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Phytochemical Profiling, Antioxidant and Antibacterial Activities of *Phyllanthus Niruri* Leaf Extracts

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ABSTRACT

Background: *Phyllanthus niruri* is a well-known medicinal plant in traditional medicine systems. This study aimed to evaluate the pharmacognostic, phytochemical, antioxidant, and antibacterial properties of *P. niruri* leaf extracts.

Methods: Antioxidant activity was evaluated using the DPPH radical scavenging assay. Antibacterial activity of the ethanol extract and its fractions (chloroform, acetone soluble, acetone insoluble) was tested against five bacterial strains using disc diffusion and minimum inhibitory concentration (MIC) assays.

Results: Phytochemical screening confirmed the presence of alkaloids, flavonoids, phenolics, tannins, and saponins. Total phenolic and flavonoid contents were 7.12 mg GAE/100mg and 16.7 mg QE/g, respectively. TLC showed major spots at Rf 0.71 and 0.77. The ethanol extract exhibited good antioxidant activity (IC50 = 8.05 ± 0.2 µg/mL). The chloroform soluble fraction (CSF) demonstrated the strongest antibacterial activity, with maximum zones of inhibition against *E. coli* (28.65 mm) and *S. aureus* (23.52 mm). The lowest MIC values were observed for CSF against *E. coli* (204.6 µg/mL).

Conclusion: *P. niruri* leaf ethanol extract is a rich source of phenolic and flavonoid compounds with significant antioxidant and broad-spectrum antibacterial activity.

1. Introduction

Phyllanthus niruri (family Phyllanthaceae), commonly known as "Bhumi amla," is a small annual herb widely used in traditional medicine systems such as Ayurveda, Unani, and Siddha for treating jaundice, diabetes, and microbial infections. The plant is known to contain a variety of secondary metabolites including lignans, flavonoids, tannins, and alkaloids.[1-3] Despite its ethnopharmacological significance, systematic pharmacognostic standardization and bioactivity profiling are essential for quality control and therapeutic

validation.[4-5] This study aimed to (1) establish pharmacognostic parameters, (2) perform qualitative and quantitative phytochemical analysis, (3) evaluate antioxidant potential via DPPH assay, and (4) assess antibacterial activity of various leaf extracts/fractions against clinically relevant bacterial strains. [6]

2. Materials and Methods

2.1 Plant Material Collection and Authentication

Fresh young and mature leaves of *Phyllanthus niruri* were collected from the local area of Alwar, Rajasthan, India, during the flowering season. The plant was authenticated by a botanist at the Department of Botany, RR College, Alwar.

2.2 Sample Preparation

Fresh leaves were washed under running water to remove soil and debris, then shade-dried at room temperature. The dried leaves were pulverized to obtain 60# powder and stored in airtight containers. [7]

2.3 Macroscopic and Microscopic Studies

Macroscopic characters (color, odor, taste, size) were recorded. Free-hand transverse sections (T.S.) of fresh leaves were stained and observed for histological features. Powdered microscopy was performed on dried, stained leaf powder. [8-9]

2.4 Physicochemical Parameters

Foreign organic matter (FOM), moisture content, extractive values (using petroleum ether, hexane, acetone, ethanol, and water), total ash, water-soluble ash, acid-insoluble ash, and sulphated ash were determined according to WHO guidelines for quality control of herbal materials. [10-11]

2.5 Preparation of Extracts and Fractions

The powdered drug was defatted with petroleum ether (60-80°C) using a Soxhlet apparatus. The marc was then extracted with 800 mL ethanol. The ethanol extract was concentrated under reduced pressure and further fractionated as follows: [12]

- **Chloroform soluble fraction (CSF)** and **chloroform insoluble fraction**
- The chloroform insoluble fraction was further partitioned with acetone to obtain **acetone soluble fraction (ASF)** and **acetone insoluble fraction (AIF)**.

Percentage yields were calculated based on the dry weight of the starting material.

2.6 Qualitative and Quantitative Phytochemical Analysis

Standard chemical tests were performed for alkaloids, flavonoids, phenolics, tannins, saponins, sterols, carbohydrates, coumarins, and glycosides.

- **Total Phenolic Content:** Determined by Folin-Ciocalteu method and expressed as mg gallic acid equivalent (GAE)/100mg extract.
- **Total Flavonoid Content:** Determined by aluminum chloride colorimetric method and expressed as mg quercetin equivalent (QE)/g extract.

2.7 Thin Layer Chromatography (TLC)

TLC was performed on precoated silica gel 60 plates (Merck) using a solvent system of Toluene: Ethyl Acetate: Formic Acid (5:3.5:0.5). Spots were visualized using vanillin-sulfuric acid reagent. Rf values were calculated.

2.8 Antioxidant Activity (DPPH Assay)

The DPPH radical scavenging activity of the ethanol extract (EEPN) was measured at concentrations ranging from 12.5 to 400 µg/mL. Ascorbic acid was used as a standard. The percentage inhibition was calculated, and IC50 values were determined by linear regression analysis. All tests were performed in triplicate. [13-14]

2.9 Antibacterial Activity

2.9.1 Test Microorganisms

Five bacterial strains were used: *Staphylococcus aureus*, *Bacillus subtilis* (Gram-positive), *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* (Gram-negative). Cultures were maintained on agar slants at 4°C. [15]

2.9.2 Disc Diffusion Assay

The Kirby-Bauer disc diffusion method was used. Fresh bacterial suspensions (100 µL, 0.5 McFarland standard) were spread on Muller-Hinton Agar (MHA) plates. Sterile discs (6 mm) were placed on the agar, and 50 µL of each extract/fraction (ethanol extract, CSF, ASF, AIF) was applied. Ciprofloxacin (5 mcg) and amoxicillin (30 mcg) were used as positive controls. Plates were incubated at 37°C for 18-24 hours. Zones of inhibition (ZOI) were measured in mm. Activity was classified as: inactive (<8 mm), active (9-15 mm), very active (>15 mm). [16-17]

2.9.3 Minimum Inhibitory Concentration (MIC)

MIC was determined using the standard broth microdilution method for the ethanol extract and the most active fraction (CSF) in triplicate. Serial two-fold dilutions of extracts were tested against each bacterial strain. Ciprofloxacin was used as a standard. [18]

3. Results and Discussion

3.1 Pharmacognostic Characterization

Macroscopically, fresh leaves were green, becoming pale green to brownish upon drying, with a characteristic taste and odorless nature (size: length 0.5–2 cm; width 2–9 mm). Microscopic examination of the leaf T.S. (Fig. 6.1) revealed a single-layered epidermis with a thin cuticle, an indistinct mesophyll (not strongly differentiated into palisade and spongy layers), and collateral vascular bundles. Stomata were predominantly abaxial. Powder microscopy (Fig. 6.2) showed diagnostic features including lignified sclereids, prisms of calcium oxalate, and simple/compound starch grains. These findings are consistent with earlier reports on *Phyllanthus* species.

3.2 Physicochemical Parameters

The extractive values (Table 1) were highest in ethanol (7.6% w/w), followed by water (6.65%), indicating the presence of polar and medium-polar bioactive compounds. The lowest extractive value was observed with hexane (1.25%). Proximate analysis (Table 2) showed total ash (8.28%), water-soluble ash (5.15%), acid-insoluble ash (1.95%), and moisture content (7.56%). The low moisture content minimizes the risk of microbial contamination during storage. The ash values were within acceptable limits for herbal drugs.

Table 1: Extractive values of *P. niruri* leaf in different solvents

Solvent	Color of Extract	Extractive value (% w/w)
Petroleum ether (60-80°C)	Pale green	2.12
Hexane	Green	1.25
Acetone	Greenish Brown	3.58
Ethanol	Greenish Brown	7.6
Aqueous/Water	Brownish green	6.65

Table 2: Proximate analysis of *P. niruri* crude drug

Physicochemical Parameter	Value (% w/w)
Total Ash Content	8.28 ± 0.45
Water-soluble ash	5.15 ± 0.18
Acid-insoluble ash	1.95 ± 0.12
Sulphated ash	9.55 ± 0.48
Moisture content	7.56
Foreign matter	1.4

3.3 Extraction Yield

The percentage yield of the petroleum ether extract was 1.75%, and the ethanol extract was 7.6% w/w. Fractionation of the ethanol extract yielded the acetone insoluble fraction as the major component (42.72% of ethanol extract), followed by chloroform soluble (22.68%) and acetone soluble (20.25%) fractions (Table 3).

Table 3: Percentage yield and consistency of *P. niruri* leaf extracts/fractions

Extract/Fraction	**Color**	**Consistency**	**Yield (% w/w)**
Petroleum ether extract	Light brown	Sticky mass	1.75% (of crude drug)
Ethanol extract	Dark brown	Sticky mass	7.6% (of crude drug)
Chloroform soluble	Dark brown	Sticky mass	22.68% (of ethanol ext.)
Acetone soluble	Dark brown	Sticky mass	20.25% (of ethanol ext.)
Acetone insoluble	Dark brown	Sticky mass	42.72% (of ethanol ext.)

3.4 Phytochemical Analysis

Qualitative analysis (Table 4) of the ethanol extract revealed the presence of alkaloids, flavonoids, phenolics, tannins, saponins, carbohydrates, and glycosides, while sterols/triterpenoids and coumarins were absent. Quantitative analysis (Table 5) demonstrated a total phenolic content of 7.12 ± 0.52 mg GAE/100mg extract and total flavonoid content of 16.7 ± 1.02 mg QE/g extract. The presence of these metabolites, particularly flavonoids and phenolics, is likely responsible for the observed antioxidant and antibacterial activities.

Table 4: Qualitative phytochemical tests of *P. niruri* ethanol extract

Phytoconstituent	Test	Result
Alkaloids	Dragendorff's / Mayer	++ / +
Flavonoids	Shinoda / Fluorescence	++ / ++
Phenolics	FeCl3 / Folin-Ciocalteu	++ / ++
Tannins	Gelatin / Lead acetate	++ / +
Saponins	Froth / Haemolytic	+ / +
Glycosides	Borntrager's / Baljet	+ / +

(++ = present, + = moderately present, - = absent)

Table 5: Quantitative phytochemical analysis

Parameter	Value
Total Phenol	7.12 ± 0.52 mg GAE/100mg
Total Flavonoid	16.7 ± 1.02 mg QE/g

3.5 Thin Layer Chromatography

TLC of the ethanol extract using toluene:ethyl acetate:formic acid (5:3.5:0.5) resolved four major spots with Rf values of 0.34, 0.50, 0.71, and 0.77. The predominant spots at Rf 0.71 (blue-black) and 0.77 (dark blue) likely correspond to flavonoids and phenolic compounds, consistent with the phytochemical analysis.

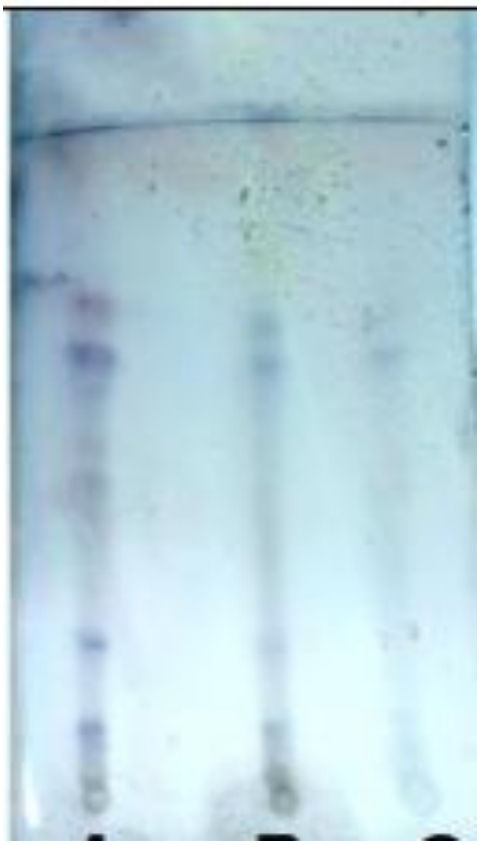

Figure 1: thin layer chromatography of ethanol extract of *Phyllanthus niruri*

3.6 Antioxidant Activity

The ethanol extract of *P. niruri* demonstrated concentration-dependent DPPH radical scavenging activity (Table 6, Fig. 6.6 & 6.7). The IC50 value of the extract was 8.05 ± 0.2 µg/mL, compared to 5.12 ± 0.54 µg/mL for ascorbic acid. This indicates potent antioxidant potential, likely attributable to the high flavonoid and phenolic content. The lower IC50 value (closer to ascorbic acid) suggests strong reducing capacity, which may contribute to its antibacterial mechanism.

Table 6: DPPH radical scavenging activity of ethanol extract vs. ascorbic acid

Concentration (µg/mL)	% Inhibition (Ethanol Extract)	% Inhibition (Ascorbic Acid)
12.5	8.1 ± 0.24	16.54 ± 0.25
25	15.5 ± 0.25	31.32 ± 0.4
50	27.52 ± 0.28	54.7 ± 0.52
100	44.3 ± 1.1	65.85 ± 1.4
200	58.7 ± 1.2	80.5 ± 1.2
400	75.7 ± 1.7	86.3 ± 1.35
IC50 (µg/mL)	**8.05 ± 0.2**	**5.12 ± 0.54**

Values are mean ± SEM, n=3.

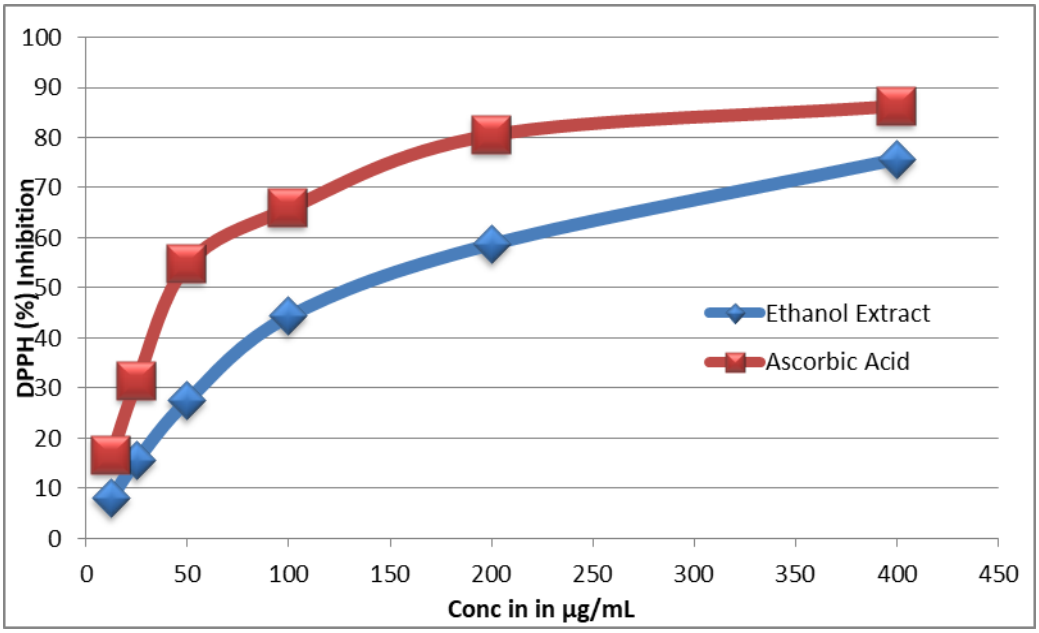

Figure 2: *in vitro* antioxidant capacity using the DPPH inhibition assay

3.7 Antibacterial Activity

3.7.1 Disc Diffusion Assay

The antibacterial activity of the ethanol extract and its fractions varied significantly (Table 7, Fig. 6.8). The chloroform soluble fraction (CSF) consistently showed the highest activity against all tested bacteria, with very large zones of inhibition (>15 mm for all strains, classified as "very active"). *E. coli* was the most sensitive organism (ZOI = 28.65 mm for CSF), followed by *S. aureus* (23.52 mm) and *B. subtilis* (18.65 mm). *K. pneumoniae* was the most resistant (ZOI = 19.20 mm for CSF). The crude ethanol extract also showed good broad-spectrum activity, particularly against *E. coli* (23.72 mm) and *B. subtilis* (22.20 mm).

The acetone soluble fraction showed moderate activity (ZOI 12.54–18.4 mm), while the acetone insoluble fraction was weakly active.

The fact that CSF was more active than the crude ethanol extract suggests that fractionation concentrated the active medium-polarity compounds (e.g., certain lignans, terpenoids, or flavonoids). The activity against both Gram-positive and Gram-negative bacteria indicates a broad-spectrum mechanism, potentially involving membrane disruption or inhibition of cell wall synthesis.

Table 7: Zone of inhibition (mm) of *P. niruri* leaf extracts and fractions

Treatment (50 µL)	*S. aureus*	*P. aeruginosa*	*E. coli*	*K. pneumoniae*	*B. subtilis*
Ethanol extract	20.15	17.82	23.72	16.54	22.2
Chloroform soluble fr.	23.52	21.87	28.65	19.2	18.65
Acetone soluble fr.	14.5	13.87	18.4	12.54	13.24
Acetone insoluble fr.	9.56	13.12	10.3	8.45	11.28
Ciprofloxacin (5 mcg)	30.2	28.54	33.38	27.12	28.6
Amoxicillin (30 mcg)	32.45	30.85	35.6	27.95	32.28

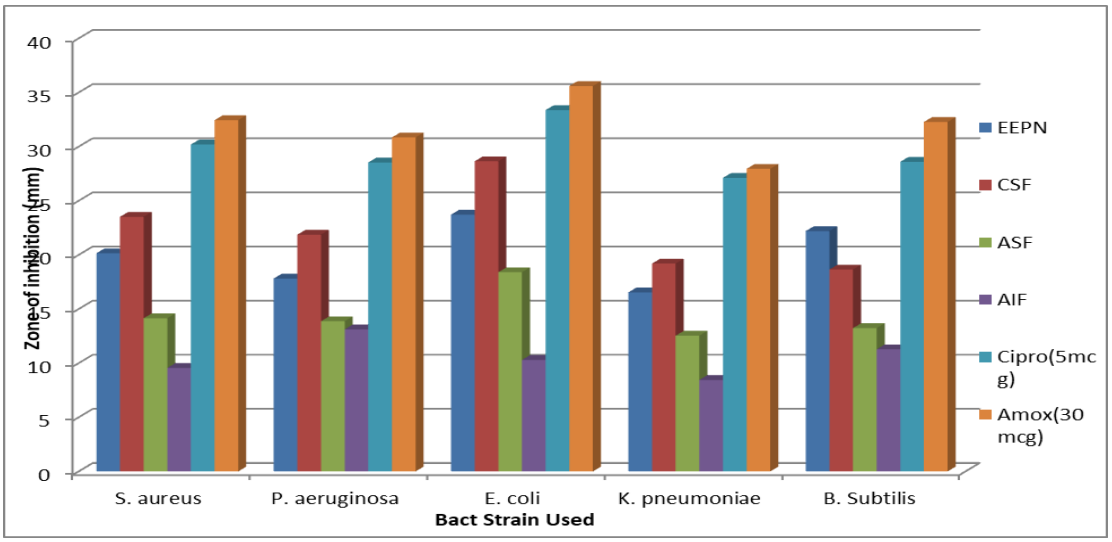

Figure 3: Antibacterial activity against a range of bacterial species is demonstrated by the ethanol extract and fractions of *Phyllanthus niruri*

3.7.2 Minimum Inhibitory Concentration (MIC)

MIC values confirmed the potency of CSF (Table 8). The lowest MIC was observed for CSF against *E. coli* (204.6 µg/mL) and *B. subtilis* (236.2 µg/mL), while the highest MIC (most resistant) was against *K. pneumoniae* (455.7 µg/mL for CSF). The ethanol extract showed higher MIC values (i.e., lower potency) compared to CSF, consistent with the disc diffusion results. These results demonstrate that *P. niruri*, particularly its chloroform soluble fraction, possesses significant antibacterial activity, especially against *E. coli*.

Table 8: Minimum Inhibitory Concentration (MIC) values (µg/mL)

Bacterial Strain	Ethanol Extract	Chloroform Soluble Fraction	Ciprofloxacin
S. aureus	282.4 ± 5.1	270.2 ± 3.6	35.5 ± 1.2
B. subtilis	252.7 ± 2.8	236.2 ± 2.6	24.3 ± 0.6
P. aeruginosa	330.5 ± 4.2	295.0 ± 4.6	32.6 ± 0.8
E. coli	242.3 ± 5.1	204.6 ± 5.0	36.4 ± 0.8
K. pneumoniae	470.4 ± 7.5	455.7 ± 7.0	52.1 ± 1.6

4. Discussion

The present study provides comprehensive pharmacognostic, phytochemical, and biological data for *Phyllanthus niruri* leaves. The microscopic features, including calcium oxalate crystals, sclereids, and starch grains, serve as reliable identification markers. The low moisture content (7.56%) ensures good storage stability.

The high extractive value in ethanol (7.6%) and water (6.65%) suggests that polar solvents are most efficient for extracting the bioactive constituents. This is consistent with the qualitative analysis, which identified polar compounds such as phenolics, flavonoids, saponins, and glycosides. The total phenolic and flavonoid contents (7.12 mg GAE/100mg and 16.7 mg QE/g, respectively) are significant and correlate well with the strong antioxidant activity (IC50 = 8.05 µg/mL).

The antibacterial results are noteworthy. The chloroform soluble fraction (CSF) exhibited superior activity compared to the crude ethanol extract against all tested strains, particularly *E. coli* (28.65 mm ZOI, MIC 204.6 µg/mL) and *S. aureus* (23.52 mm). This indicates that the active principles are of medium polarity and were concentrated during fractionation. *K. pneumoniae* was the most resistant organism, with the highest MIC values. The susceptibility of *E. coli* to *P. niruri* extracts has therapeutic implications, as *E. coli* is a common cause of urinary tract infections, a condition for which *P. niruri* is traditionally used. The observed antibacterial activity may be mediated through multiple mechanisms, including disruption of cell membrane integrity by flavonoids, inhibition of nucleic acid synthesis by tannins, or chelation of essential metals by phenolics. [19-20]

5. Conclusion

This study successfully established pharmacognostic standards for *Phyllanthus niruri* leaves. The ethanol extract is rich in phenolics and flavonoids, demonstrating potent antioxidant activity (IC50 = 8.05 µg/mL). The chloroform soluble fraction of the ethanol extract showed the strongest and broadest antibacterial activity, especially against *E. coli* and *S. aureus*, with MIC values as low as 204.6 µg/mL. These findings support the traditional medicinal use of *P. niruri* and provide a scientific basis for its standardization and potential development as a phytopharmaceutical for treating bacterial infections and oxidative stress-related conditions.

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