

Quantitative phytochemical composition and antibacterial potential of *Phyllanthus amarus* Linn leaf extracts

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Abstract

Public health experts keep warning that bacteria are slipping out of reach of our last-line antibiotics. In that urgent context, researchers have turned another set of plant leaves, this time the small, unassuming *Phyllanthus amarus* Linn. This study explores the qualitative phytochemicals and antibiotic potential of *P. amarus* extract on *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi*. Quantitative phytochemical analysis revealed higher concentrations of bioactive compounds—including alkaloids, flavonoids, glycosides, tannins, phenols, saponins, and terpenoids—in the ethanolic extract. Antibacterial assays using disc diffusion showed dose-dependent inhibition by both extracts, with ethanolic extracts exhibiting significantly higher antibacterial activity. At 210 mg/mL, ethanolic extracts achieved inhibition zones comparable to ciprofloxacin, especially against *E. coli* (33.26 mm) and *S. typhi* (31.4 mm). Aqueous extracts were less potent but still showed measurable activity at higher concentrations. These results highlight the therapeutic potential of *P. amarus*, particularly its ethanolic extract, as a natural antibacterial agent, with implications for developing phytopharmaceuticals to combat drug-resistant infections.

Keywords: Antibacterial; Antibiotics; *Phyllanthus amarus*; Phytochemicals

1. Introduction

Increasing antimicrobial resistance (AMR) in bacteria hinders years of continuous progress in ameliorating global infectious diseases (Ahmed *et al.*, 2024). Available and approved antibiotics might lose their efficacy due to the surge in multidrug-resistant bacteria; it is thus imperative to develop everlasting therapeutic alternatives (Aslam *et al.*, 2024). Many therapeutic drugs have been derived from medicinal plants and continue to be an area of study (Falade *et al.*, 2025; El-Saadony *et al.*, 2025; Agarwal *et al.*, 2016; Pratima *et al.*, 2025). *Phyllanthus amarus* is one of the available worldwide medicinal plants with limited medicinal activities, including being antimicrobial, antioxidant, hepatoprotective, and anti-inflammatory (Rocejanasaroj *et al.*, 2025; Falade *et al.*, 2025; Ajibade *et al.*, 2015; Agarwal *et al.*, 2016). Therefore, the medicinal plant *P. amarus* is of scientific interest due to research efforts focused on plants belonging to the family Euphorbiaceae, and global and general accessibility of the species in almost any tropical and subtropical biome or geographic region of the world, including tropical Africa, tropical Asia and tropical South America (Falade *et al.*, 2025). *P. amarus* has folklore medicinal uses, and Indigenous folk medicines have traditional ethnomedical uses for medicinal purposes. As with other countries and indigenous peoples, *P. amarus* have been used to treat jaundice, diabetes, dysentery, skin infections, gastrointestinal disorders, etc. *P. amarus* may offer an essential medicinal plant for human health due to the abundance of active bioactive phytochemicals, including alkaloids, flavonoids, tannins, lignans, and phenolic compounds.

There is a current demand for other antibacterial agents and documented pharmacological potential from *P. amarus*. More systematic studies are warranted to provide both the extracts' qualitative and quantitative phytochemical profiles and their antibacterial activity. The phytochemical profile of the extract needs to be understood to define further how

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these extracts act. Knowing the phytochemical profile will also be helpful when discussing the standardization of herbal products. Additional significance will be knowing how to extract plant metabolites with similar or favourable antibacterial potential efficiently; thus, comparing ethanolic and aqueous extracts will be useful. Previous works have reported on the quantitative phytochemical and antibacterial properties of ethanolic and aqueous extract of *P. amarus* on some clinical isolates (*Staphylococcus aureus*, *E. coli* and *Salmonella typhi*) separately. These bacterial pathogens are of significant public health concern as they are the cause of a large number of infections with increasing multi-class resistance to numerous antibiotics. *S. aureus* is a gram-positive pathogen responsible for skin, respiratory, and blood infections. In particular, Methicillin-resistant *S. aureus* (MRSA) is typically an infection control issue in health care. *Escherichia coli*, a Gram-negative bacterium, is a frequent microbiological agent associated with urinary tract infections, neonatal meningitis, and gastrointestinal diseases. *S. typhi*, the agent of typhoid fever, continues to be a significant pathogen in low and middle-income countries with major morbidity and mortality. By measuring significant phytochemicals and evaluating the antimicrobial activity of its extracts, this study explores the possible use of *P. amarus* as a source of antibacterial agents. This study aims to determine the optimal extraction technique for boosting the therapeutic potential of this widely used medicinal species by contrasting the effectiveness of an ethanolic and aqueous extraction. The findings are expected to provide important information for rationally designing phytopharmaceuticals for resistant bacterial illnesses and optimizing herbal formulations for apparent biological activity.

2. Material and methods

2.1. Plant Collection and Authentication

For phytochemical investigation, samples of *P. amarus* Linn. plants were taken from a low grassy region in a rural environment, offering the best conservation and the least contamination. Taxonomist who worked at the University of Ilorin, Kwara State, Nigeria's Herbarium Unit of the Department of Plant Biology, where he stored his herbarium specimens as voucher specimens, confirmed that the plant was correctly identified.

2.2. Preparation of Plant Extract

Each gathered plant was rinsed separately under running water to remove dirt and other contaminants. After being cleaned, the plants were allowed to air dry in the shade at room temperature ($25 \pm 2^\circ\text{C}$) until no moisture was left on them. Using a mechanical grinder, washed and dried samples were ground into a fine powder and then sieved for consistency so they could be stored in sealed containers for controlled environmental extraction.

2.3. Extraction Procedure

The powdered *P. amarus* was macerated using a methanol solvent to create a crude extract. After adding 100 g of *P. amarus* to 500 mL of methanol, the mixture was allowed to sit at room temperature for 72 hours. Throughout the holding period, there was constant shaking. After the maceration procedure, the extract was poured into a funnel. Whatman No. 1 filter paper was then used to filter the extract. Rotating evaporators concentrated the remaining dissolved extract, opening at a 45-degree angle. Until it was needed for additional analysis, the resultant extract was kept in sterile amber bottles at 4°C (Oyekanmi *et al.*, 2023).

2.4. Quantitative Determination of the Phytochemicals in the Plant Extracts

The quantitative analysis for determining the total phytochemicals of the *P. amarus* extracts were carried out using standard procedures (with some modifications). A spectrophotometer was used to determine the Tannins and the Phenol contents based on UV spectra through absorption maxima at individual wavelengths of every biocomponent (The total Phenol contents were obtained using a standard calibration curve). Others (Alkaloids, saponins, Tannins and tannins) were determined as described earlier (Harborne 1973; Obadoni and Ochuko 2002; Burden and Robinson 1981; Bohm and Kocipai-Abyazan 1994).

2.5. Antibacterial study

The standard disc dilution technique, previously published, was used to test the antibacterial activity of *P. amarus* ethanolic and aqueous extracts using strains of *S. typhi* (ATCC 27,870), *E. coli* (ATCC 25,922), and *S. aureus* (ATCC 12,598). Gram-negative and Gram-positive bacteria, respectively, *E. coli* and *S. aureus*, were chosen as model organisms for antibiotic investigations. On Luria-Bertani (LB) medium, bacterial cultures were cultivated and incubated for 24 hours at 25°C . They were then refrigerated at 4°C . to generate 10^6 colony-forming units (CFU) per millilitre. Both bacterial pathogens were cultivated on nutrient agar for an entire night.

The LB agar plates were covered with 100 microliters of each bacterial culture. Before being soaked in 30, 60, 90, 120, 150, 180, and 210 mg/mL of *P. amarus* ethanolic and aqueous extract, a disc (sterile filter paper) was made. Ciprofloxacin (5 mg/mL) and water served as the control for this assessment. The prepared discs were carefully positioned on the agar plate surface, making sure they stuck securely and were sufficiently separated from one another. The zone of inhibition was measured in millimetres (mm) after the plates were incubated for 18 to 24 hours at 37 °C. Three duplicates of each experiment were conducted, and the average was calculated.

2.6. Statistical analysis

Every experiment was conducted in triplicate, and the mean \pm standard deviation was used to report the results. Student t-test and One-way analysis of variance (ANOVA) were used for all statistical analyses, and the Duncan post hoc test was used to assess significance. A difference was deemed statistically significant if the *P-value* was less than 0.05. All statistical analyses in this investigation were conducted using GraphPad Prism 9 (GraphPad Software Inc.; San Diego, CA, USA) and the Statistical Package for Social Sciences (SPSS, Chicago, IL, USA, version 25.0).

3. Results

The quantitative phytochemical analysis of *P. amarus* leaves, as shown in Table 1, revealed significant variations in the content of bioactive compounds between aqueous and ethanolic extracts. Ethanolic extracts consistently exhibited higher concentrations of all assessed phytochemicals compared to the aqueous extracts. Alkaloids, flavonoids, glycosides, phenols, saponins, tannins, and terpenoids were significantly more abundant in the ethanolic extract, indicating ethanol's higher efficiency in extracting these compounds. For instance, alkaloids were present at 0.45 ± 0.03 mg/g in the ethanolic extract compared to 0.24 ± 0.02 mg/g in the aqueous counterpart. Similarly, glycosides measured 0.61 ± 0.06 mg/g in ethanol and 0.31 ± 0.04 mg/g in water extracts.

Table 1 Quantitative phytochemical properties of *Phyllanthus amarus* leaves

Phytochemicals (mg/g)	Aqueous extracts of <i>Phyllanthus amarus</i>	Ethanolic extracts of <i>Phyllanthus amarus</i>
Alkaloids	0.24 ± 0.02 b	0.45 ± 0.03 a
Flavonoids	0.12 ± 0.03 b	0.25 ± 0.02 a
Glycosides	0.31 ± 0.04 b	0.61 ± 0.06 a
Phenol	0.02 ± 0.01 b	0.06 ± 0.01 a
Saponins	0.07 ± 0.01 b	0.21 ± 0.02 a
Tannins	0.17 ± 0.04 b	0.26 ± 0.03 a
Terpenoids	0.15 ± 0.02 b	0.31 ± 0.04 a

Values on the row with the same superscript letter are not significantly different from each other at a 0.05 level of significance

Antibacterial testing showed that both extracts exhibited concentration-dependent zones of inhibition against *S. aureus*, *E. coli*, and *S. typhi*. Ciprofloxacin, used as a positive control, showed the highest inhibition across all pathogens (25.85 mm for *S. aureus*, 30.68 mm for *E. coli*, and 29.6 mm for *S. typhi*). Ethanolic extracts at 210 mg/mL reached inhibition zones of 25.95 mm, 33.26 mm, and 31.4 mm, respectively—comparable to the standard antibiotic (Table 2).

Aqueous extracts also showed increasing efficacy with concentration, though with generally lower inhibition zones. At 210 mg/mL, the aqueous extract produced inhibition zones of 18.92 mm (*S. aureus*), 21.35 mm (*E. coli*), and 21.43 mm (*S. typhi*).

The lowest inhibition zones were observed at 30 mg/mL concentration for both extracts, with ethanolic extracts performing better (e.g., 4.82 mm for *S. aureus*) than aqueous (3.91 mm). Aqueous extracts demonstrated limited antibacterial effects at low concentrations but improved performance at higher doses. Notably, ethanolic extracts at 180–210 mg/mL demonstrated zones statistically indistinguishable from ciprofloxacin, especially against *E. coli* and *S. typhi*.

Overall, the results indicate strong antibacterial potential of *P. amarus*, especially when extracted with ethanol. The increased effectiveness with higher concentrations also suggests a dose-dependent mechanism.

Table 2 Zones of Growth Inhibition (mm) Showing Antibacterial Activity for both ethanolic and aqueous extracts of *Phyllanthus amarus*

	Inhibition zone in diameter (mm \pm SD) around the discs					
	<i>S. aureus</i>	Range	<i>E. coli</i>	Range	<i>S. typhi</i>	Range
Control	0 \pm 0 g	0 - 0	0 \pm 0 h	0 - 0	0 \pm 0 k	0 - 0
Ciprofloxacin (5 mg/mL)	25.85 \pm 2.42 ^a	22.55 - 28.27	30.68 \pm 2.82 ^b	27.81 - 34.33	29.6 \pm 0.54 ^{ab}	28.99 - 30.11
Et <i>P. amarus</i> (30 mg/mL)	4.82 \pm 0.84 ^f	3.77 - 5.81	7.85 \pm 0.68 ^f	6.88 - 8.44	8.53 \pm 1.27 ^h	7.44 - 10.37
Et <i>P. amarus</i> (60 mg/mL)	8.92 \pm 0.63 ^e	8.33 - 9.69	13.44 \pm 1.76 ^e	10.85 - 14.74	14.32 \pm 2.47 ^g	10.93 - 16.44
Et <i>P. amarus</i> (90 mg/mL)	12.08 \pm 1.12 ^d	10.77 - 13.38	18.18 \pm 1.62 ^d	16.29 - 20.06	16.94 \pm 1 ^{ef}	16.12 - 18.26
Et <i>P. amarus</i> (120 mg/mL)	15.54 \pm 1.12 ^c	14.24 - 16.84	22.2 \pm 1.27 ^c	21.08 - 23.82	18.62 \pm 1.01 ^{de}	17.3 - 19.43
Et <i>P. amarus</i> (150 mg/mL)	19.01 \pm 1.12 ^b	17.71 - 20.31	21.22 \pm 1.04 ^c	20.1 - 22.33	19.05 \pm 1.23 ^d	17.61 - 20.48
Et <i>P. amarus</i> (180 mg/mL)	25.23 \pm 2.38 ^a	22.05 - 27.18	31.74 \pm 2.02 ^{ab}	29.12 - 33.86	29.23 \pm 0.58 ^b	28.66 - 29.75
Et <i>P. amarus</i> (210 mg/mL)	25.95 \pm 3 ^a	22.52 - 29.39	33.26 \pm 2.36 ^a	30.63 - 36.37	31.4 \pm 2.4 ^a	29.84 - 34.92
Aq <i>P. amarus</i> (30 mg/mL)	3.91 \pm 0.08 ^f	3.82 - 3.99	5.54 \pm 0.06 ^g	5.47 - 5.62	5.69 \pm 0.71 ^{ij}	4.86 - 6.51
Aq <i>P. amarus</i> (60 mg/mL)	4.33 \pm 0.36 ^f	3.99 - 4.81	5.71 \pm 0.06 ^g	5.64 - 5.78	4.6 \pm 0.78 ^j	3.77 - 5.61
Aq <i>P. amarus</i> (90 mg/mL)	4.22 \pm 0.04 ^f	4.16 - 4.25	5.89 \pm 0.06 ^g	5.82 - 5.95	6.82 \pm 2.36 ^{hi}	4.55 - 9.79
Aq <i>P. amarus</i> (120 mg/mL)	16.18 \pm 1.91 ^c	13.39 - 17.45	11.88 \pm 0.48 ^e	11.24 - 12.33	13.68 \pm 0.77 ^g	12.58 - 14.25
Aq <i>P. amarus</i> (150 mg/mL)	16.01 \pm 1.14 ^c	15.31 - 17.71	13.83 \pm 0.39 ^e	13.47 - 14.36	16.35 \pm 1.39 ^f	14.48 - 17.83
Aq <i>P. amarus</i> (180 mg/mL)	16.64 \pm 0.62 ^c	15.91 - 17.33	20.86 \pm 1.25 ^c	19.39 - 22.17	21.42 \pm 1.39 ^c	19.93 - 23.11
Aq <i>P. amarus</i> (210 mg/mL)	18.92 \pm 1.74 ^b	16.99 - 21.16	21.35 \pm 1.26 ^c	19.96 - 22.91	21.43 \pm 1.17 ^c	20.18 - 22.81

Note: Inhibition zone in diameter (mm) around the discs (6mm) impregnated with 100 μ g/disc of extract; Ciprofloxacin (5 mg/mL) was used as positive reference standard antifungal discs

Means followed by the same letters in each column are not significantly different at $p = 0.05$ based on Duncan's multiple range test.

4. Discussion

The results obtained from the phytochemical and antibacterial evaluation of *P. amarus* show its promising therapeutic value. The study shows that *P. amarus* is a reservoir of diverse phytochemicals, including alkaloids, terpenoids, steroids, polyphenolic compounds and lignans, responsible for various medicinal effects (Patel *et al.*, 2011). This is in line with recent studies that supported this claim (Falade *et al.*, 2025; Rocejanasaroj *et al.*, 2025; Agarwal *et al.*, 2016; Zubair *et al.*, 2017; Pratima *et al.*, 2025).

Previous findings of bioactive compounds having greater phytochemical and alkaloids, flavonoids, glycosides, and terpenoids in ethanolic extracts is true as ethanol is a superior solvent towards extracting compounds from plants (Falade *et al.*, 2025). Ethanol's intermediate polarity increases the solubility of both polar and nonpolar compounds. Thus, ethanolic extracts that have more phytochemicals support that claim (Chuo *et al.*, 2022). Ethanol's superiority over aqueous extracts emphasizes the different approaches of pharmacological solvents (Chuo *et al.*, 2022). The phytochemical screening results indicate alkaloids, flavonoids, glycosides, phenol, saponins, tannins and terpenoids in both aqueous and ethanolic *P. amarus* extracts. The result corroborates previous reports that suggest that phytochemicals are responsible for the activities of *P. amarus* (Zubair *et al.*, 2017; Falade *et al.*, 2025). But slightly different from report by Zubair *et al.*, (2017) where saponins was not detected in the methanolic extract.

The antiviral, anti-inflammatory, and antioxidant properties of alkaloids and flavonoids are well known. Antibacterial activity is more prominent due to the presence of large amounts of these compounds. Membrane disruption, inhibition of DNA and RNA, and metal ion chelation such as enzymes vital for bacterial metabolism are major traits of certain flavonoids.

Both aqueous and ethanolic extracts of *P. amarus* exhibited antimicrobial activity against the tested organisms (*S. aureus*, *E. coli* and *S. typhi*), with the average diameter of the zone of inhibition ranging from 3.77-33.26, which is similar to a recent report that reported zones of inhibition between 19 and 21 mm against these bacteria pathogens (Marappa and Gunashree 2025). This is also in line with a report of the zone of inhibition ranging from 14 to 25 mm against *S. aureus* (Zubair *et al.*, 2017). This antimicrobial property might be due to both Glycosides and saponins. Glycosides and saponins' antimicrobial traits are contributed to by decreasing cell size and diffusing through the barrier. Together, these compounds generate a stronger antibacterial effect than the extract alone (Saboora *et al.*, 2019; Suresh *et al.*, 2025).

P. amarus exhibits a wide range of antibacterial activity to positive gram (*S. aureus*) and negative gram (*E. coli* and *S. Typhi*) bacteria. This is extremely important because negative gram tend to be more resistant because of their outer membrane barrier. The efficacy observed on *E. Coli* and *S. Typhi* indicates that the extracts may have some compounds that evade or destroy this layer (Ajibade *et al.*, 2015; Rocejanasaroj *et al.*, 2025; Onocha *et al.*, 2003). Ethanol extracts surpassed aqueous extracts at every concentration and all considered bacteria, achieving inhibition zones nearly equal to ciprofloxacin at higher doses.

It can be inferred that the extracts have an antibacterial effect in a proportional dose-response manner, ensuring that these compounds accumulate above minimum effective levels. These considerable increments in potency alongside increases in concentration of ethanolic extracts demonstrates this. Additionally, some of the higher concentrations of ethanolic extracts showed little significant difference from ciprofloxacin, suggesting that these extracts stand as effective natural substitutes or complements to clinically used antimicrobial drugs (Falade *et al.*, 2025; Patel *et al.*, 2011; Ghosh *et al.*, 2022).

The results show that even though aqueous extracts are less potent, they still possess antibacterial activity at higher concentrations. This finding is useful for regions where ethanol might be inaccessible, emphasizing the hypothesis regarding the use of water-based preparations. However, the lower effectiveness compared to ethanolic extracts needs to be considered for therapeutic formulation purposes.

These findings are crucial in the search for plant-based antibacterial agents. With increasing resistance to antibiotics, natural products such as *P. amarus* provide useful focuses for alternative therapeutic approaches. Further studies should bulk up on the active constituents, constructing a thorough toxicity profile, and planning in vivo efficacy trials. Studies aimed at combining conventional antibiotics may also show some additive or synergistic effects, which could enhance the efficacy of the antibiotics while simultaneously lowering the dosage of synthetic drugs required.

In conclusion, the abundant phytochemicals and pronounced antibacterial activity of *P. amarus*, particularly its ethanolic extract, substantiate its inclusion in traditional medicine and warrant more sophisticated and rigorous standards for products developed from it.

5. Conclusion

In this study, it was found that *P. amarus*-especially its ethanol extract-packs a serious punch against bacteria. Alkaloids, flavonoids, glycosides, and terpenoids all seem to join the brew, letting it take on familiar foes such as *S. aureus*, *E. coli*, and *S. typhi*. At higher doses, the ethanolic preparation matches ciprofloxacin, which gives lab techs an obvious benchmark to think about. A water-only version lags behind but still does something, so it may help where nobody can get near a bottle of vodka. The results play by the old dose-response rule: crank up the concentration and the kill rate climbs. That pattern lines up neatly with folk wisdom about using the plant for wounds and gut trouble, nudging scientists to start talking about a green alternative to prescription pills. Further studies are needed to isolate active compounds, determine toxicity profiles, and evaluate synergistic potential with conventional antibiotics.

Compliance with ethical standards

Disclosure of conflict of interest

Authors have declared that no conflict of interest

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