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Research Article

Phytochemical Constituents and Antimicrobial Properties of Differential Solvent Fractions of *Phyllanthus amarus* leaves (stone breaker)

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Abstract

Background: Medicinal plants are rich in bioactive molecules known as phytochemicals, which play a key role in the antimicrobial properties of plants and plant-based products. *Phyllanthus amarus*, widely regarded as an important therapeutic plant in tropical and subtropical regions traditionally used to treat ailments affecting the stomach, liver, kidneys, and spleen
Aims: This study aimed to identify phytochemical constituents and antimicrobial properties of differential solvent fractions of ethanolic leaf extract of *Phyllanthus amarus*
Method: The powdered leaf sample was extracted using ethanol at a sample-solvent ratio of 1:3. The resulting crude extract was then fractionated using four different solvents: petroleum ether, chloroform, acetone, and methanol. Each of the four fractions was subsequently subjected to phytochemical screening and antimicrobial activity evaluation.
Results: Phytochemical profiles and antimicrobial activities of petroleum ether, chloroform, acetone, and methanol fractions of *Phyllanthus amarus*. Phytochemical screening revealed that alkaloids and glycosides occurred in all fractions, while other metabolites were selectively extracted based on solvent polarity. Antimicrobial tests against *Staphylococcus aureus*, *Escherichia coli*, *Rhizopus* spp., and *Aspergillus* spp. showed that the chloroform fraction had the strongest inhibitory activity. The results indicate that moderately non-polar compounds are chiefly responsible for the plant's antimicrobial properties and identify the chloroform fraction as the most promising source of bioactive agents.
Conclusion: The study shows that solvent polarity strongly influences the phytochemical content and antimicrobial activity of *Phyllanthus amarus*. Overall, the findings highlight the importance of solvent selection and identify the chloroform fraction as the most promising source of potent antimicrobial agents.

Keywords: Antimicrobial; Fraction; *Phyllantus amarus*; Phytochemial screening

1. INTRODUCTION

Medicinal plants are rich in bioactive molecules known as phytochemicals, which play a key role in the antimicrobial properties of plants and plant-based products(El-Saadony *et al*., 2025; Hochma *et al*., 2021; Nguyen *et al*., 2022).These compounds are naturally produced as primary and secondary metabolites in the leaves, fruits, and roots of medicinal plants, and they help protect the plant against various diseases (Kandar, 2021a; Kaur & Ahmed, 2021; Pandita & Pandita, 2021). Common phytochemicals include flavonoids, phenols, terpenoids, alkaloids, glycosides, and tannins, each contributing to the plant's therapeutic potential (Rahman *et al*., 2018).

Over the years, there has been growing global interest in the therapeutic benefits of medicinal plants as safe, affordable, and effective alternatives for developing medicines, foods, and nutritional supplements. Long before the discovery of microorganisms, humans recognized the healing power of plants (Danjuma *et al*., 2025). Even today, despite advances in modern healthcare and increased life expectancy in regions like Europe and the United States, many people are returning to herbal remedies to manage illnesses and combat the rise of drug-resistant pathogens. In fact, about 25% of all currently prescribed drugs are derived from plant sources (Anyiam *et al*., 2025). Antimicrobial resistance the ability of microorganisms such as bacteria, viruses, fungi, and parasites to survive drugs designed to kill them poses a serious health challenge, often leading to infections that are harder to treat and carry a higher risk of severe illness or death (Salam *et al*., 2023).

One medicinal plant that has attracted considerable attention is *Phyllanthus amarus*, widely regarded as an important therapeutic plant in tropical and subtropical regions. Traditionally, it has been used to treat ailments affecting the stomach, liver, kidneys, and spleen. Its leaves contain bitter bioactive compounds such as phyllanthin, hypophyllanthin, and niranthin (Adebisi *et al*., 2021). Beyond these, it is employed in managing menstrual disorders, gonorrhea, diabetes, urinary and intestinal infections, kidney and gallbladder stones, influenza, measles,

and tuberculosis. It is also used for liver-related conditions like hepatitis, jaundice, liver cancer, and hepatotoxicity, as well as to boost the immune system and combat hepatitis A (Bekoe *et al*., 2020; Ameen *et al*., 2021).

Scientific studies support these traditional uses. For example, leaf extract fractions containing alkaloids, flavonoids, carbohydrates, and anthraquinones have been shown to protect liver and brain tissues in mice infected with *Plasmodium berghei*, demonstrating its potential in combating malaria-associated oxidative stress (Uzuegbu *et al*., 2025). Another study revealed that *P. amarus* leaf extracts can reduce viral titers of Newcastle disease virus in embryonated chicken eggs in a dose-dependent manner, highlighting its antiviral properties (Faeji *et al*., 2025).

This study aimed to identify phytochemical constituents and antimicrobial properties of petroleum ether, chloroform, acetone, and methanol fractions of ethanol crude extract of *Phyllanthus amarus* leaves grow within the premises of the Federal Polytechnic, Idah, Kogi State

Fig 1: *Phyllanthus amarus*

2. Material and Method

The instruments and reagents used were of analytical grade. All materials like glass wares, bijou bottles were thoroughly cleaned with distilled water, detergent and sterilized to remove dirt and impurities.

2.1 Identification and Collection of Sample

Fresh leaves of *Phyllanthus amarus* were collected from the premises of the Federal Polytechnic, Idah, Kogi State. The plant sample was authenticated at the Department of Science Laboratory Technology, Federal Polytechnic, Idah, Nigeria. The leaves were thoroughly washed with running tap water, dried at room temperature, and ground into a fine powder using a mortar and pestle. The powdered sample was then kept for further analysis (Danjuma *et al*., 2025; Mustapha *et al*., 2025)

2.2 Extraction and Fractionation

A 200 g portion of the sample was maceration in 600 mL of 95% ethanol inside volumetric flask at room temperature for one week, with regular shaking. After the maceration period, the mixture was filtered through Whatman filter paper, and the filtrate was allowed to evaporate to dryness. The resulting crude extract was then sequentially fractionated using four solvents of increasing polarity: petroleum ether, chloroform, acetone, and methanol (Enechi *et al*., 202; Abubakar & Haque 2020).

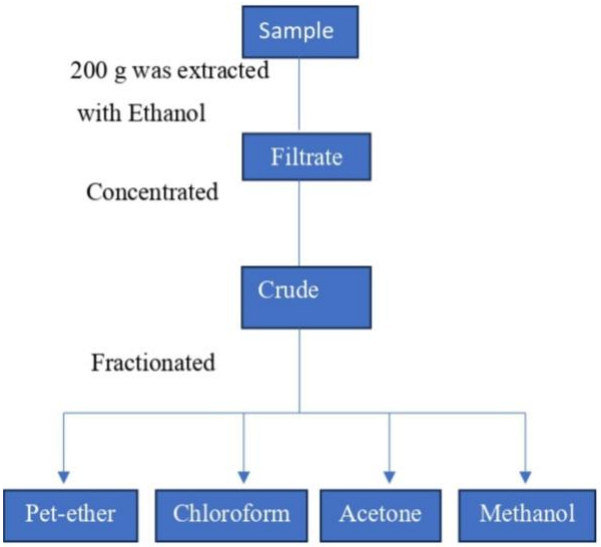

Fig 2: Extraction Flowchart

:2.3 Phytochemical Screening

Phytochemical screening of the extract was performed to identify the presence of various phytochemicals using standard laboratory methods

2.2.1 Test for alkaloids (Wagner's Reagent)

A small portion of each fraction was neutralized by adding one or two drops of dilute H_2SO_4. The neutralized solution was then treated with Wagner's reagent (iodine in potassium iodide), and the appearance of a brownish-black precipitate indicated the presence of alkaloids (Al-Wajih *et al*., 2022).

2.2.2 Test for Flavonoids (Alkaline Reagent Test)

A few drops of NaOH were added to each fraction, resulting in an intense yellow colour. Subsequent addition of HCl caused the yellow coloration to disappear (Ibrahim and Birniwa 2024).

2.2.3 Test for Saponins (Frothing test)

A 0.5 mL portion of each fraction was vigorously shaken with water in a test tube. The formation of stable froth lasting 1–2 minutes, even upon warming, indicated the presence of saponins (Al-Wajih *et al*., 2022).

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2.2.4 Test for tannins (Lead acetate test)

Approximately 5 mL of an aqueous solution of each fraction was boiled in a test tube, and a few drops of 1% lead acetate solution were added. Formation of a yellow or red precipitate indicated the presence of tannins (Saha *et al*., 2020).

2.2.5 Test for Polyphenols (Salkowski Test).

3 drops of 5 % solution of lead acetate were added to 1 ml of each fraction. Yellow precipitate indicated the presence of polyphenols (Ibrahim and Birniwa 2024)

2.2.6 Test for Glycosides (Killer-killiantest)

2mL of each fraction with 3 mL of chloroform and 3drops of ammonium solution was added in drops. Appearance of pink color confirmed the presence of glycoside (Danjuma *et al*., 2025).

2.2.7 Terpenoids (Salkowski)

1 mL of chloroform and 1 mL of concentrated H_2SO_4 were added to 3 mL of each fraction. A reddish-brown color confirmed the presence of terpenoids (Danjuma *et al*., 2025).

2.3 Antimicrobial Test (Disc Diffusion Method)

The antimicrobial activity was evaluated using the well diffusion method, following the Kirby-Bauer procedure as described by Raveesha et al. (2021). Standard aseptic microbiological methods were followed throughout the study.

2.3.1 Preparation of Sensitivity Disc

Whatman No. 1 filter paper discs (6 mm in diameter) were prepared using a paper pouch, with 20 discs placed in each Bijou bottle. The bottles were then sterilized by autoclaving at 112 °C for 15 minutes and allowed to cool before use (Kebede *et al* 2021)

2.3.2 Preparation of Stock Solution and Serial Dilution

1 mL of dimethyl sulfoxide (DMSO) was used to dissolve 0.06 g (60 mg) of each fraction to prepare stock solution. From this solution, 0.5 mL was transferred into a sterilized Bijou bottle containing 20 punched filter paper discs to obtain a concentration of 60 mg/mL. Another 0.5 mL of DMSO was then added to the remaining stock solution, and 0.5 mL of this diluted mixture was placed into another sterilized Bijou bottle containing 20 filter paper discs, labelled as 30 mg/mL. The process was repeated by adding an additional 0.5 mL of DMSO to the stock solution, and 0.5 mL was again transferred into a sterilized Bijou bottle containing 20 discs, labelled as 15 mg/mL. The same procedure was followed for each fraction (Ibrahim & Birniwa 2024).

2.3.3 Preparation of Nutrient Agar (NA)

Nutrient agar was used as the growth medium for *Escherichia coli* and *Staphylococcus aureus*. It was prepared following the manufacturer's instructions by dissolving 16.25 g of nutrient agar in 125 mL of distilled water in a 250 mL volumetric flask. After complete dissolution, the medium was sterilized by autoclaving at 121 °C for 15 minutes and then allowed to cool to room temperature for approximately 45 minutes. The sterilized medium was poured into sterile Petri dishes and left to solidify (Lawan and Ismail., 2024)

2.3.4 Sabouraud Dextrose Agar (SDA)

Sabouraud Dextrose Agar (SDA) was used as the culture medium for *Aspergillus niger* and *Rhizopus* spp. The medium was prepared according to the manufacturer's instructions by dissolving 14 g of SDA in 250 mL of distilled water. It was then sterilized by autoclaving at 121 °C for 15 minutes and allowed to cool at room temperature for 45 minutes. The sterilized medium was subsequently poured into sterile Petri dishes and left to solidify (Lawan and Ismail 2024).

2.3.5 Source of microorganisms used for anti-microbial activities

Staphylococcus aureus and *Escherichia coli* were sourced from the clinical laboratory of Our Lady Specialist Hospital, Idah, Kogi State, Nigeria. *Aspergillus* and *Rhizopus* species were isolated from bread and onion samples cultured on SDA plates and subsequently identified using microscopic examination.

2.3.6 Bioassay Procedure

The test organisms were inoculated into sterile nutrient agar and Sabouraud agar using the pour plate method. Antimicrobial discs containing different concentrations (60 mg/mL, 30 mg/mL, and 15 mg/mL) were aseptically placed on the prepared plates with sterile forceps, ensuring adequate spacing to avoid overlapping zones of inhibition. Standard antibiotic discs ofloxacin for bacteria and ketoconazole for fungi were placed at the centre of the inoculated plates. The bacterial plates were incubated at 37 °C for 24 hours, while the fungal plates were incubated for 72 hours, after which the zones of inhibition were observed and measured (Ewelike *et al*., 2021)

3.1 Results

Table 1: Weights and Physical Properties of the fractions

S/N	Extract	Weight(g)	Percentage yield (%)	Colour	Texture
1	Petroleum ether	2.30	30.26	Dark green	Oily
2	Chloroform	2.2	28.95	Dark green	Gummy
3	Acetone	1.8	23.68	Dark green	Gummy
4	Methanol	1.3	17.11	Brown	Gummy
5	Crude extract	7.6	3.8	Brown	Gummy

Table 2: Phytochemical screening

S/N	Phytochemical	Petroleum Ether	Chloroform	Methanol	Acetone
1	Alkaloids	+	+	+	+
2	Tannins	+	-	-	+
3	Saponin	+	+	-	-
4	Flavonoids	+	-	-	+
5	Steroids	+	-	+	+
6	Polyphenols	+	+	+	-
7	Glycosides	+	+	+	+
8	Terpenes	+	-	-	-

+ = Present, - = Absent

Table 3: Antimicrobial activity of Petroleum Ether fraction

S/N	Concentration (mg/mL)	*Staphylococcus aureus*	*E. coli*	*Rhizopus spp.*	*Aspergillus*
1	60	6.0	4.0	5.0	3.0
2	30	6.0	2.0	6.0	R
3	15	3.0	2.0	R	R
4	C	1.0	13.0	1.0	0.0

Key: R = Resistance, C = Control

Table 4: Antimicrobial activity of Acetone fraction

S/N	Concentration (mg/ml)	*Staphylococcus aureus*	*E. coli*	*Rhizopus spp.*	*Aspergillus*
1	60	5.0	6.0	6.0	3.0

2	30	4.0	3.0	3.0	1.0
3	15	2.0	0.01	1.0	R
4	C	16.0	0.09	14.0	13.0

Key: R = Resistance, C = Control

Table 5: Antimicrobial activity of methanol fraction

S/N	Concentration (mg/ml)	*Staphylococcus aureus*	*E. coli*	*Rhizopus*	*Aspergillus*
1	60	3.0	5.0	4.0	5.0
2	30	2.0	2.0	2.0	R
3	15	R	1.0	R	R
4	C	12.0	19.0	12.0	14.0

Key: R = Resistance, C = Control

Table 6: Antimicrobial activity of hloroform fraction

S/N	Concentration (mg/ml)	*Staphylococcus aureus*	*E. coli*	*Rhizopus*	*Aspergillus*
1	60	8.0	4.0	5.0	4.0
2	30	3.0	R	3.0	1.0
3	15	R	R	1.0	R
4	C	14.0	13.0	9.0	11.0

Key: R = Resistance, C = Control

3.2 Discussions

3.2.1 Implications

The extraction of phytochemical substances from the plant material are affected by various factors including time, temperature, solvent concentration and polarity of solvent (Nawaz *et al*., 2020). Successive solvent fractionation of the crude extract yielded petroleum ether, chloroform, acetone, and methanol fractions with varying recoveries and physical characteristics. The total crude extract obtained was 7.60 g with percentage yield 3.8% relative to the crude drug. The percentage yields of the respective fractions were calculated relative to the crude extract weight.

The results presented in Table 1 showed the weight, colour, and texture of the various solvent fractions. The petroleum ether fraction produced the highest yield (30.26%) and appeared as a dark green oily extract. The chloroform fraction yielded 28.95% and was dark green with a gummy texture. The acetone fraction accounted for 23.68% and was also dark green and gummy in nature. The methanol fraction gave the lowest yield (17.11%) and appeared brown with a gummy consistency. All values reported represent a single determination (n = 1).

The phytochemical screening revealed the presence of several important bioactive

compounds across the four solvent fractions, indicating that the plant contains a diverse range of secondary metabolites with potential antimicrobial properties. The distribution of these compounds varied with solvent polarity, suggesting that different classes of phytochemicals were selectively extracted based on their solubility characteristics. Table 2 reveals that, Alkaloids were found in all four fractions (petroleum ether, chloroform, methanol, and acetone), indicating that they are widely distributed within the plant material. Tannins were detected only in the petroleum ether and acetone fractions, but absent in chloroform and methanol extracts. Tannins are astringent compounds capable of precipitating microbial proteins, which may partially explain the antimicrobial activity observed in the petroleum ether and acetone fractions. Saponins appeared in the petroleum ether and chloroform fractions, but were absent in methanol and acetone. Flavonoids were detected in petroleum ether and acetone fractions but were absent in chloroform and methanol.
Steroids were present in petroleum ether, methanol, and acetone fractions, Polyphenols were detected in all fractions except acetone. Glycosides were widely present in all fractions, indicating that the plant contains significant amounts of glycosylated compounds. Terpenes were detected only in the petroleum ether fraction. As highly non-polar molecules.
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The antimicrobial activities of the different solvent fractions revealed clear differences in effectiveness, reflecting how the plant's bioactive compounds are distributed based on solvents polarity as shown in Table 3. The maximum zone of inhibition observed in this study was 6.0 mm (for *Staphylococcus aureus* at 60 mg/mL and *Rhizopus* at 30 mg/mL). While this confirms antimicrobial presence, it is significantly lower than the 18.2 mm reported by Ali & Kannike (2025) for *Klebsiella* and the 14.2 mm noted by Ukwubile & Odugu (2018) for *Staphylococcus aureus* at 100 mg/mL.

The petroleum ether fraction showed moderate activity, with its highest inhibition observed at 60 mg/mL against *Staphylococcus aureus* (6.0 mm), *E. coli* (4.0 mm), *Rhizopus* spp. (5.0 mm), and *Aspergillus* spp. (3.0 mm). Its effectiveness declined with decreasing concentration, and resistance was apparent at 15 mg/mL for *Rhizopus* and *Aspergillus*. This pattern suggests that the antimicrobial components extracted by petroleum ether may be non-polar compounds such as lipophilic terpenoids, steroids, or fatty acid derivatives.
In contrast, the acetone fraction in Table 4 demonstrated stronger and broader antimicrobial activity than petroleum ether fraction, particularly at 60 mg/mL, where inhibition zones ranged between 5.0 and 6.0 mm for *S. aureus*, *E. coli*, and *Rhizopus* spp., with *Aspergillus* spp. showing moderate sensitivity (3.0 mm). The noticeable decline in activity at lower concentrations showed the effect of concentration. The higher performance of this fraction may be linked to the presence of semi-polar compounds such as phenolics, flavonoids, and alkaloids which acetone is known to extract efficiently.
Table 5: The methanol fraction showed the weakest antimicrobial effect among all the fractions tested. Although inhibition values at 60 mg/mL ranged from 3.0 to 5.0 mm, a steep decline occurred at lower concentrations, where most organisms exhibited resistance except *E. coli* (1.0 mm). This trend indicates that the methanol-soluble components typically tannins, saponins, and phenolic glycosides may be present in lower concentrations or possess limited antimicrobial strength in this particular plant.
Overall, the chloroform fraction in table 6, emerged as the most effective. At 60 mg/mL, it produced inhibition zones of 8.0 mm for *S. aureus*, 4.0 mm for *E. coli*, 5.0 mm for *Rhizopus*, and 4.0 mm for *Aspergillus*. Even at 30 mg/mL, its activity remained substantial, with resistance occurring only in *E. coli*. The observed inhibition zones of 6.0 mm for Gram-positive *S. aureus* compared to 4.0 mm for Gram-negative *E. coli* suggests a higher susceptibility in Gram-positive strains. This is consistent with the findings of Imran *et al*. (2021), who argued that the outer membrane of Gram-negative bacteria acts as a more effective permeability barrier against the polar metabolites (Methanol/Acetone fractions) of *P. amarus*. Interestingly, the sample showed a unique peak in activity against *Rhizopus spp*. At 30 mg/mL (6.0 mm), which actually exceeded its activity at 60 mg/mL (5.0 mm). This may suggest a "pro-zone" effect or specific phytochemical interactions at moderate concentrations. In contrast, *Aspergillus* showed high resistance (R) at concentrations below 60 mg/mL, aligning with Nisar *et al*. (2018), who noted that Aspergillus species often require higher phenolic concentrations for membrane disruption.

The superior performance of the chloroform fraction suggests that the most potent antimicrobial constituents are moderately non-polar, possibly including chlorophyll derivatives, aglycone flavonoids, alkaloids, or certain terpenoids that are readily extracted by chloroform.

3.2.2 Research Contribution

This research demonstrates that *Phyllanthus amarus* contains diverse bioactive phytochemicals whose extraction varies with solvent polarity. Petroleum ether produced the highest crude yield and extracted mainly non-polar metabolites, while methanol yielded the lowest and contained more polar compounds. Phytochemical screening confirmed the presence of important secondary metabolites including alkaloids, tannins, saponins, flavonoids, steroids, polyphenols, glycosides, and terpenes with their distribution differing across fractions.

Antimicrobial testing revealed notable variations in activity among the fractions. The chloroform fraction exhibited the strongest broad-spectrum antimicrobial effect, indicating that moderately non-polar compounds contribute most significantly to the plant's bioactivity. The acetone fraction showed intermediate but consistent antimicrobial potency, while petroleum ether displayed only moderate activity. Methanol was the least effective. Overall, the study highlights that solvent polarity strongly influences both phytochemical extraction and antimicrobial performance, identifying the chloroform fraction as the most promising source of potent antimicrobial agents in *Phyllanthus amarus*.

3.2.4 Suggestions

According to the results of the phytochemical activities conducted on the leaves, every phytochemical that was tested was present in the leaves. Their effectiveness on the test organisms demonstrated their importance in the traditional treatment of the diseases mentioned in the literature. In order to facilitate the production of their synthetic forms for broader use as antimicrobial medications and potential therapeutic agents, I suggest separating the bioactive compounds from the most active component (chloroform) and then elucidating their structures.

3.2.3 Limitation

This research is restricted to only leaves of the plant with which ethanol was used which may not fully capture all possible phytochemicals or their optimal extraction efficiencies. The antimicrobial activity was tested against only four organisms using disc diffusion method. At higher concentrations, the disc diffusion method is mainly restricted by the solubility and the rate of diffusion of the bioactive metabolites present in the agar medium. With increasing concentrations of the *Phyllanthus amarus* extract, the agar medium surrounding the disc gets saturated, resulting in the so-called 'plateau' effect, where the extent of inhibition does not increase proportionally with the increase in the concentration of the test material; the slow rate of passage of the high-molecular-weight compounds, such as tannins and saponins, through the microscopic pores of the agar medium also contributes to the effect

4. CONCLUSION

The study shows that solvent polarity strongly influences the phytochemical content and antimicrobial activity of *Phyllanthus amarus*. All fractions contained important metabolites, though in varying distributions. The chloroform fraction displayed the highest antimicrobial activity, suggesting that moderately non-polar compounds are mainly responsible for the plant's bioactivity. Overall, the findings highlight the importance of solvent selection and identify the chloroform fraction as the most promising source of potent antimicrobial agents.

5. ACKNOWLEDGMENT

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AUTHORS' CONTRIBUTIONS

Imrana Lawan (Federal Polytechnic, Idah) Conceptualization of the study, methodology design, extraction and fractionation procedures, data analysis, manuscript drafting, and overall practical supervision.

Suwaiba Abba Ismail (Kano State Polytechnic) Performed phytochemical screening, assisted with antimicrobial assays, contributed to data interpretation, and participated in manuscript reviewing and editing.

Aminu Baita (Northwest University, Kano) Conducted antimicrobial activity evaluation, supported laboratory experimentation, and assisted in compiling research results.

Zeenatu Ali Baba (Northwest University, Kano) Contributed to data organization, literature review, result validation, and critical revision of the final manuscript.

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