

Phytochemical Screening and Comparative Antimicrobial Activity of *Phyllanthus niruri* L. (Phyllanthaceae) against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*

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Abstract

Phyllanthus niruri L. is a medicinal plant widely used in traditional medicine in Mozambique, yet its comparative antimicrobial spectrum remains to be fully elucidated through scientific research. This study evaluated the phytochemical profile and antimicrobial activity of the crude leaf extract of *P. niruri* L. collected in Nampula, Mozambique. Qualitative phytochemical screening was performed to identify secondary metabolites. Antimicrobial potential was assessed using the Kirby-Bauer disk diffusion method and the Minimum Inhibitory Concentration (MIC) via the broth microdilution method, with 0.5% triphenyltetrazolium chloride (TTC) as a metabolic indicator. Phytochemical analysis revealed the presence of phenols, flavonoids, and tannins, while alkaloids and saponins were absent. The crude leaf extract of *P. niruri* L. demonstrated high potency against *Staphylococcus aureus* and *Candida albicans*, both yielding an MIC of 12.5 mg/mL. In the disk diffusion assay, a plateau effect was observed for *Staphylococcus aureus* ($P=0.587$), whereas *Candida albicans* exhibited a robust dose-dependent response ($P < 0.001$). Notably, at concentrations ≥ 25 mg/mL, the crude leaf extract of *P. niruri* L. outperformed the clinical standard Fluconazole against the yeast. *Escherichia coli* exhibited significant resistance (MIC >100 mg/mL). The *P. niruri* L. extract possesses selective antimicrobial properties, standing out as a potent natural antifungal agent. These findings validate its ethnomedicinal use and offer a promising alternative to address antimicrobial resistance.

Introduction

Phytotherapy represents an ancestral and essential practice in primary healthcare within developing countries, where approximately 80% of the global population relies on traditional medicine for treating various pathologies (Elachouri et al., 2021; Tamo, Parfait and Lawrence, 2023; Aremu, Luo and Mussarat, 2024; Latif and Nawaz, 2025). In Mozambique, the recognition of local ethnobotanical knowledge is fundamental, particularly in regions where conventional sanitary coverage is limited (Manuel et al., 2020; Razão et al., 2024). Among the diverse species used to manage infectious diseases in Mozambican flora, *P. niruri* L., (Phyllanthaceae) stands out. Popularly known as "quebra-pedra" in Portuguese and "opwecha malukhu" in Emakua, a local language spoken in Nampula province. *Phyllanthus niruri* L. is widely employed in traditional medicine to treat urinary and hepatic disorders, as well as infectious and inflammatory processes (Jantan et al., 2019; Barbosa et al., 2020; Razão et al., 2024; Tiwana and Cock, 2024; Dahiru et al., 2025).

The fundamental basis of the multi-target pharmacological profile of *P. niruri* L., lies in its complex phytochemical constitution. The plant synthesizes a diverse array of secondary metabolites with distinct therapeutic properties. Among the primary isolated groups, the chemical structures of the secondary metabolites from the crude leaf extract of *P. niruri* L. are shown below (Fig. 1), as reported in the literature, polyphenols and tannins, such as gallic acid (6), geraniin (7), and corilagin (8), ensure astringent activity alongside robust antiviral and antibacterial functions (Keyvani-Ghamsari, Rahimi and Khorsandi, 2023; Hadidi et al., 2024; Mandal and Domb, 2024).

Regarding the phytochemical profile with bacteriostatic and bactericidal potential, quercetin (3), rutin (4), and astragalín (5) stand out, alongside biologically relevant flavonoids such as epicatechin (13), vitexin (18), kaempferol (23), and apigenin (24), which provide significant antioxidant properties and contribute to the inhibition of microorganisms (Rusmana et al., 2017; Osonga et al., 2019; Liang et al., 2021; Obistioiu et al., 2021; Faith Robert Owabhel and Abraham Sisein Eboh, 2022; Ivanov et al., 2022; Zhong et al., 2022; Veiko et al., 2023; Wang et al., 2023; Tiwana and Cock, 2024).

These polyphenols constitute the primary secondary metabolites isolated from *P. niruri* L., with reported antibacterial activity, often acting through the inhibition of protein synthesis or the disruption of bacterial cell wall integrity, (Araújo et al., 2024; Hasan et al., 2024; Labu et al., 2026).

Additionally, alkaloids such as norsecurinine (9) and nirurine (10) play a crucial role through their analgesic and antispasmodic effects (Jantan et al., 2019), while terpenes, including lupeol (11) and limonene (12), exhibit pharmacological activities focused on reducing inflammatory processes and exerting cytotoxic effects against abnormal cells (Jantan et al., 2019; Wróblewska-Luczka et al., 2023; Dalimunthe et al., 2024; Intan Wiratmini et al., 2025).

Regarding the antimicrobial activity documented in the literature for the genus *Phyllanthus*, global scientific evidence frequently correlates biological efficacy with the presence of diverse classes of secondary metabolites. In many tropical regions, the antifungal response is predominantly associated with specific lignans and alkaloids, such as phyllanthin (1), hypofyllantin (2), lintetralin (25), and securinolines including securinine (26), securinol A (27), and securinol B (28) (Shilpa et al., 2018; Ferrante et al., 2020; Bose Mazumdar Ghosh, Banerjee and Chattopadhyay, 2022; Sulaiman et al., 2022; Windayani et al., 2022; Pratima et al., 2025; Saket et al., 2025). These metabolites demonstrate selective inhibitory capacity against filamentous fungi and yeasts, exhibiting distinct mechanisms of action from those observed in polyphenols. The segregation of these biological activities across different chemical classes, where polyphenols frequently dominate the antibacterial response and lignans or alkaloids drive the antifungal response highlights the complexity of the secondary metabolism of *P. niruri* L., and reinforces the necessity of bio-guided isolation approaches for the development of new antimicrobial agents (Shilpa et al., 2018; Ferrante et al., 2020; Windayani et al., 2022; Tiwana and Cock, 2024; Labu et al., 2026). However, recent investigations demonstrate that intra-specific chemical variability can determine distinct phototherapeutic profiles. It is hypothesized that fluctuations in biogeographical and adaptive barriers induce the preferential biosynthesis of alternative metabolic pathways, establishing the need to map local chemotypes whose pharmacological mechanisms do not rely exclusively on these minority or absent classes. Taxonomically, *P. niruri* L., formerly classified under Euphorbiaceae is a small annual herb native to the tropical and subtropical regions of the Americas and Asia, with a significant presence in India and the Amazon basin. In Mozambique, however, its occurrence is characterized as introduced and naturalized. The species has demonstrated high adaptability to the local tropical climate, frequently proliferating as a weed in moist soils, cultivated areas, and roadsides (Namuddu et al., 2025; Preeti Rani & Pooja b, 2025). It is noteworthy that specific ethnopharmacological studies regarding *P. niruri* L., in the Mozambican context remain scarce.

Nevertheless, existing research from other tropical regions provides significant ecological and ethnomedicinal relevance to this study. Parallel to this traditional use, antimicrobial resistance (AMR) has emerged as a global public health crisis. This scenario is particularly critical in Mozambique, where resistance rates exceed 50% for *E. coli* and 40% for *S. aureus* (Garrine et al., 2023; Gahimbare et al., 2024). Such an epidemiological landscape necessitates an urgent search for effective therapeutic alternatives. However, despite the widespread accessibility and use of *P. niruri* L., by the Mozambican population, a significant scientific gap remains regarding the experimental validation of its activity against local pathogens.

Furthermore, concerns persist regarding the efficacy of artisanal preparation methods and the safety of indiscriminate use, especially against microorganisms such as *C. albicans*, which frequently exhibit resistance to conventional antifungals (Santos et al., 2018; Branda et al., 2025; Hetta et al., 2025). The absence of systematic data correlating the phytochemical profile of this species with its inhibitory action limits its safe integration into formal healthcare systems and the development of standardized phytopharmaceuticals (Tiwana and Cock, 2024).

Given this critical context, the present study aimed to characterize the qualitative phytochemical profile and evaluate the antimicrobial activity of the crude leaf extract of *P. niruri* L., collected in Nampula, Mozambique. Specifically, this research sought to identify the predominant bioactive compounds and determine the inhibitory potential, including the Minimum Inhibitory Concentration (MIC), against *Staphylococcus aureus*, *E. coli*, and *C. albicans*, providing scientific evidence to support the ethnomedicinal use of the species while contributing to the prospection of novel plant-derived antimicrobial agents.

MATERIAL AND METHODS

1.1 Plant Material and Extraction

Phyllanthus niruri L., leaves were collected in Nampula, Mozambique (15°05'23.5"S, 39°25'16.5"E), in November 2025. Botanical identification was confirmed, and a voucher specimen was deposited in the Eduardo Mondlane Herbarium registration Number, 8561. The plant material was shade-dried and ground in a knife mill. The crude leaf extract of *P. niruri* L was prepared by static maceration using 70% ethanol (1:12 w/v ratio) for seven days at the Laboratory of Bioactive Natural Products of the Rovuma University. After filtration, the solvent was removed under reduced pressure using a rotary evaporator at 40°C to yield the crude dry extract. The extract was stored in amber glass flasks under refrigeration (4°C), (Zhang, Lin and Ye, 2018; Hasan et al., 2024).

1.2 Phytochemical Screening

Qualitative phytochemical screening was performed to identify the main classes of secondary metabolites. The presence of phenolic compounds and tannins was verified using the ferric chloride test (1% FeCl₃; Sigma-Aldrich, St. Louis, MO, USA). For the identification of flavonoids, the Shinoda reaction

was employed, using magnesium ribbon (Merck KGaA, Darmstadt, Germany) and concentrated HCl (reagent grade, 37%, Honeywell, Charlotte, NC, USA). Alkaloids were screened through precipitation tests with Dragendorff's and Mayer's reagents (Sigma-Aldrich, St. Louis, MO, USA). The presence of saponins was determined by the foam index test, based on the capacity to form persistent foam after mechanical agitation, (Shaikh and Patil, 2020; Maheshwaran et al., 2024; Matseke, Mapfumari and Mothibe, 2025).

1.3 Antimicrobial Assays

Antimicrobial activity was evaluated using the disk diffusion method (Kirby-Bauer), following Clinical and Laboratory Standards Institute (CLSI) guidelines (Afhami et al., 2021; Gaur et al., 2023). Strains of *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), and *Candida albicans* (ATCC 10231) were bred at the Microbiology Laboratory of Nampula Central Hospital. Microbial suspensions were standardized to the 0.5 McFarland scale (approximately 1.5×10^8 CFU/mL). Sterile filter paper disks (6 mm) were impregnated with the extract at concentrations of 3.125, 6.25, 12.5, 25, 50, 75, and 100 mg/mL and placed on Mueller-Hinton agar (bacteria) and Sabouraud agar yeast. Vancomycin (30µg), Tobramycin (25µg), and Fluconazole (25µg) were used as positive controls, while 70% ethanol served as the negative control. Plates were incubated at 37°C for 24 h (bacteria) and 48 h yeast. Inhibition zones (mm) were measured using a digital caliper. (Spengler et al., 2022; Worku et al., 2023; Lopez Venditti et al., 2025).

1.4 Determination of Minimum Inhibitory Concentration (MIC)

The MIC was determined by the broth microdilution method in 96-well microtiter plates. The crude extract was subjected to serial dilutions in Mueller-Hinton broth (100 to 3.125 mg/mL). Each well received a standardized inoculum of 5×10^5 CFU/mL. Sterility, growth, and solvent controls were included. After incubation at 37°C, the MIC was defined as the lowest concentration capable of visually inhibiting growth, confirmed by the addition of 0.5% triphenyltetrazolium chloride (TTC), following the procedure described by (Parvekar et al., 2020; Kowalska-Krochmal and Dudek-Wicher, 2021; Kadeřábková, Mahmood and Mavridou, 2024).

1.5 Statistical Analysis

All assays were performed in technical and biological triplicate. A Shapiro - Wilk normality test was performed, and the results ($p > 0.05$) supported the use of parametric statistical methods. One-way analysis of variance (ANOVA), followed by Tukey's post-hoc test, was used to determine statistically significant differences ($P < 0.05$). Analyses were processed using GraphPad Prism version 8.0, and all the data were expressed as mean $\pm$ SD (standard deviation).

RESULTS

2.1 Phytochemical Profile and Antimicrobial Activity

The extraction process of *P. niruri* leaves yielded 5.94% (w/w) of crude leaf extract relative to the initial dry biomass. This yield is consistent with the maceration method employed, which aims to maximize the recovery of polar bioactive constituents. The qualitative phytochemical screening of the crude leaf extract of *P. niruri* L (Table 1) revealed a predominance of phenolic compounds, flavonoids, and tannins, while alkaloids, saponins, and terpenes were notably absent. The antimicrobial susceptibility profiles across the tested pathogens (Table 2) suggest that this polyphenolic triad drives the observed biological activity through mechanisms modulated by the structural characteristics of each microorganism (Fig. 2).

Table 1
Qualitative phytochemical screening of the ethanolic leaf
crude extract of *Phyllanthus niruri* L.

Secondary metabolites	Leaf crude ethanolic extract
Alkaloids	-
Phenols	+
Flavonoids	+
Saponins	-
Tannins	+
Terpenes	-

Legend

(+) Presence of the metabolite; (-) Absence of the metabolite. Phytochemical tests were performed in triplicate to ensure the reproducibility of the colorimetric and precipitation reactions.

These results are consistent with previous reports for the genus *Phyllanthus*, which identify polyphenols as the primary secondary metabolites responsible for its pharmacological properties (Nisar et al., 2018; Kosnayani et al., 2022; Albrecht et al., 2023; Ghazali, Tarmizi and Yusof, 2024; Hasan et al., 2024; Nikitasari et al., 2024; Prajapati, Maitreya and Rawal, 2025).

The presence of tannins and phenols is particularly relevant to the potent antifungal activity observed against *Candida albicans* in this study (Shilpa et al., 2018; Gizaw et al., 2022; Khan et al., 2023). Tannins are known to reach the fungal cell membrane and induce lipid peroxidation, leading to the leakage of intracellular contents (Hasan et al., 2024; Parvatham et al., 2026). Furthermore, flavonoids can inhibit fungal growth by interfering with the synthesis of essential enzymes and cell wall components (Windayani et al., 2022; Nawfetrias et al., 2024). The synergy between these classes of compounds likely explains why the 100 mg/mL extract concentration outperformed the commercial antifungal Fluconazole, as the crude leaf extract of *P. niruri* L, offers a complex matrix of bioactive molecules acting through multiple inhibitory mechanisms.

2.2 Antimicrobial Dynamics and Physicochemical Considerations

The antimicrobial evaluation of the crude leaf extract of *P. niruri* L., revealed distinct susceptibility profiles among the tested strains, demonstrating that efficacy is modulated by both the pathogen's cell wall structure and the physicochemical properties of the extract (Table 2). Regarding the Gram-positive bacterium *Staphylococcus aureus*, a consistent inhibitory activity was observed, with zones ranging from 9.7 ± 1.5 to 13.3 ± 3.2 mm. The lack of a strong linear correlation ($r = 0.0721$) indicates a plateau effect, where maximum inhibitory potential is reached at an early stage. Notably, the 12.5 mg/mL concentration yielded halos statistically comparable to those at 100 mg/mL, suggesting high potency without the requirement for elevated dosages. This behavior is attributed to the presence of tannins and phenols (Table 1), which form irreversible complexes with proline-rich proteins in the peptidoglycan layer, leading to cell lysis (Villanueva et al., 2023). On the other hand, the extracts showed moderate activity against *E. coli*, with inhibition zones ranging from 7 to 10 mm, which is to be expected, given that the outer membrane, rich in lipopolysaccharides, acts as a selective barrier against large polyphenolic molecules (Fig. 2). However, the most significant finding was the robust, dose-dependent activity against *Candida albicans* ($r = 0.9148$). At 100 mg/mL, the extract (24.3 ± 1.15 mm) significantly outperformed the positive control fluconazole (16.0 ± 0.0 mm, $p < 0.05$). This suggests a potent synergistic effect between flavonoids and tannins against fungal ergosterol and cell wall components (Lobiuc et al., 2023). It is important to note that the non-linear dose-response observed for bacterial strains at higher concentrations (75–100 mg/mL) may be influenced by the physicochemical dynamics of the disk diffusion assay. High concentrations of tannins and polar macromolecules can increase the viscosity of the extract, potentially limiting the radial diffusion of active constituents through the agar matrix (Tiwana and Cock, 2024). Consequently, the recorded zones of inhibition may represent a conservative estimate of the extract's true pharmacological potential, particularly at higher concentrations where molecular crowding within the agar might occur.

Table 2

Antibacterial activity of *Phyllanthus niruri* ethanolic leaf crude extract against *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922) and *Candida albicans* (ATCC 10231) determined by the disk diffusion assay.

Concentration of leaf crude extract (mg/ml)	<i>S. aureus</i>	<i>E. coli</i>	<i>C. albicans</i>
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
3.125	11.3 ^a $\pm$ 0.6	7 ^a $\pm$ 0.0	12.3 ^a $\pm$ 1.2
6.25	12 ^a $\pm$ 0.0	8 ^a $\pm$ 0.0	12.7 ^{ab} $\pm$ 1.5
12.5	13.3 ^a $\pm$ 0.6	7 ^a $\pm$ 0.0	14.3 ^{bc} $\pm$ 1.5
25	11.7 ^a $\pm$ 1.7	10 ^{ab} $\pm$ 3	17.3 ^{cd} $\pm$ 0.6
50	13.7 ^a $\pm$ 1.2	7.3 ^a $\pm$ 1.2	20 ^{de} $\pm$ 1.0
75	9.7 ^a $\pm$ 1.5	10 ^a $\pm$ 0.0	22 ^{ef} $\pm$ 1.0
100	13.3 ^a $\pm$ 3.2	9.7 ^{ab} $\pm$ 2.9	24.3 ^{fg} $\pm$ 1.2
Vancomycin (30 μ g) (PC)	15 ^a $\pm$ 1.0		
Tobramycin (25 μ g) (PC)	-	13.7 ^b $\pm$ 1.2	-
Fluconazole (25 μ g) (PC)	-	-	16 ^{gh} $\pm$ 0.0
70% Ethanol (NC)	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0

Values are expressed as mean $\pm$ SD (n = 3). Different superscript letters in the same column indicate statistically significant differences between groups (one-way ANOVA followed by Tukey's post-hoc test, P < 0.05). PC: Positive Control; NC: Negative Control. (-) indicates that the clinical standard was not tested against that specific non-target microbial strain, *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922) and *Candida albicans* (ATCC 10231).

2.3 Minimum Inhibitory Concentration (MIC)

The quantitative susceptibility of the tested strains was established through the MIC assay, with metabolic viability confirmed by the TTC redox indicator (Table 3). The extract exhibited high potency against *S. aureus* and *C. albicans*, both yielding an MIC of 12.5 mg/mL. For *S. aureus*, this value explains the plateau effect observed in the disk diffusion assays (Table 2); since the lowest tested dose (3.125 mg/mL) already initiated inhibition and the MIC was reached at 12.5 mg/mL, further increases in concentration did not yield proportionally larger halos, suggesting a saturation of molecular targets within the Gram-positive peptidoglycan layer.

In contrast, *C. albicans* demonstrated high susceptibility, with the MIC of 12.5 mg/mL aligning with the robust, dose-dependent inhibition zones observed in the Kirby-Bauer test. The lack of red formazan precipitate indicates complete metabolic arrest, likely due to polyphenolic interference with the fungal respiratory chain or membrane integrity (Lobiuc et al., 2023). Conversely, *E. coli* showed significant resistance (MIC > 100 mg/mL), confirming that the lipopolysaccharide-rich outer membrane effectively restricts the influx of the extract’s bioactive tannins and flavonoids to sub-therapeutic levels. These findings suggest that the mechanism of action involves the precipitation of membrane proteins and enzymatic inhibition in the electron transport chain (Sunitha et al., 2017), establishing *P. niruri* as a potent candidate for natural antimicrobial development.

Table 3

Minimum Inhibitory Concentration of *P. niruri* leaf crude extract against bacterial and fungal strains.

Microorganism	MIC Range Tested (mg/mL)	MIC Value (mg/mL)	Classification
<i>S. aureus</i> (ATCC 25923)	3.125–100	12.5	High Potency
<i>E. coli</i> (ATCC 25922)	3.125–100	> 100	Low Potency
<i>C. albicans</i> (ATCC 10231)	3.125–100	12.5	High Potency

Classification based on ethnopharmacological potency thresholds. The range tested was adjusted to reflect the concentrations used in the disk diffusion assay for direct correlation.

DISCUSSION

The qualitative phytochemical screening of the *Phyllanthus niruri* ethanolic leaf extract revealed a robust presence of phenolic compounds, flavonoids, and tannins, consistent with previous reports for the genus *Phyllanthus* (Sunitha et al., 2017; Hasan et al., 2024). These secondary metabolites are critical determinants of the observed biological activity. The absence of alkaloids and saponins suggests that the antimicrobial potential is primarily derived from the synergistic action of this polyphenolic triad, which is recognized for its ability to complex with cell wall proteins and destabilize cytoplasmic membranes (Habeeb Rahuman et al., 2022; Anshary et al., 2026).

Although the preliminary phytochemical screening performed in this study was qualitative in nature, it successfully delineated a distinct chemical signature for the *Phyllanthus niruri* L. chemotype native to Nampula, characterized by a robust and exclusive polyphenolic triad (phenols, flavonoids, and tannins). We acknowledge that the absence of quantitative determinations, such as Total Phenolic Content (TPC) or Total Flavonoid Content (TFC), represents a limitation regarding fine chemical profiling. However, the primary focus and core scientific contribution of this manuscript reside not in isolation chemistry, but rather in the comparative pharmacological dynamics, kinetic behavior, and selective potency of the crude extract against critical clinical and resistant pathogens. By demonstrating a clear dose-dependent efficacy against *Candida albicans* that significantly outperforms the clinical standard Fluconazole at higher concentrations, this work provides immediate ethnopharmacological relevance. Qualitative

screening was therefore robust enough to establish that this exceptional antimicrobial profile is driven by a non-alkaloidal, purely polyphenolic synergy, setting a solid baseline for future bio-guided isolation and subsequent quantitative chromatographic characterization of this specific geographical variant.

Regarding antibacterial activity, the results demonstrated a marked selectivity between Gram-positive and Gram-negative pathogens. The extract showed significant potency against *Staphylococcus aureus*, achieving consistent inhibition even at the lowest concentration tested, 3.125 mg/mL (11.33 ± 0.58 mm). The observed plateau effect, where increasing concentrations did not result in significantly larger inhibition zones ($r = 0.0721$; $P = 0.587$), indicates early saturation of binding sites within the thick peptidoglycan layer. This phenomenon aligns with findings describing the relative ease with which tannins and phenols penetrate the cell wall of Gram-positive bacteria, leading to the precipitation of vital proteins and leakage of intracellular contents (Albrecht et al., 2023; Tiwana and Cock, 2024). Furthermore, flavonoids may contribute to metabolic exhaustion by inhibiting ATP synthase and nucleic acid synthesis (Firdaus et al., 2025). Conversely, the resistance observed in *Escherichia coli* highlights the role of the lipopolysaccharide (LPS) outer membrane as an efficient permeability barrier (Nikolic and Mudgil, 2023). This asymmetric structure restricts bulky polar molecules, such as the condensed tannins identified in this extract, from reaching the periplasmic space. The presence of modest inhibition zones (7.00 to 10.00 mm) only at specific concentrations reinforces the protective role of the Gram-negative cell envelope (Štumpf et al., 2020; Villanueva et al., 2023). The most significant finding was the exceptional antifungal potency against *Candida albicans*. The extract demonstrated a clear dose-dependent effect ($P < 0.001$), starting with 12.33 mm at 3.125 mg/mL and reaching 24.33 mm at 100 mg/mL. Notably, at concentrations above 25 mg/mL, the crude extract outperformed the clinical standard, fluconazole (16.0 ± 0.0 mm), suggesting a potent multi-target mechanism. While fluconazole specifically inhibits the enzyme 14 α -demethylase, the polyphenols from *Phyllanthus niruri* L., likely act synergistically; flavonoids may interfere with ergosterol biosynthesis, while tannins form irreversible complexes with fungal cell wall proteins and chitin (Gizaw et al., 2022; Khan et al., 2023). Metabolic confirmation via the triphenyltetrazolium chloride (TTC) assay reinforces that the inhibition is not merely fungistatic but leads to the cessation of microbial viability. The absence of red formazan coloration at 12.5 mg/mL for both *S. aureus* and *C. albicans* indicates that the extract constituents directly interfere with the respiratory chain or enzymatic integrity. In summary, the integration of phytochemical and biological data positions the *Phyllanthus niruri* L. ethanolic extract as a promising candidate for developing phytomedicines, providing scientific validation for its traditional use against candidiasis and Gram-positive infections.

Conclusion

The findings of this study provide scientific validation for the ethnopharmacological use of *Phyllanthus niruri* L., demonstrating a potent and selective antimicrobial profile linked to its rich polyphenolic content. The extract exhibited high efficacy against *S. aureus* and exceptional antifungal activity against *C. albicans*, outperforming fluconazole and achieving an MIC of 12.5 mg/mL. In contrast, the structural barriers of *E. coli* conferred resistance to the extract within the tested range. These results highlight *P.*

niruri as a superior candidate for the development of new antifungal phytopharmaceuticals and encourage further investigation into the isolation of its bioactive synergistic molecules.

Declarations

Author Contributions

IJ: Investigation, Methodology, Writing – original draft. GMCG: Investigation (Laboratory support). JA: Investigation (Laboratory support). GJH: Conceptualization, Methodology. JDLD: Statistical analysis and review. ASD: Formal analysis, Software (Analytical data processing). LGC: Formal analysis, Software (Analytical data processing). JTE: Formal analysis, Software (Analytical data processing), Writing – review & editing.

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Data Availability Statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical Disclosures

The authors declare that no experiments involving human participants or animals were performed for this study. The authors declare that no patient data are included in this article. The authors declare that no patient data or identifiable information are presented in this manuscript.

Conflicts of Interest The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Figures

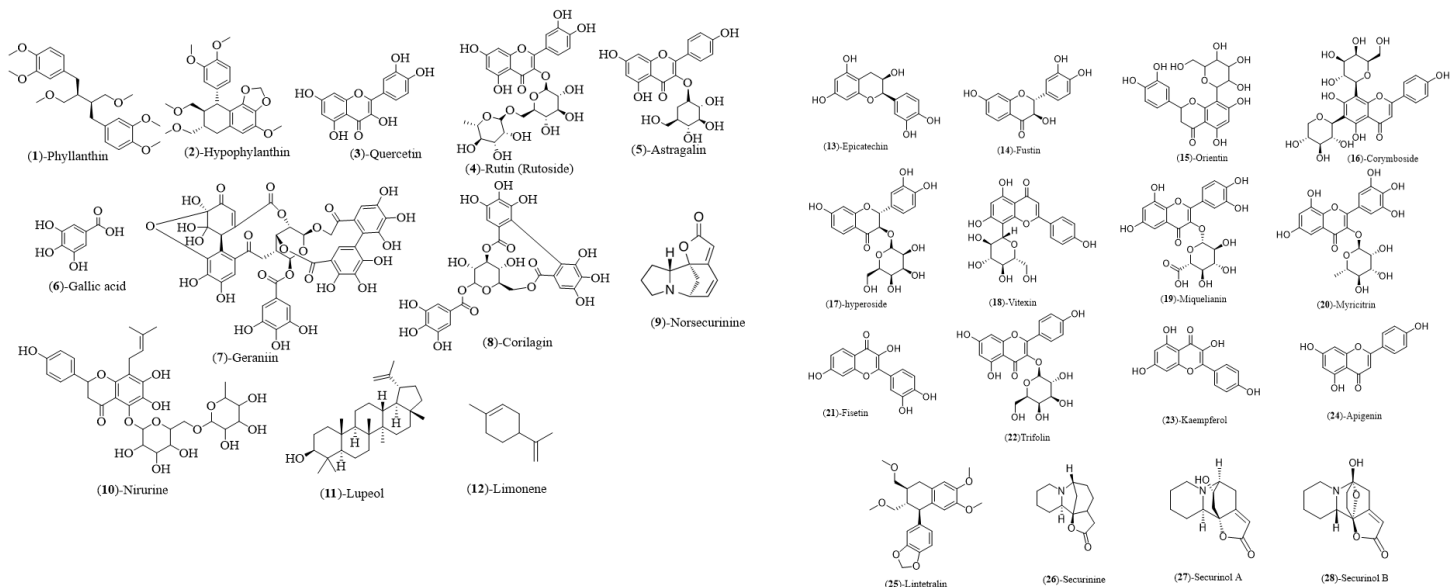


Figure 1

Chemical structures of secondary metabolites isolated from leaf crude extract of *Phyllanthus niruri* L., reported in the literature.

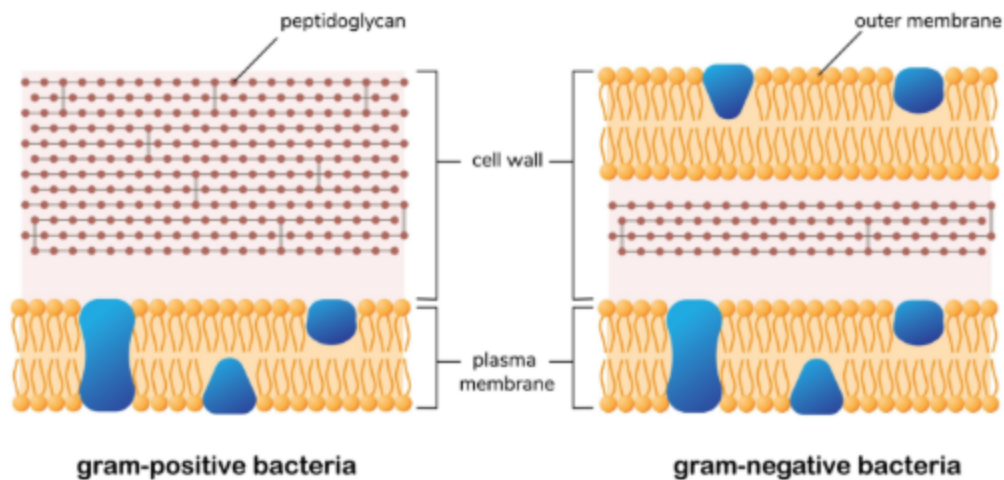


Figure 2

Structural differences between Gram-positive and Gram-negative bacterial cell walls and their influence on the permeation of *Phyllanthus niruri* secondary metabolite.

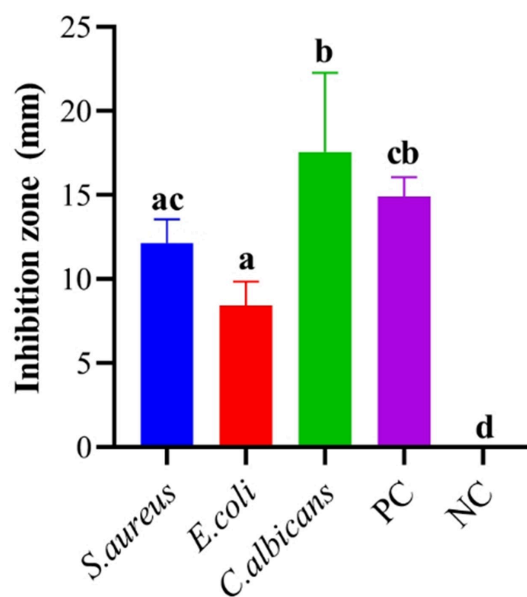
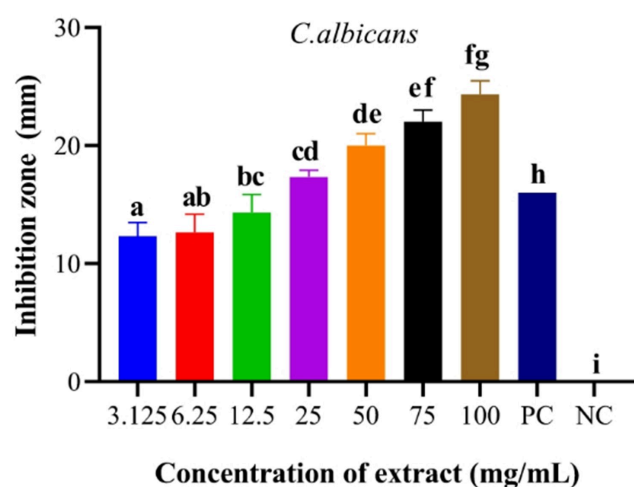
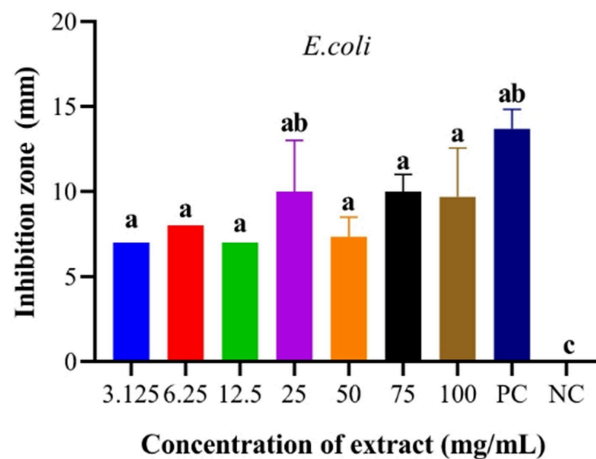
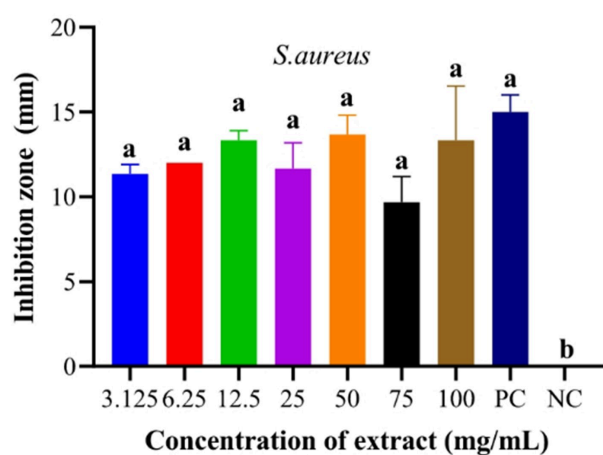


Figure 3

Antimicrobial activity of *Phyllanthus niruri* ethanolic leaf crude extract against *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922) and *Candida albicans* (ATCC 10231).

Supplementary Files

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