemicals, such as flavonoids, tannins, and alkaloids, are influenced by diurnal and seasonal changes.[12] Environmental parameters, including sunlight, humidity, and temperature, were considered to ensure the preservation of the plant's biochemical integrity.

The collected plant material was authenticated by a certified taxonomist at the Department of Botany, Lagos State University. The stem bark samples were subjected to a controlled drying process in a hot-air oven set at 45°C until all moisture was removed. The drying temperature was maintained below 50°C to preserve thermolabile compounds, including phenolics and terpenoids. The dried samples were pulverized into fine powder using a pre-sterilized electric grinder to avoid microbial or cross-contamination. The powdered material was stored in sterile, air-tight polyethylene bags at ambient room temperature (25-27°C) in a cool, dry, and dark environment to prevent oxidative degradation and to maintain phytochemical stability.

Extraction of Bioactive Compounds

The extraction of bioactive compounds from the powdered stem bark of *T. avicennioides* was performed using cold maceration, a method chosen for its simplicity, cost-effectiveness, and ability to preserve thermolabile phytochemicals. A total of 250 g of powdered stem bark was immersed in a hydroalcoholic solvent system comprising methanol (analytical grade, commercial laboratory supplier) and distilled water mixed in a 3:2 ratio (v/v). This solvent system was selected based on its efficacy in extracting a broad spectrum of phytochemicals, including alkaloids, tannins, flavonoids, and saponins, due to methanol's high polarity and water's compatibility with traditional medicinal preparations.[13]

The extraction process was conducted at ambient room temperature (25-28°C) over a period of 96 hr (4 days). The mixture was intermittently agitated every 8-12 hr to enhance mass transfer between the plant matrix and the solvent. After the 96 hr extraction period, the plant residue was filtered under aseptic conditions using Whatman No. 1 filter paper to separate the liquid extract (filtrate) from the solid plant material. The filtrate was then concentrated using a rotary evaporator under reduced pressure at 40-45°C. This temperature range was carefully maintained to prevent the thermal decomposition of volatile and thermolabile phytochemicals, which could compromise the biological activity of the extract.[14]

After preliminary concentration, any remaining moisture or residual solvent was removed by air-drying the extract under a gentle stream of air in a clean environment. To protect the phytochemicals from photo-oxidation and degradation, the dried extract was transferred into sterile, amber-coloured glass bottles and stored at 4°C.

Antibacterial Sensitivity Testing

Antibacterial sensitivity testing was conducted to assess the *in vitro* antibacterial potential of the hydroalcoholic stem bark extract of *T. avicennioides* against clinically relevant bacterial strains. The antibacterial assay was performed against clinically significant bacterial species commonly implicated in human infections, including Gram-positive bacteria: *Staphylococcus aureus, Staphylococcus epidermidis*, and *Enterococcus* spp.; and Gram-negative bacteria: *Escherichia coli, Klebsiella* spp., *Chromobacterium* spp., *Serratia* spp., *Pseudomonas aeruginosa*, and *Enterobacter* spp. These organisms were selected based on their medical importance, high prevalence in hospital-acquired infections, and known resistance to conventional antibiotics. Notably, several of the organisms investigated in this study, including *Staphylococcus aureus, Klebsiella* spp., *Pseudomonas aeruginosa*, and *Enterobacter* spp., belong to the ESKAPE group of pathogens, which are widely recognized for their ability to evade the effects of antimicrobial agents and cause difficult-to-treat infections.[15] The isolates were sourced from the laboratory stock culture of the Department of Microbiology from the Lagos State University, and were previously authenticated through standard microbiological identification procedures, including Gram staining and biochemical profiling (e.g., catalase, oxidase, indole, citrate utilization, and sugar fermentation tests).[16]

Two micrological media were used in the study: Mueller-Hinton Agar (MHA) was selected for its standardized composition and suitability for antibacterial susceptibility testing, including the well diffusion method, as recommended by the Clinical and Laboratory Standards Institute.[17] Nutrient agar (NA) was used for routine cultivation, maintenance, and sub-culturing of bacterial isolates. The media were prepared by dissolving the appropriate amount of powdered medium in distilled water, followed by sterilization via autoclaving.

To ensure consistency and reproducibility in the antimicrobial assay, bacterial inocula were standardized according to the 0.5 McFarland turbidity standard, which corresponds to approximately 1.5×10^8 colony-forming units per milliliter (CFU/mL).[18] Fresh cultures of each test organism were sub-cultured on nutrient agar plates and incubated at 37°C for 18-24 hr to obtain actively growing colonies. Well-isolated colonies were suspended in a 0.85% sterile saline solution (from commercial laboratory supplier) and the turbidity of the bacterial suspension was adjusted to visually match the 0.5 McFarland standard.

The crude hydroalcoholic extract of *T. avicennioides* stem bark was reconstituted to evaluate its antimicrobial activity. A stock solution was prepared by dissolving 350 mg of the dried extract in 10 mL of a methanol-distilled water mixture (1:1, v/v), yielding a concentration of 35 mg/mL. An additional working concentration of 30 mg/mL was subsequently prepared from the stock solution. All solutions were freshly prepared prior to each assay to minimize degradation of bioactive compounds. The prepared extracts were transferred into sterile, amber-coloured vials to reduce photo-oxidation and stored at 4°C until use. A solvent control consisting of methanol-distilled water (1:1, v/v) without plant extract was included in all antimicrobial assays to ensure that the solvent system did not contribute to antibacterial activity.

Antibacterial Activity Assay (Agar Well Diffusion Method)

The antibacterial activity of the *T. avicennioides* stem bark extract was assessed using the agar well diffusion method, a widely accepted and cost-effective technique for screening the antimicrobial potential of plant-derived substances.[19] This method allows for direct observation of diffusion-based inhibition of microbial growth by bioactive compounds present in the extract.

Inoculation of Agar Plates

Mueller-Hinton agar (MHA) plates were inoculated by evenly swabbing the entire surface with standardized bacterial suspensions (adjusted to 0.5 McFarland standard, ~1.5×10^8 CFU/mL). The swabbing was performed in three directions using a sterile cotton swab to ensure a uniform lawn of bacterial growth.[20]

Well Formation

Using a sterile cork borer, wells of uniform diameter (6 mm) were aseptically punched into the solidified agar. The wells were spaced adequately apart to prevent overlapping of zones of inhibition.[21] A volume of 100 µL from each prepared extract concentration (ranging from 30 mg/mL to 35 mg/mL) was carefully dispensed into the respective wells under aseptic conditions.[22]

Controls

Positive controls: Wells were loaded with the standard antibiotics, streptomycin and ampicillin (analytical grade; supplied by Tunnex Laboratories Engineering Limited, Mile 12, Lagos, Nigeria) at final concentrations of 10 µg/mL each. These antibiotics served as reference standards for benchmarking the antibacterial activity of the plant extract.[23]

Negative control: Wells containing only the extraction solvent were included to ensure that any observed inhibition was attributable solely to the plant extract. The solvent system consisted of a 1:1 mixture of methanol and distilled water (50% methanol). Preliminary control assays confirmed that this solvent mixture did not produce inhibitory effects under the assay conditions, validating its suitability as a negative control. For future repetitions of the assay, lower percentage methanol-water mixtures should also be evaluated to determine the optimal solvent composition for establishing a robust negative control setup.[24]

Incubation

All inoculated and treated plates were incubated at 37°C for 24 hr, a standard time and temperature regime that supports optimal growth of mesophilic bacteria and allows for effective diffusion and interaction between the extract and microbial cells.[25] After incubation, the plates were examined for clear zones of inhibition surrounding the wells, indicating areas where bacterial growth was suppressed due to the antimicrobial action of the extract.

Measurement of Zones of Inhibition

The diameter of each inhibition zone was measured in millimeters (mm) using a standard transparent ruler or Vernier caliper to ensure accuracy and precision.[26] Each measurement was recorded in triplicate for every extract concentration and test organism, using separate plates to maintain experimental independence and account for variability. The mean zone of inhibition was calculated and expressed as mean±standard deviation (SD) for each treatment group. Larger zone diameters corresponded to greater antibacterial activity, reflecting the potency of the extract against the respective bacterial strain.[27]

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the plant extract was determined using a two-fold serial dilution method.[28] The crude extract was diluted to obtain concentrations ranging from 0.05 mg/mL to 35 mg/mL. Each diluted extract was added to pre-sterilized, molten nutrient agar cooled to 45°C, mixed thoroughly, and poured into sterile Petri dishes. After solidification, the surface of the agar was allowed to dry. Each plate was streaked with a standardized 18-hr-old broth culture of the test bacterial strains. The plates were incubated at 37°C for 72 hr. After incubation, plates were examined for the presence or absence of visible microbial growth. The lowest concentration that completely inhibited visible growth was recorded as the MIC of the extract.[29]

Determination of Minimum Bactericidal Concentration (MBC)

To determine the minimum bactericidal concentration (MBC), samples were taken from MIC plates that showed no visible growth. These samples were sub-cultured onto freshly prepared nutrient agar plates (without extract) and incubated at 37°C for 48 hr. The lowest concentration of the extract that yielded no growth on the nutrient agar plate was considered the MBC.[30]

Brine Shrimp Lethality Assay (BSLA)

The brine shrimp lethality assay (BSLA) was employed to evaluate the cytotoxicity of the hydroalcoholic stem bark extract of *T. avicennioides*. This assay is a simple, rapid, and cost-effective bioassay used for preliminary assessment of cytotoxicity in plant extracts, synthetic compounds, and environmental samples.[31] It is based on the ability of a test substance to kill nauplii (larval stage) of *Artemia franciscana*, a non-selective invertebrate model organism.

Dry cysts of *Artemia franciscana* were incubated in a conical container filled with 1 L of artificial seawater under constant illumination (24-28°C) and aeration using an aquarium pump, which ensured adequate oxygenation and gentle agitation of the medium to prevent cysts from settling at the bottom. The cysts were allowed to hatch over 48 hr. Actively swimming nauplii were collected from the illuminated portion of the container using a sterile Pasteur pipette, avoiding unhatched eggs and debris. Freshly hatched nauplii (<24 hr old) were used for all experiments.[32] The *Artemia franciscana* cysts used for hatching were sourced from AfriShop Online (Nigeria), a Lagos based supplier that distributes brine shrimp eggs within the region.

The plant extract was initially dissolved in a small volume of dimethyl sulfoxide (DMSO, analytical grade; supplied by Tunnex Laboratories Engineering Limited, Mile 12, Lagos, Nigeria) to facilitate solubilization. The resulting stock solution was subsequently diluted with artificial seawater (also supplied by Tunnex Laboratories Engineering Limited, Mile 12, Lagos, Nigeria) to prepare the required test concentrations. Serial dilutions were performed to obtain concentrations of 35, 17.5, 8.75, 4.38, 2.19, 1.09, and 0.55 µg/mL. Care was taken to ensure that the final concentration of DMSO in each test solution did not exceed 1% (v/v), a level generally considered nontoxic to brine shrimp nauplii. Artificial seawater containing the same concentration of DMSO (1% v/v) but without plant extract served as the solvent control.[33]

Approximately 8-15 brine shrimp nauplii were transferred into each well containing 5 mL of the prepared test solution using a sterile Pasteur pipette. Care was taken to avoid unhatched cysts or debris. Each concentration was tested in quadruplicate ($n = 4$) to ensure reproducibility and reliability of the results. A negative control group was prepared with 1% DMSO in artificial seawater, whilst a positive control group was exposed to potassium dichromate at a concentration of 10 µg/mL, a known cytotoxic reference compound. The assay was performed in sterile 48-well plates, with each well containing a total volume of 800 µL of test solution. The plates were incubated under ambient laboratory conditions (25-28°C) with continuous illumination for 24 hr. No feeding was provided during the exposure period to avoid external influences on mortality. After 24 hr, the number of dead and surviving nauplii in each vessel was counted manually using a low-power dissecting microscope. Nauplii were considered dead if they exhibited no movement during a 10-sec observation period, even after gentle agitation.[34]

Calculation of Mortality and LC_{50} Determination

The percentage mortality for each replicate was calculated using the formula:

$$\text{Percentage Mortality} = \frac{\text{Number of Dead Nauplii}}{10} \times 100$$

The mean mortality and standard deviation (SD) were calculated from the quadruplicates. Data from the positive and negative controls were used to validate the assay. A mortality rate exceeding 90% in the positive control and ≤10% in the negative control was considered indicative of a valid test.[35]

To estimate the median lethal concentration (LC_{50}), which represents the concentration required to kill 50% of the test organisms, mortality percentages were converted to probit values using Finney's method or a probit transformation table. A linear regression analysis was performed by plotting probit mortality (Y) against the logarithm (base 10) of extract concentration (X), producing the regression equation:

Probit values were calculated from the mean % mortality values of the quadruplicates. Regression analysis was applied to estimate the LC_{50} value. The regression model demonstrated a significant linear fit (R^2=0.981), described by the equation:

$$Y = 1.401x + 4.063$$

Where *Y* is the probit value and *X* is $\log_{10}$ concentration.

LC_{50} Calculation: To find the LC_{50} (probit = 5.0) : 5.0 = 1

$$Y = AX + b$$

Where: *Y* is the probit value, *X* is $\log_{10}$ concentration, *A* is the slope, and *b* is the intercept.

The LC_{50} was calculated by solving for *X* at Probit = 5.0, corresponding to 50% mortality. The resulting $\log_{10}$ (LC_{50}) value was then transformed back into normal concentration units. The coefficient of determination (R^2) was used to assess the goodness-of-fit of the regression model. The 95% confidence interval (CI) for LC_{50} was also reported.[36]

The toxicity potential of the extract was interpreted according to previously proposed brine shrimp lethality assay (BSLA) toxicity classifications.[37] In this system, LC_{50} values ≤10 µg/mL are regarded as highly cytotoxic, values between 10-100 µg/mL are considered strongly cytotoxic, values between 100-1000 µg/mL indicate moderate cytotoxicity, while LC_{50} values greater than 1000 µg/mL are regarded as non-toxic.[38] Based on this classification, the LC_{50} values obtained in the present study were interpreted accordingly.

Statistical Analysis

All experimental data were analysed using SPSS statistical software (version 26) and GraphPad Prism (version 9). The mean values and standard deviations were calculated for all replicates. One-way analysis of variance (ANOVA) was performed to determine significant differences between groups, followed by Tukey's *post hoc* test for pairwise comparisons.[39] Homogeneity of variance was confirmed using Levene's test ($p>0.05$).[40] Probit regression analysis was applied to the mortality data from the brine shrimp lethality assay to estimate LC_{50} values.[41]The regression model was assessed for goodness-of-fit using the coefficient of determination (R^2) and standard error of the estimate. Statistical significance was set at $p<0.05$.[42]

RESULTS

Percentage Yield of Plant Extracts

A total of 250 g of oven-dried (45°C) and pulverized stem bark was subjected to extraction using a combination of 900 mL of methanol and 600 mL of distilled water as the extracting solvents. After complete solvent removal and drying, the weight of the crude extract recovered was 9.6 g.

Weight of plant material: 250 g; Weight of extract yield: 9.6 g.

$$\text{Percentage Yield} = 3.84\%$$

Antimicrobial Sensitivity Results

The antibacterial activity of the hydroalcoholic stem bark extract is summarised in Table 1, which presents the inhibition zones produced against all tested Gram positive and Gram negative bacteria at 30 mg/mL and 35 mg/mL. At 35 mg/mL, the extract produced inhibition zones ranging from 17 to 22 mm against the tested isolates. The solvent control produced no inhibition, while the standard antibiotics were active against several isolates.

The combined MIC and MBC results summarised in Table 2, shows that the extract exhibited its strongest inhibitory and bactericidal effects against *Chromobacterium* spp. (MIC 0.05 mg/mL; MBC 0.25 mg/mL) and several *Staphylococcus aureus* strains, whilst markedly higher concentrations were required for less susceptible organisms such as *Staphylococcus epidermidis, Klebsiella* spp., and *Serratia* spp. The close alignment between MIC and MBC values for most isolates further supports the predominantly bactericidal nature of the extract, further suggests a rapid transition from growth suppression to complete bacterial eradication across a broad spectrum of organisms.

Toxicity Assessment Using Brine Shrimp (*Artemia franciscana*) Lethality Assay

In Table 3, the BSLA revealed a clear concentration-dependent cytotoxic effect of the hydroalcoholic stem bark extract of *T. avicennioides* on *A. franciscana* nauplii. Mortality was negligible in the negative control (≤5%), confirming the solvent had no toxic influence. The positive control (potassium dichromate at 10

Table 1: Summary of antibacterial activity of *T. avicennioides* stem bark extract.

Bacterial Isolate	Zone of Inhibition (35 mg/mL)	Zone of Inhibition (30 mg/mL)	Streptomycin (1 mg/mL)	Ampicillin (1 mg/mL)	Negative Control (Methanol:Water)
Escherichia coli	18±0.4 mm	20±0.6 mm	21±1.3 mm	29±1.5 mm	0 mm
Pseudomonas aeruginosa	18±0.4 mm	19±0.3 mm	17±0.3 mm	17±0.3 mm	0 mm
Klebsiella spp.	17±0.3 mm	19±0.3 mm	19±0.3 mm	30±1.0 mm	0 mm
Staphylococcus aureus	18±0.4 mm	21±1.3 mm	15±0.7 mm	17±0.3 mm	0 mm
Chromobacterium spp.	20±0.6 mm	18±0.4 mm	18±0.4 mm	7±0.0 mm	0 mm

µg/mL) produced almost complete lethality (96%), validating the assay.

Table 3 summarizes the cytotoxic effects of the extract on *Artemia franciscana* nauplii, showing a progressive increase in mortality from 10% at 0.55 µg/mL to 90% at 35 µg/mL. The negative control produced no mortality, whereas the positive control resulted in 97.5% lethality, confirming the validity of the assay. The table also presents the raw replicate counts and calculated mean mortality values, which were used to perform probit analysis and estimate the LC_{50} value. At lower concentrations (100-250 µg/mL), mortality remained relatively modest (12-25%) but increased sharply to 90% at the highest tested concentration of 2000 µg/mL. The dose-response trend is illustrated in Supplementary Figure 1, while the probit regression output used to derive the LC_{50} is presented in Supplementary Figure 2. Probit analysis yielded an estimated LC_{50} of approximately 820 µg/mL, placing the extract within the category of moderate toxicity according to Meyer's toxicity scale.[43] This quantitative profile highlights a clear concentration dependent response and provides a robust basis for classifying the extract's acute toxicological potential.

Table 2: MIC and MBC exhibited *T. avicennioides* hydroalcoholic stem bark extract against susceptible bacterial test organisms.

Microorganism	MIC (mg/mL)	MBC (mg/mL)
Chromobacterium spp.	0.05	0.25
Staphylococcus aureus	0.50	0.50
Staphylococcus aureus	0.25	0.25
Staphylococcus aureus	1.08	1.08
Staphylococcus epidermidis	17.50	17.50
Chromobacterium spp.	2.185	2.185
Klebsiella spp.	8.75	8.75
Enterobacter spp.	2.185	2.185
Escherichia coli	2.185	2.185
Staphylococcus aureus	4.375	8.75
Serratia spp.	1.08	8.75

DISCUSSION

The overall experimental workflow and the relationship between the antibacterial and toxicity components of this study are summarised in Figure 1, providing a visual context for interpreting the observed bioactivity patterns. This schematic highlights the sequential approach, from plant material preparation and extraction to antibacterial assays and brine shrimp lethality testing, which collectively supports the integrated evaluation of the extract's therapeutic potential and safety profile.

The hydroalcoholic stem bark extract of *Terminalia avicennioides* exhibited notable antibacterial activity against both Gram positive and Gram negative bacteria, producing inhibition zones of 16-22 mm at 30-35 mg/mL. The extract exhibited its strongest activity against *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*, maintaining comparable activity across all tested isolates (Table 2). These observations support the ethnomedicinal relevance of the species.

The MIC and MBC results further confirm the extract's efficacy, with the lowest values recorded against *Chromobacterium* spp. (MIC: 0.05 mg/mL, MBC: 0.25 mg/mL). The close correlation between MIC and MBC values for most organisms suggests that the extract exhibits bactericidal activity. However, some strains, including *Serratia*, displayed a higher MBC than MIC, indicating a bacteriostatic effect at lower concentrations.

These findings align with the traditional use of *T. avicennioides* in treating bacterial infections and highlight its potential as a source of novel antimicrobial agents.[44] The presence of diverse phytochemicals, including tannins, flavonoids, and alkaloids may contribute to the extract's antibacterial properties.

The BSLA results revealed that the hydroalcoholic stem bark extract of *T. avicennioides* exhibits moderate cytotoxicity, with an LC_{50} value of approximately 820 µg/mL, classifying it as moderate toxicity according to Meyer's toxicity scale.[45] The cytotoxicity was concentration-dependent, with higher concentrations leading to increased mortality of *Artemia franciscana* nauplii.

Table 3: Mortality of *Artemia franciscana* nauplii exposed to varying concentrations of *T. avicennioides* Hydroalcoholic stem bark extract after 24 hr. Target number of nauplii per well was 10; the actual number varied slightly between 8-12 to account for transfer variability.

Concentration (µg/mL)	Repl. 1	Repl. 2	Repl. 3	Repl. 4	Mean Dead	% Mortality±SD	Control Type
35	9	10	9	8	9.0	90%±8.16	
17.5	8	9	7	8	8.0	80%±4.08	
8.75	6	7	5	6	6.0	60%±8.16	
4.375	5	6	4	5	5.0	50%±8.16	
2.1875	3	4	2	3	3.0	30%±8.16	
1.09375	2	2	1	3	2.0	20%±8.16	
0.546875	1	1	1	1	1.0	10%±0.00	
0.0	0	0	0	0	0.0	0%	Negative
10.0	10	10	9	10	9.75	97.5%±2.5	Positive

Whilst the cytotoxicity suggests potential applications in anticancer or pesticidal therapies, it also raises concerns about the safety of the extract for medicinal use. The findings emphasize the need for further toxicological studies, particularly in mammalian models, to establish safe therapeutic windows and assess potential risks.

The antibacterial activity of *T. avicennioides* stem bark extract may be due to its tannins, flavonoids, alkaloids, and saponins.[46] These compounds are known to possess antimicrobial properties through mechanisms such as disrupting bacterial cell membranes, inhibiting enzymatic pathways, and interfering with DNA replication.[47]

The broad-spectrum activity observed in this study supports the ethnomedicinal use of *T. avicennioides* for treating infections.[48] The extract's ability to inhibit both Gram-positive and Gram-negative bacteria is particularly promising, as Gram-negative bacteria are often more resistant to antibiotics due to their complex cell wall structure.[49]

The MIC and MBC results validate the extract's antibacterial potential, with particularly low values observed against *Chromobacterium* spp. and *Staphylococcus aureus*. *Chromobacterium* species are opportunistic pathogens associated with severe infections such as septicemia and abscess formation, particularly in immunocompromised individuals. *Staphylococcus aureus* is a major human pathogen responsible for a wide range of infections, including skin and soft tissue infections, pneumonia, bacteremia, and food poisoning, and is well known for its ability to develop resistance to multiple antibiotics. Therefore, the observed antibacterial activity of the extract against these organisms highlights its potential therapeutic relevance. These findings suggest that the extract contains potent bioactive compounds that could be developed into effective antimicrobial agents. However, the variability in MIC and MBC values across bacterial strains highlights the need for further studies to understand the specific mechanisms of action and identify the active compounds. These MIC and MBC trends are tabled in Table 2, which provides a comparison of inhibitory and bactericidal thresholds across the tested isolates. The table highlights that the low MIC and MBC values for *Chromobacterium* spp. and *Staphylococcus aureus*, contrasting with the higher concentrations required for *Staphylococcus epidermidis* and *Klebsiella* spp. This pattern reinforces the extract's strongest activity against highly susceptible organisms and supports the interpretation that its antimicrobial effect is predominantly bactericidal for most strains. Significantly, the pronounced difference in susceptibility between *S. aureus* and *S. epidermidis* is noteworthy, as both belong to the same genus. *Staphylococcus epidermidis* is well documented to possess multiple resistance mechanisms, including biofilm formation and reduced permeability to antimicrobial agents, which may contribute to its decreased susceptibility compared with *S. aureus*. Previous studies have also reported that *S. epidermidis* strains frequently exhibit broader resistance profiles to conventional antibiotics, which may partly explain the higher MIC and MBC values observed in this study.

The extract exhibited a concentration-dependent increase in nauplii mortality in the brine shrimp lethality assay. Probit analysis of the mortality data yielded an LC_{50} value of approximately 820

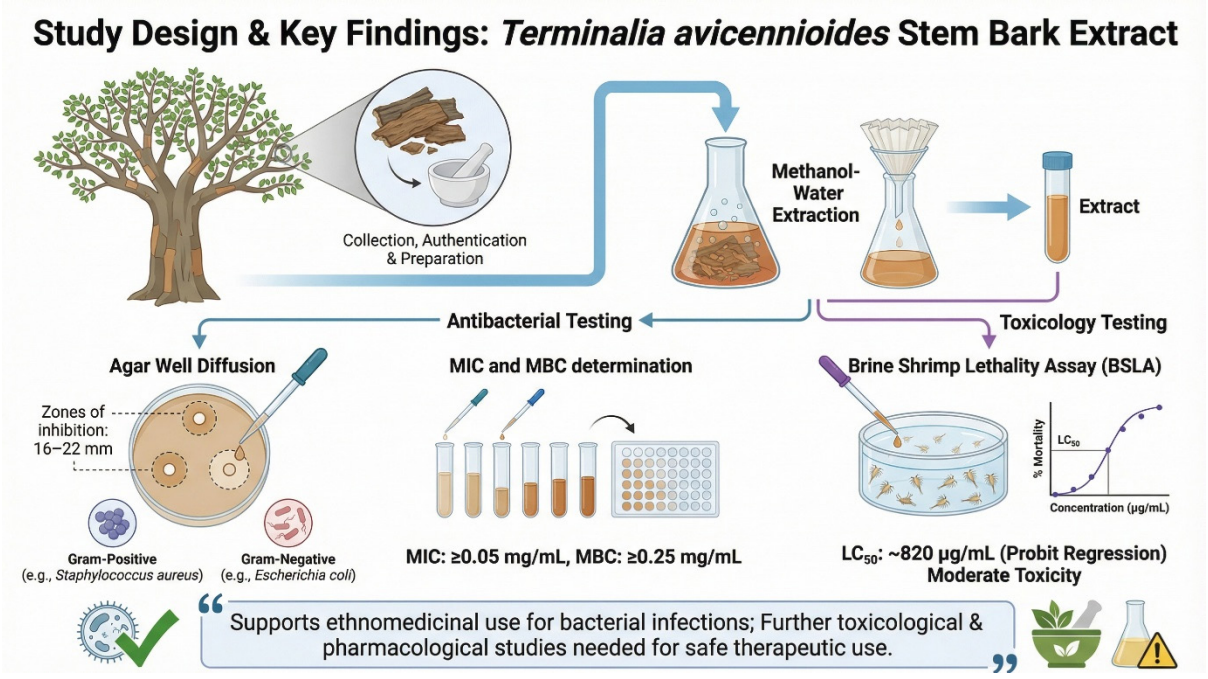

Figure 1: Schematic overview of the study design and key findings. A visual summary of the extraction workflow, antibacterial assays (agar well diffusion, MIC, MBC), and brine shrimp lethality testing used to evaluate the bioactivity and toxicity of *Terminalia avicennioides* stem bark extract.

µg/mL, indicating relatively moderate toxicity under the assay conditions.

This study has several limitations; the *in vitro* assays do not account for the complexities of living systems, such as metabolism, bioavailability, and immune responses. The study tested the extract against a limited number of bacterial strains, which may not fully represent the diversity of pathogens encountered in clinical settings. The use of only hydroalcohol as the extracting solvent may have excluded other bioactive compounds with different polarities. The BSLA provides preliminary toxicity data but does not directly reflect the extract's safety in mammalian systems.

To address the limitations of the present study and to further explore the therapeutic potential of *T. avicennioides*, several research directions are warranted. Advanced analytical techniques such as HPLC, LCMS, and NMR should be employed to isolate and characterize the specific bioactive constituents responsible for the antibacterial and cytotoxic effects. Broader antibacterial screening, including against multidrugresistant clinical isolates, will help define the full antimicrobial spectrum of the extract. Comprehensive *in vivo* toxicity studies in mammalian models are essential to establish safe dosage ranges and evaluate potential systemic risks. Parallel efforts should focus on developing standardized extract formulations that preserve antimicrobial potency while minimizing toxicity. Investigating synergistic interactions with conventional antibiotics may further enhance therapeutic efficacy and contribute to strategies for combating antimicrobial resistance. Finally, sustainable harvesting and conservation practices must be prioritized to ensure longterm availability of *T. avicennioides* for research and potential clinical development.

CONCLUSION

The hydroalcoholic stem bark extract of *T. avicennioides* demonstrated strong antibacterial activity and moderate cytotoxicity, lending scientific support to its traditional use in managing infectious diseases. Its broadspectrum efficacy suggests the presence of potent bioactive constituents with therapeutic promise. However, there is a need for rigorous safety assessment before clinical translation. Advancing *T. avicennioides* as a viable therapeutic candidate will require comprehensive *in vivo* studies to establish efficacy and safe dosage ranges, alongside detailed phytochemical investigations to isolate and characterise the compounds responsible for its biological effects. The development of standardised formulations will be essential to ensure consistent potency and reproducibility, whilst exploring synergistic interactions with conventional antibiotics may enhance antimicrobial performance and contribute to strategies addressing antibiotic resistance. Finally, sustainable harvesting and conservation measures must be prioritised to protect this valuable medicinal species and ensure its longterm availability for research and potential therapeutic development.

ACKNOWLEDGEMENT

None.

ABBREVIATIONS

AMR: Antimicrobial resistance; **MIC:** Minimum inhibitory concentration; **MBC:** Minimum bactericidal concentration; **BSLA:** Brine Shrimp Lethality Assay; **LC_{50}:** Lethal concentration causing 50% mortality; **DMSO:** Dimethyl sulfoxide; **MHA:** Mueller-Hinton Agar; **NA:** Nutrient agar; **CFU/mL:** Colony-forming units per milliliter; **CLSI:** Clinical and Laboratory Standards Institute; **ANOVA:** Analysis of variance; **SD:** Standard deviation; **SPSS:** Statistical Package for the Social Sciences; **CI:** Confidence interval; **WHO:** World Health Organization.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization was carried out by T.A.N. and O.O.O; V.A. and I.E.C. transformed T.A.N.'s bachelor's data work into a full manuscript data. Methodology was developed by T.A.N. and O.O.O., with image software support provided by V.A. Validation was performed by T.A.N., O.O.O., V.A. and I.E.C. Formal analysis and investigation were conducted by T.A.N. and O.O.O. Resources and data curation were provided by T.A.N and V.A. The original manuscript draft was written by T.A.N., with subsequent review and editing undertaken by T.A.N., V.A. and I.E.C. Visualization was completed by V.A. and I.E.C, while supervision was provided by O.O.O., V.A. and I.E.C. Project administration was managed by V.A. All authors have read and approved the final version of the manuscript.

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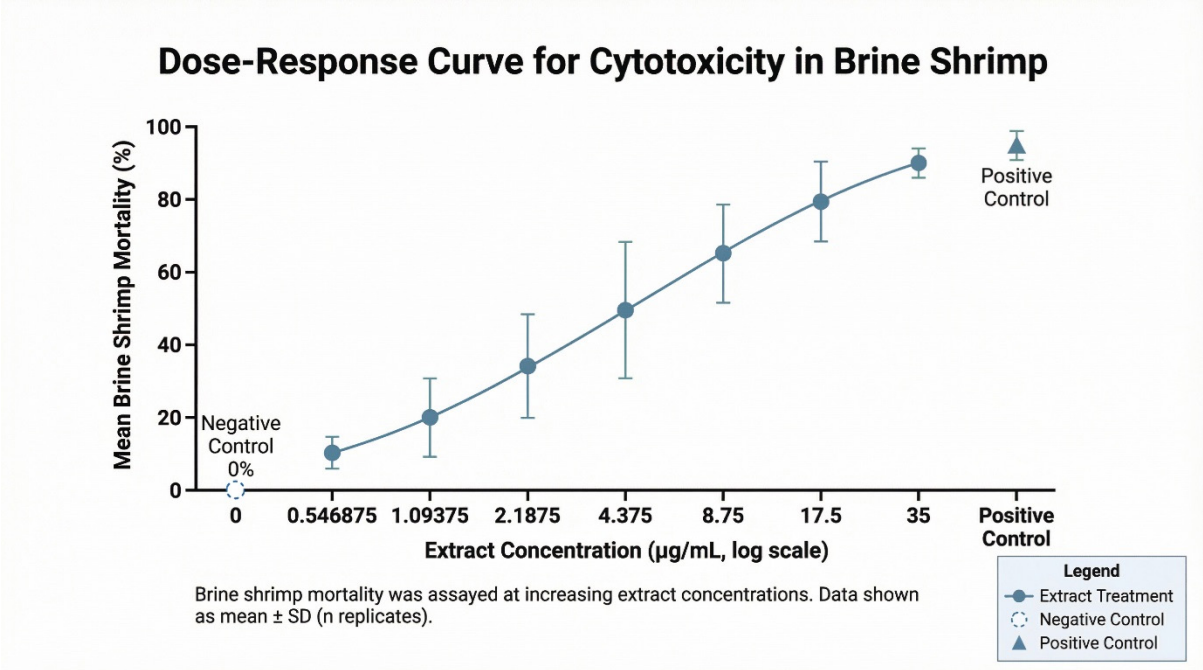
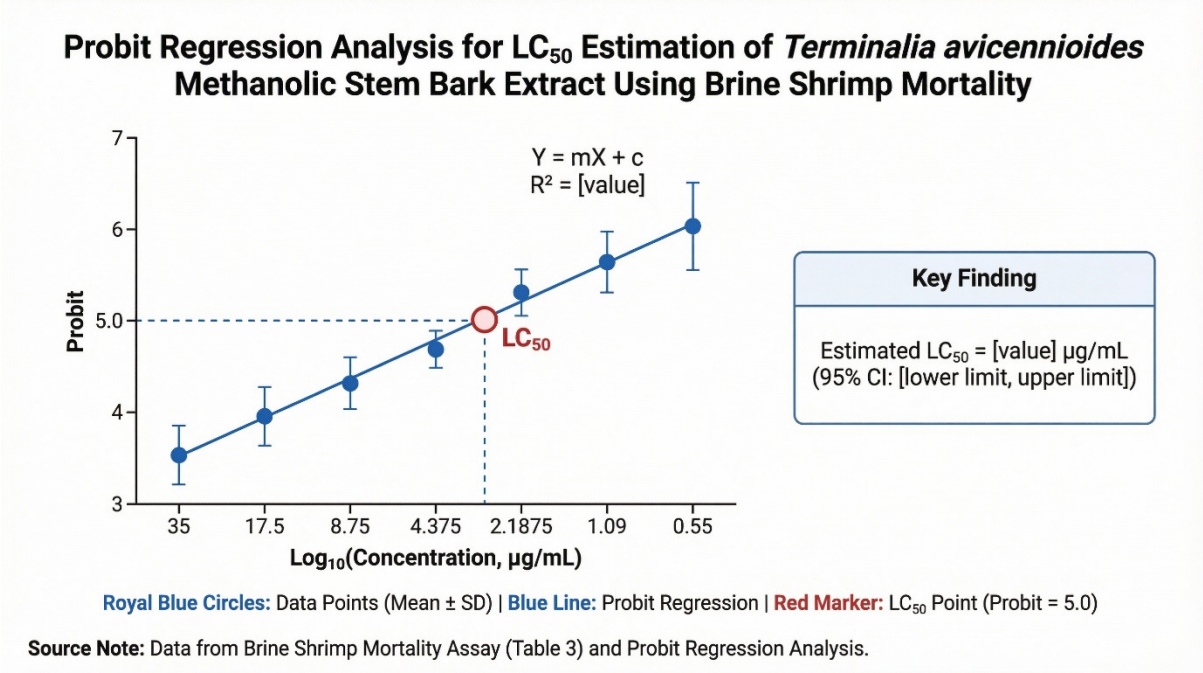
Cite this article: Ngwu TA, Olasunkanmi OO, Akpe V, Cock IE. Antibacterial Efficacy and Cytotoxicity of *Terminalia avicennioides* Guill. and Perr. Stem Bark Extract Against Resistant Pathogens. Pharmacognosy Communications. 2026;16(2):85-94.



Supplementary Figure 1: Dose-response curve for cytotoxicity in *Artemia franciscana*. Mean mortality (%) of brine shrimp nauplii exposed to increasing concentrations of *Terminalia avicennioides* stem bark extract, with negative and positive controls included for assay validation.



Supplementary Figure 2: Probit regression analysis for LC_{50} estimation. Probit transformed mortality values plotted against extract concentration to determine the LC_{50} of *Terminalia avicennioides* stem bark extract. The linear regression model demonstrates a strong fit ($R^2 = 0.981$), supporting the reliability of the LC_{50} estimate.