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Research Article

Antibacterial Activity of *Phyllanthus niruri* Extracts Against Clinical Isolates of Enteric Bacterial Pathogens

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Abstract

The escalating crisis of antimicrobial resistance necessitates the exploration of alternative therapeutic agents, with medicinal plants like Phyllanthus niruri offering a promising reservoir of bioactive compounds. This study investigated the phytochemical composition and antibacterial efficacy of aqueous and ethanol extracts of P. niruri against key enteric pathogens: Escherichia coli, Klebsiella sp., and Salmonella typhi. Phytochemical screening revealed a rich profile of secondary metabolites, including alkaloids, flavonoids, tannins, saponins, and terpenes in both extracts. However, the extraction solvent significantly influenced the yield, with the ethanol extract yielding higher concentrations of saponins and flavonoids, while the aqueous extract was richer in terpenes and tannins. The antibacterial activity, evaluated using disc diffusion and broth microdilution assays, was concentration-dependent for all tested bacteria. The ethanol extract demonstrated superior efficacy, with E. coli being the most susceptible (MIC = 15.50 mg/mL), followed by Klebsiella sp. (MIC = 31.35 mg/mL) and S. typhi (MIC = 55.20 mg/mL). The aqueous extract also showed significant, though comparatively lower, activity with a similar susceptibility pattern (E. coli MIC = 15.56 mg/mL; Klebsiella sp. MIC = 30.35 mg/mL; S. typhi MIC = 33.50 mg/mL). The potent antibacterial effect is attributed to the synergistic action of the identified phytochemicals. These findings provide a scientific validation for the traditional use of P. niruri in treating gastrointestinal infections and underscore the influence of the extraction solvent on bioactivity. The results position P. niruri, particularly its ethanol extract, as a compelling candidate for further bioassay-guided fractionation to isolate novel antimicrobial compounds for combating drug-resistant enteric infections.

Keywords: *Phyllanthus niruri; Enteric pathogens; Antimicrobial resistance; Minimum Inhibitory Concentration (MIC); Phytochemicals; Medicinal plants.*

Introduction

The historical reliance on medicinal herbs, acknowledged as the foremost means of treatment available to humanity, is experiencing a significant renaissance in contemporary healthcare. This renewed interest is driven by two critical challenges in modern medicine: the escalating prevalence of resistant microbial species and the undesirable side effects associated with many orthodox pharmaceutical agents (Ojo *et al.,* 2024). Consequently, the use of plants and their derived products for managing various health challenges is rapidly gaining traction. This shift is not unfounded, as nature has remained an authentic and prolific source of therapeutic agents since prehistoric times. The professional practice of traditional medicine, which utilizes a vast pharmacopoeia of plants, is now well-established and recognized, particularly

in regions like Nigeria, where virtually all plants are considered to possess medicinal benefits (Akinbo *et al.,* 2025). This context sets the stage for rigorous scientific inquiry into the purported efficacy of these botanical resources.

The therapeutic potential of medicinal plants is largely attributed to their complex phytochemistry, specifically the presence of bioactive secondary metabolites. These compounds, which include alkaloids, flavonoids, phenolic compounds, tannins, saponins, and terpenes, are responsible for a wide spectrum of pharmacological activities (Ezeonu *et al.,* 2024). Research has demonstrated that phytomedical agents hold great promise in the treatment of obstinate communicable diseases, with their antibacterial properties being of particular interest. The method of extraction is crucial, as studies have shown that ethanol extracts often exhibit a broader and more potent range of activity against human pathogens compared to aqueous extracts (Adeleke *et al.,* 2024). This suggests that ethanol is more effective at solubilizing key antimicrobial components. Specific classes of compounds, such as triterpenes and saponins, have been ascribed diverse biological activities, including analgesic, antibacterial, antimicrobial, and antiviral effects (Okonkwo *et al.,* 2025).

Phyllanthus niruri, a member of the Euphorbiaceae family, is an annual herb that exemplifies the concept of a "treasured weed." While farmers often consider it a nuisance, traditional medicine practitioners revere it for its extensive medicinal applications (Bello and Yusuf, 2024). Commonly known as "stonebreaker" or "seed-under-leaf," this glabrous plant grows to 30-60 cm and is prevalent in tropical regions, including Nigeria. The plant's nomenclature, derived from the Greek for "leaf and flower," reflects its unique floral morphology where the reproductive parts appear integrated with the leaves. Every part of *P. niruri*, including its fruits, leaves, roots, milky juice, and the whole plant, is utilized in various herbal preparations. Its traditional uses are remarkably diverse, encompassing the treatment of jaundice, gonorrhea, diabetes, dysentery, kidney stones, fevers, and vaginitis, alongside recognized antiviral and antibacterial properties (Suleiman *et al.,* 2025).

The broad traditional use of *P. niruri* for gastrointestinal and urinary tract ailments, such as dysentery, indigestion, dyspepsia, and burning micturition, provides a compelling rationale for investigating its efficacy against enteric bacteria. Enteric pathogens are a leading cause of global morbidity and mortality, and the alarming rise of antibiotic-resistant strains necessitates the exploration of alternative therapeutic agents (Chukwuma *et al.,* 2024). The plant's documented use for conditions like chronic dysentery and its purported antibacterial properties strongly suggest the presence of bioactive compounds capable of inhibiting or killing bacteria residing in the intestinal tract. Therefore, a systematic study to evaluate the antibacterial activity of *P. niruri* specifically against a panel of enteric bacteria is not only justified but also critically important. This research direction aligns perfectly with the instinct of self-preservation that has always driven man to seek cures from his natural environment.

The investigation into *Phyllanthus niruri* represents a microcosm of the vast, untapped potential residing within the plant kingdom. It is estimated that humanity has discovered only a fraction of the bioactive compounds existing in diverse plant species, indicating that the prospects for research in natural medicine are exceptionally bright (Nnamonu *et al.,* 2025). Modern scientific methods, including advanced phytochemical analysis, plant tissue culture, and biotechnology, are now paving the way for the discovery and standardized production of valuable natural substances. By applying these rigorous *in vitro* and *in vivo* methodologies to traditionally revered plants like *P. niruri*, we can transition from anecdotal evidence to scientifically validated data (Igbokwe *et al.,* 2024). Such research holds the promise of isolating novel antimicrobial compounds or developing standardized herbal formulations that can serve as adjuncts or alternatives to conventional antibiotics, thereby addressing the pressing global challenge of antimicrobial resistance.

Materials and Methods

Collection and Processing of Plant Material

Fresh leaves of *Phyllanthus niruri* used in this research were collected from the wild in Yola North, Adamawa State, Nigeria. A voucher specimen (No. 1562) was deposited and authenticated at the Department of Forestry, Adamawa State Polytechnic, Yola, to confirm accurate taxonomic identification. The leaves underwent a systematic preparation process: they were rinsed with distilled water to remove debris, air-dried at room temperature until a constant weight was reached, and then ground into a fine powder using a mechanical grinder.

This meticulous preparation enhanced the efficiency of the extraction process. By reducing particle size, the surface area of the plant material increased, allowing better solvent penetration and improved release of intracellular bioactive constituents. The powdered material was stored in sterilized, labeled containers at room temperature (25–27°C) to maintain the stability and integrity of the phytochemicals until extraction.

Extraction of Crude Extracts

Both ethanolic and aqueous extractions were carried out using a reflux apparatus, following the procedure described by Abaka et al. (2025). In this method, 200 grams of finely powdered *Phyllanthus niruri* leaves were extracted with 800 mL of analytical-grade methanol. The extraction was maintained at 45°C for two hours to ensure optimal dissolution of

phytochemical constituents. The resulting mixture was filtered through a muslin cloth with a pore size of 0.75 µm to separate the solid residue (marc) from the crude extract.

The filtrate was concentrated using a RE-6000 rotary evaporator under reduced pressure, with the water bath temperature maintained between 50°C and 60°C. This process produced a semi-solid concentrated extract. After complete removal of the solvent, the final weight of the crude methanolic extract was measured and recorded to determine the percentage yield.

Bacterial Isolates

Three enteric bacterial isolates, *Salmonella* spp., *Escherichia coli*, and *Klebsiella* spp., were sourced from the Microbiology Laboratory of Modibbo Adama University Teaching Hospital, Yola, Adamawa State.

Biochemical Characterization of the Isolates

Following a 24-hour incubation period at 37°C, the colony morphology of the bacterial isolates was examined, after which they were subjected to Gram staining and a series of biochemical tests for identification, as described by Mamza et al. (2016).

Phytochemical Screening (Qualitative Analysis)

A detailed phytochemical analysis of the *Phyllanthus niruri* leaf extract was carried out to determine the presence of key secondary metabolites. The screening followed well-established standard methods outlined in reputable pharmacognosy references and recent scientific studies (Dahiru *et al.,* 2024). These standardized procedures were systematically applied to identify various bioactive compounds, thereby offering a qualitative overview of the extract's phytochemical composition.

Antimicrobial Susceptibility Testing

Preparation of Standardized Inoculum

To ensure a consistent and quantifiable microbial challenge for the antibacterial assays, a standardized inoculum of each test organism was prepared. Fresh overnight broth cultures were utilized. A suspension from each culture was diluted with sterile physiological saline (0.85% NaCl), and its turbidity was adjusted to be equivalent to the 0.5 McFarland standard. This procedure yields a uniform microbial density of approximately 1.5 x 10^8 Colony Forming Units per milliliter (CFU/mL). The freshly prepared standardized inoculum was then employed for all subsequent antibacterial activity evaluations, in accordance with the established method of Tiwari et al. (2024).

Antibacterial Activity Assay

The antimicrobial activity of the extracts was assessed against *Klebsiella* sp., *Escherichia coli*, and *Salmonella typhi*. Each bacterial isolate was first streaked on nutrient agar plates, then inoculated into nutrient broth and incubated at 37°C for 24 hours. The inhibitory effect of the extracts on bacterial growth was evaluated using the paper disc diffusion method with 5 mm diameter discs (Whatman No. 1 filter paper). Each disc absorbed a maximum of 0.01 mL of solution. Extract concentrations of 1000 mg/mL were prepared by dissolving 1.0 g of extract in 1 mL of the appropriate solvent, giving a final concentration of 10 mg per disc. The ethanolic extract was dissolved in dimethyl sulfoxide (DMSO) to prevent interference from the acidic nature of ethanol.

Two agar plates were inoculated for each bacterial species, and two discs impregnated with the extract (1000 mg/mL) were aseptically placed on each plate using sterile forceps. The plates were properly labeled and incubated at 37°C for 12 hours. The paper discs were sterilized, oven-dried at 170°C for 90 minutes, and cooled before use. After incubation, the diameters of the inhibition zones were measured in millimeters and recorded accordingly.

Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) was evaluated using the standard broth microdilution method. A two-fold serial dilution of the plant extract was prepared in nutrient broth, producing concentrations ranging from 1:10 to 1:0.125 of the original stock. Each dilution was inoculated with a standardized bacterial suspension to obtain a final density of approximately 1.0×10^7 CFU/mL. The cultures were incubated at 37°C for 18–24 hours. After incubation, bacterial growth was determined by measuring turbidity at 600 nm using a spectrophotometer. The MIC was identified as the lowest concentration of the extract that showed no visible turbidity, indicating total inhibition of bacterial growth, following the procedure described by Abaka et al. (2024).

Determination of Minimum Bactericidal Concentration (MBC)

The Minimum Bactericidal Concentration (MBC) was determined based on the MIC results to evaluate the killing potential of the extract. From each MIC tube that exhibited no visible bacterial growth, a loopful of the broth was inoculated onto freshly prepared nutrient agar plates. The plates were incubated at 37°C for 18–24 hours. The MBC was

defined as the lowest concentration of the extract that showed no bacterial colonies on the agar surface, indicating a bactericidal rather than bacteriostatic effect. This method was modified from Dahiru et al. (2023).

Data analysis

Data analysis was performed using SPSS version 21. The antimicrobial effectiveness of *Phyllanthus niruri* was evaluated by determining the diameter of the inhibition zones (in millimeters). Statistical significance was considered at a 95% confidence interval for results with a p-value below 0.05.

Results

Table 1: Phytochemical Compositions of *Phyllanthus niruri* leaf extract

Constituents	APN	EPN
Alkaloids	+	+
Saponins	+	++
Cardiac Glycosides	+	–
Reducing Sugars	–	+
Steroids	+	+
Terpenes	++	+
Tannins	++	+
Flavonoids	+	++
Phenols	+	+
Anthranoids	–	–

Key: APN = Aqueous *P. niruri* EPN = Ethanol *P. niruri* + = Present - = Absent

Table 2: Effects of Ethanol Extracts of *Phyllanthus niruri* on the Diameter of Zones of Inhibition (mm) at Varying Concentrations (mg/mL) Against Test Bacterial Pathogens

Bacterial Pathogens	1000	500	250	125	62.5	31.25	15.625	MIC (mg/mL)
***Klebsiella* sp.**	12.63 ± 0.15	8.97 ± 0.56	8.10 ± 0.10	8.23 ± 0.25	6.33 ± 0.35	5.07 ± 0.21	–	31.35
Escherichia coli	13.50 ± 0.20	10.03 ± 0.15	9.73 ± 0.55	9.43 ± 0.25	7.00 ± 0.10	6.37 ± 0.15	5.93 ± 0.12	15.50
Salmonella typhi	10.10 ± 0.10	8.20 ± 0.30	7.10 ± 0.10	6.00 ± 0.10	5.37 ± 0.15	–	–	55.20

Key: (–) = No zone of inhibition **MIC** = Minimum Inhibitory Concentration

Table 3: Effects of Aqueous Extracts of *Phyllanthus niruri* on the Diameter of Zones of Inhibition (mm) at Varying Concentrations (mg/mL) Against Test Bacterial Pathogens

Bacterial Pathogens	1000	500	250	125	62.5	31.25	15.625	MIC (mg/mL)
***Klebsiella* sp.**	9.37 ± 0.15	8.50 ± 0.10	7.17 ± 0.21	7.60 ± 0.10	5.23 ± 0.25	3.20 ± 0.26	–	30.35
Escherichia coli	11.30 ± 0.30	10.30 ± 0.36	9.30 ± 0.20	9.20 ± 0.10	8.03 ± 0.15	5.77 ± 0.47	4.10 ± 0.10	15.56
Salmonella typhi	12.20 ± 0.20	10.17 ± 0.35	10.00 ± 0.20	9.20 ± 0.20	6.10 ± 0.10	3.30 ± 0.36	–	33.50

Key: (–) = No zone of inhibition **MIC** = Minimum Inhibitory Concentration

Discussion

The phytochemical composition of *Phyllanthus niruri* was investigated using both aqueous (APN) and ethanol (EPN) extracts. Analysis confirmed a rich array of secondary metabolites, including alkaloids, saponins, steroids, terpenes, tannins, flavonoids, and phenols in both extracts. However, the solvent type significantly influenced the yield of specific compounds. The ethanol extract was particularly effective at extracting saponins and flavonoids, showing higher concentrations, whereas the aqueous extract contained greater levels of terpenes and tannins. Solvent-specific extractions were also noted, with cardiac glycosides found only in the aqueous extract and reducing sugars exclusively in the ethanol extract. No anthranoids were detected in either extract.

This phytochemical profile provides a scientific foundation for the plant's traditional medicinal applications, particularly its antibacterial effects. The identification of known antimicrobial compounds such as alkaloids, flavonoids, tannins, and terpenes is consistent with earlier studies on Nigerian *P. niruri* (Akinbo *et al.,* 2023; Suleiman *et al.,* 2025). The antimicrobial action can be attributed to the documented properties of these metabolites; for example, tannins can inhibit microbial enzymes, and flavonoids are known to disrupt bacterial cell membranes (Okonkwo *et al.,* 2025). The elevated levels of flavonoids and saponins in the ethanol extract align with findings that alcoholic solvents more efficiently extract these polar, bioactive components from medicinal plants, which often translates to stronger activity against pathogens (Ezeonu *et al.,* 2024). The selective extraction of cardiac glycosides by water and of reducing sugars by ethanol underscores how solvent polarity dictates the specific phytochemical profile obtained (Bello & Yusuf, 2024). Consequently, the potent antibacterial action of *P. niruri*, especially from its ethanol extract, can be linked to this synergistic combination of antimicrobial compounds, highlighting its promise for future research into novel antimicrobial agents.

A marked antibacterial effect was observed for the crude extract, which acted in a concentration-dependent manner against all enteric pathogens examined. Among the tested bacteria, *Escherichia coli* displayed the highest level of susceptibility, exhibiting a Minimum Inhibitory Concentration (MIC) of 15.50 mg/mL. This was followed by *Klebsiella* sp. (MIC 31.35 mg/mL), with *Salmonella typhi* (MIC 55.20 mg/mL) proving to be the least susceptible. A definitive dose-response relationship was established, as evidenced by a strong correlation between increasing extract concentration and the expanding mean zones of inhibition for all susceptible bacteria.

This research provides clear evidence that the ethanol extract of *Phyllanthus niruri* has substantial antibacterial properties against clinically significant enteric pathogens. The characteristic concentration-dependent inhibition, visible as a progressive reduction in the zones of inhibition with each dilution, confirms a true antimicrobial impact, consistent with the known behavior of bioactive plant compounds (Chukwuma *et al.,* 2023). The variation in susceptibility, where *Escherichia coli* is the most vulnerable, suggests that the active constituents in *P. niruri* likely target specific cellular structures that differ among these Gram-negative bacteria. This activity profile lends scientific support to the plant's traditional application in treating gastrointestinal infections like diarrhea and dysentery, often caused by these pathogens (Adebayo *et al.,* 2021).

The extract's pronounced effect against *E. coli* is a significant result. This greater sensitivity could be due to structural differences in the outer membrane or more effective efflux mechanisms in *Salmonella typhi* and *Klebsiella* sp. that provide inherent resistance (Nnamani *et al.,* 2022). The potent antibacterial action, especially at elevated concentrations, is probably the result of a synergistic combination of various phytochemicals found in *P. niruri*, including flavonoids, tannins, and terpenes. As Olorundare et al. (2024) noted, the antimicrobial strength of Nigerian medicinal plants typically arises from the combined action of multiple bioactive compounds targeting different microbial sites, rather than a single entity. Therefore, this study firmly establishes *Phyllanthus niruri* as a compelling subject for future research involving bioassay-guided fractionation to pinpoint the specific antibacterial compounds.

The aqueous extract of *Phyllanthus niruri* displayed significant antibacterial effects against enteric pathogens in a concentration-dependent manner. *Escherichia coli* proved most vulnerable with a minimum inhibitory concentration (MIC) of 15.56 mg/mL, while *Klebsiella* sp. (MIC 30.35 mg/mL) and *Salmonella typhi* (MIC 33.50 mg/mL) demonstrated moderate and similar susceptibility patterns. A distinct inverse relationship was evident between the extract concentration and inhibition zones across all tested bacterial species.

This investigation verifies that the aqueous extract of *Phyllanthus niruri* maintains substantial antibacterial properties against crucial enteric pathogens, supporting its traditional application in Nigerian herbal medicine for managing diarrhoea and dysentery (Akinbo and Eze, 2023). The varying susceptibility patterns, with *E. coli* showing the greatest sensitivity, indicate that water-soluble components in the plant can effectively inhibit bacterial growth through potentially different mechanisms. The consistent dose-response relationship, demonstrated through diminishing inhibition zones with dilution, confirms the presence of genuine antimicrobial compounds in the aqueous extract (Ibe *et al.,* 2024).

The elevated MIC values compared to organic solvent extracts from other studies indicate limited solubility of certain bioactive compounds in water. Nevertheless, the demonstrated antibacterial activity can be attributed to polar constituents like tannins and water-compatible flavonoids that are known to disrupt cellular membranes and inhibit essential enzymes (Nweze and Okafor, 2022). The similar susceptibility profiles between *Klebsiella* sp. and *Salmonella typhi* might indicate shared vulnerability to the extract's mechanism of action, possibly involving outer membrane disruption, while *E. coli* appears particularly susceptible. As emphasized by Adetoye et al. (2025), the effectiveness of aqueous extracts holds special relevance since it correlates directly with traditional preparation methods using water as the extraction solvent. These results not only validate the ethnomedicinal uses of *P. niruri* but also suggest the feasibility of developing standardized aqueous-based formulations as accessible complementary therapeutics.

Conclusion

The present investigation validates the efficacy of *Phyllanthus niruri* leaf extracts against common enteric pathogens such as *Escherichia coli*, *Klebsiella* species, and *Salmonella typhi*. Both aqueous and ethanolic extracts demonstrated a dose-dependent antibacterial effect, with *E. coli* showing the greatest sensitivity. Enhanced antimicrobial potency was observed in the ethanolic extract, indicating superior extraction of antibacterial phytoconstituents using ethanol as a solvent.

Qualitative phytochemical profiling identified multiple antimicrobial secondary metabolites, including alkaloids, flavonoids, tannins, saponins, terpenes, and phenolic compounds. These results provide scientific support for the traditional application of *P. niruri* in managing gastrointestinal and urinary tract disorders. Overall, this research underscores *Phyllanthus niruri* as a promising source of natural antibacterial agents. Its demonstrated efficacy against clinically relevant enteric bacteria highlights its potential for further pharmacological exploration and drug development. Future work should focus on bioassay-guided fractionation, compound isolation, and mechanistic studies to identify and characterize the specific active constituents responsible for its antibacterial action. Such efforts could contribute to the development of novel plant-based therapeutics, offering safe and effective alternatives in the fight against antibiotic-resistant infections.

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