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Phytochemical and Nutritional Evaluation of *Phyllanthus niruri* Leaves: Implications for Ethnomedicinal Use

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ABSTRACT
The global resurgence of interest in plant-based therapeutics has intensified scientific efforts toward the identification and validation of medicinal plants as sustainable sources of bioactive compounds. Consequently, a thorough biochemical characterization of such traditional plants is essential to support drug discovery and development. This study investigated the phytochemical composition and proximate profile of the leaves of *Phyllanthus niruri* (*P. niruri*), a plant widely recognized in traditional medicine for the management of ailments such as hepatitis, urolithiasis, and diabetes. Fresh *P. niruri* leaves were extracted using 100% ethanol, and standard analytical procedures were employed for phytochemical and proximate analyses. The phytochemical screening indicated a rich presence of key secondary metabolites, including flavonoids, saponins, tannins, alkaloids, phenols, terpenoids, cardiac glycosides, anthraquinones, and phlobatannins, whereas steroids and quinones were absent. Proximate analysis revealed a high moisture content (39.33 ± 0.48%) and appreciable levels of carbohydrates (31.47 ± 0.58%) and crude fibre (15.26 ± 0.37%), alongside moderate quantities of crude fat (6.60 ± 0.57%), total ash (8.60 ± 0.28%), and crude protein (2.35 ± 0.17%). The abundance of polyphenolic compounds (phenols, tannins, flavonoids) and alkaloids suggests a biochemical basis for the plant's reported antioxidant, anti-inflammatory, and antiviral activities. Additionally, its considerable fibre and carbohydrate contents indicate potential value as a dietary supplement. Overall, these findings substantiate the ethnopharmacological relevance of *P. niruri*, reinforcing its potential as a nutraceutical resource and promising candidate for future drug development.

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INTRODUCTION

Medicinal plants remain indispensable to global healthcare and drug discovery. Over 80 % of the world's population relies on plant-derived medicines for primary healthcare, reflecting their accessibility, affordability, and pharmacological diversity (WHO, 2010). The World Health Organization defines a medicinal plant as any species containing bioactive substances that can be used therapeutically or serve as precursors for the synthesis of useful drugs (WHO, 2002). In recent decades, scientific interest in traditional plants has intensified as a

sustainable approach for identifying novel therapeutic compounds with fewer side effects than synthetic agents. Among the vast repertoire of ethnomedicinal species, *P. niruri* L. (family *Euphorbiaceae*) has gained prominence due to its wide distribution across tropical and subtropical regions and its extensive folkloric applications (Nielsen, 2010; Kumar *et al*., 2020; Kumara *et al*., 2023; Pratima *et al*., 2025) . *P. niruri,* commonly known as "stone breaker" or "chanca piedra", is a small annual herb (30–40 cm tall) with glabrous stems and alternate, oblong leaves. It is traditionally used in the management of jaundice, diabetes, urolithiasis, dysentery, malaria, and inflammatory disorders (Kiemer *et al*., 2003; Taylor, 2003; Harikrishnan *et al*., 2018). Decoctions and infusions of its whole plant are employed to treat gastrointestinal disturbances, wounds, and hepatic dysfunctions, and to promote diuresis and detoxification (Cheema and Singh, 2021). The medicinal attributes of *P. niruri* are associated with its abundant phytoconstituents, including lignans (phyllanthin, hypophyllanthin, niranthin), flavonoids (quercetin, kaempferol derivatives), terpenoids, phenolic acids, tannins, saponins, and alkaloids (Rastogi, 1991; Singh and Panda, 2005; Bagalkotkar *et al*., 2006; Prananda *et al*., 2023). These bioactive metabolites confer antioxidant, anti-inflammatory, hepatoprotective, and antimicrobial activities, as well as potential anticancer effects. Several reports have demonstrated that *P. niruri* extracts can modulate oxidative stress, inhibit viral replication, and protect hepatocytes from toxic injury (Harish and Shivanandappa, 2006; Chen and Zhang, 2023; Wu *et al*., 2015)

Beyond its pharmacological profile, *P. niruri* also represents a potential nutraceutical resource. Proximate composition analysis—encompassing carbohydrate, protein, lipid, fibre, ash, and moisture contents—provides an estimate of nutritional value and informs its suitability as a dietary supplement. Moreover, quantifying mineral and trace-element contents enhances understanding of their physiological importance, as many minerals act as enzyme cofactors, electrolytes, or structural constituents critical for homeostasis and cellular integrity (Malik *et al*., 2023).

Despite extensive traditional use, systematic biochemical characterisation of *P. niruri* grown in Nigeria remains limited. Therefore, this study aimed to perform phytochemical and proximate analyses of the ethanolic extract of *P. niruri* whole plant to establish its bioactive composition, evaluate its nutritional potential, and provide baseline data supporting its future development as a phytopharmaceutical and nutraceutical agent.

MATERIALS AND METHODS

Chemicals

All reagents and solvents used were of analytical grade and obtained from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA), unless otherwise specified. Distilled and deionised water was used throughout the experimental procedures. Glassware and instruments were thoroughly cleaned and calibrated prior to use to ensure analytical accuracy.

Plant Material Collection and Identification

Fresh whole-plant samples of *P. niruri* L. (*Euphorbiaceae*) were collected from an open field within the University of Benin, Benin City, Edo State, Nigeria (Latitude 6.41°N, Longitude 5.61°E). The species was identified and authenticated at the Department of Plant Biology and Biotechnology, University of Benin. A voucher specimen (No. UBH–P406) was deposited in the departmental herbarium for future reference.

Preparation of Plant Extract

The collected whole plant were thoroughly rinsed with distilled water to remove surface impurities and subsequently air-dried under ambient laboratory conditions (25 ± 2 °C) for 14 days to constant weight. The dried leaves were then pulverized using a mechanical grinder to obtain a uniform fine powder (Thomas Wiley Mini-Mill Model 4 (Thomas Scientific, USA)). Approximately 950 g of the powdered material was subjected to continuous extraction in a Soxhlet apparatus using 100% ethanol for 12 h. The resulting extract was concentrated to dryness under reduced pressure at 40 °C using a rotary evaporator (Büchi Rotavapor R-300, Switzerland). The dried ethanolic extract (designated *P. niruri* extract) was weighed, transferred into airtight amber glass vials, and stored at 4 °C until subsequent phytochemical and proximate analyses.

Weight of powdered material = 950 g

Weight of the dried extract obtained after evaporation is = 50 g

$$\text{Yield (\%)} = \left(\frac{50\text{ g}}{950\text{ g}}\right) \times 100 \approx 5.26\%$$

Phytochemical Examination (Qualitative)

Glycoside

The technique established by Sofowora (1996) was employed to ascertain the existence of glycosides. A 0.5 g part of the sample was combined with 2 mL of glacial acetic acid and a drop of ferric chloride solution, followed by the addition of 1 mL of concentrated sulfuric acid. The reaction was monitored for the creation of a brown ring.

Flavonoids

The Harborne method (Harborne, 1998) was employed to ascertain the presence of flavonoids. A sample (0.5 g) of powdered plant material was subjected to heating with 10 mL of ethyl acetate in a test tube over a steam bath for 3 minutes. The filtrate was subjected to filtration, then 4 mL was combined with 1 mL of weak ammonia solution and agitated. The yellow tint noticed showed the presence of flavonoids.

Tannins

The Harborne method (Harborne, 1998) was employed to ascertain the presence of tannins. In this procedure, 0.5 g of the desiccated powdered sample was subjected to boiling in 20 mL of distilled water within a test tube and subsequently filtered. A 0.1% solution of ferric chloride (FeCl3) was added to the filtrate. The manifestation of a brownish-green or blue-black hue signifies the presence of tannins in the test samples.

Saponins

The presence of saponins was quantified utilizing the methodology established by Obadoni and Ochuko (2002). A 2.0 g quantity of the powdered sample was subjected to boiling in 20 mL of distilled water within a test tube placed in a boiling water bath, followed by filtration. Ten millilitres of the filtrate were combined with five millilitres of distilled water and agitated vigorously to produce stable, persistent foam. Approximately three drops of olive oil were incorporated into the foam and agitated forcefully to facilitate the creation of an emulsion characteristic of saponins.

Alkaloids

The detection of alkaloids was conducted utilizing the methodologies of Harborne, Trease and Evans. Exactly 0.5 g of the extract was agitated with 5 mL of 1% aqueous HCl on a steam bath. Several drops of picric acid solution were introduced to 2 mL of the extract. The emergence of a reddish-brown precipitate was seen as initial proof for the existence of alkaloids (Harborne, 1976; Trease and Evans, 1978).

Steroid

The detection of steroids was conducted utilizing the methodology established by Finar (1986). Approximately 2 mL of acetic anhydride was combined with 0.5 g of the sample and 2 mL of H2SO4. Positive tests yield specific colorimetric changes (e.g., pink, blue, green, or reddish-brown), signifying the presence of steroidal chemicals.

Terpenoids

Terpenoids were identified utilizing the methodology established by Edeoga *et al*. (2005). Five milliliters of the extract were combined with two milliliters of chloroform, followed by the cautious addition of three milliliters of strong sulfuric acid to create a distinct layer. The emergence of a reddish-brown coloration near the contact signifies the presence of terpenoids.

Quantitative Phytochemical Analysis

The phytochemicals that are present in the methanol extracts of *P. niruri* were determined and quantified by standard procedures. Quantitative estimation of the major phytochemical constituents including total phenols, flavonoids, tannins, saponins, alkaloids, terpenoids, cardiac glycosides, phlobatannins, reducing sugars, and xanthoproteins—was performed on the ethanolic extract of *P. niruri* using standard spectrophotometric and gravimetric procedures as previously described by Harborne (1998), Obadoni and Ochuko (2002), and the Association of Official Analytical Chemists (AOAC, 2000) with minor modifications.

Total Phenolic Content (TPC)

Determined using the Folin–Ciocalteu method. Briefly, 1 mL of extract (1 mg/mL) was mixed with 2.5 mL of 10% Folin–Ciocalteu reagent and 2.0 mL of 7.5% sodium carbonate. The mixture was incubated for 30 min at room temperature in the dark, and absorbance was read at 760 nm using a UV–visible spectrophotometer (Shimadzu UV-1800, Japan). Total phenolic content was expressed as mg gallic acid equivalent (GAE) per g of dry extract.

Total Flavonoid Content (TFC)

Quantified by the aluminium chloride colorimetric method. A 1 mL aliquot of extract was mixed with 4 mL of distilled water and 0.3 mL of 5% $NaNO_2$. After 5 min, 0.3 mL of 10% $AlCl_3$ was added, followed by 2 mL of 1 M NaOH after 6 min. Absorbance was recorded at 510 nm, and results were expressed as mg quercetin equivalent (QE) per g of dry extract.

Tannin Content

Determined following Harborne (1998). A 1 mL aliquot of the extract was mixed with 5 mL of 0.1 M ferric chloride solution, and the absorbance was measured at 720 nm. Tannin concentration was expressed as mg tannic acid equivalent (TAE) per g of extract.

Saponin Content

Evaluated using the gravimetric method of Obadoni and Ochuko (2002). Ten grams of sample were refluxed with 20% aqueous ethanol for 4 h, filtered, and re-extracted. The combined filtrate was reduced to 40 mL and transferred into a separatory funnel containing 20 mL diethyl ether to remove impurities. The aqueous layer was then partitioned with n-butanol, evaporated to dryness, and the residue was weighed to obtain total saponin content (mg/g dry weight).

Alkaloid Content

Determined using Harborne (1973) with slight modification. Five grams of extract were dispersed in 200 mL of 10% acetic acid in ethanol, covered, and allowed to stand for 4 h. The mixture was filtered, concentrated, and precipitated with concentrated ammonium hydroxide. The precipitate was collected, dried, and weighed to calculate the total alkaloid content.

Terpenoid Content

Quantified using the Edeoga *et al*. (2005) method. Five milliliters of extract were mixed with 2 mL of chloroform, followed by the careful addition of 3 mL of concentrated sulfuric acid to form a distinct layer. The reddish-brown coloration was read at 538 nm, and results were expressed as mg ursolic acid equivalent (UAE) per g of dry extract.

Cardiac Glycosides

Determined by the Keller–Killiani test following Sofowora (1996). 1 mL of extract was mixed with 2 mL glacial acetic acid containing $FeCl_3$ and layered with concentrated H_2SO_4. The formation of a brown ring indicated the presence of deoxy-sugars, and absorbance was read at 530 nm.

Phlobatannins and Reducing Sugars

Quantified according to AOAC (2000) methods by colorimetric estimation at 510 nm (phlobatannins) and 700 nm (reducing sugars) using glucose as a standard.

All measurements were performed in triplicate, and the results were expressed as mean ± standard deviation (SD) in mg/g of dry extract. Calibration curves for each standard compound exhibited $R^2 > 0.995$, ensuring analytical reliability.

Proximate analysis

The proximate analysis encompasses moisture content, ash content, crude protein, crude fibre, crude fat, and carbohydrates. The moisture level was assessed, and the desiccated samples were further tested for crude protein, crude fibre, ash content, and crude fat utilizing the micro-Kjeldahl method as outlined by AOAC Chemists and Chemists (1920), while carbohydrate determination was conducted according to (Chemists, 2000)

Statistical Analysis

A descriptive statistical analysis was performed on the data collected in this study. Experimental variables are reported as Mean ± Standard deviation.

RESULTS AND DISCUSSION

Qualitative Phytochemical Screening of the Ethanol Extract

Phytochemical analysis of *P. niruri* revealed the presence of flavonoids, tannins, cardiac glycosides, saponins, terpenoids, alkaloids, anthraquinone, phenols, reducing sugars, xanthoprotein, phlobatannins, while steroid and quinones proved negative, as presented in Table 1.

Table 1: Phytochemical Screening (Qualitative) of *P. niruri* ethanol extract

Phytochemicals	Ethanol Extract
Flavonoids	+
Tannins	+
Cardiac glycosides	+
Saponins	+
Steroids	-
Terpenoids	+
Alkaloids	+
Anthraquinone	+
Phenols	+
Reducing sugars	+
Xanthoprotein	+
Phlobatannins	+
Quinones	-

Key: '+' = Present, '-' = Absent

Quantitative Analysis of Phytochemicals in *P. niruri* Ethanol Extracts

Quantitative profiling of major phytochemical constituents in the ethanolic extract of *P. niruri* (Table 2) revealed considerable variability in the abundance of bioactive compounds, reflecting the plant's biochemical complexity. The concentrations of the quantified metabolites ranged from 1.2 ± 0.1 mg/g for xanthoproteins to 120.5 ± 5.1 mg/g for total phenolics, indicating a polyphenol-dominated phytochemical profile.

Table 2: Phytochemical Screening (Quantitative) of *P. niruri* ethanol extract

Phytochemical Class	Concentration (mg/g of Dry Extract)
Total Flavonoids	85.2±3.9
Total Phenols	120.5±5.1
Total Tannins	45.8±2.5
Total Saponins	32.1±1.8
Total Alkaloids	18.9±1.1
Total Terpenoids	22.7±1.5
Anthraquinones	Not Detected / Below Limit
Cardiac Glycosides	4.1±0.2
Reducing Sugars	15.3±1.0
Phlobatannins	6.5±0.4
Xanthoprotein	1.2±0.1
Steroids	Not Detected / Below Limit
Quinones	Not Detected / Below Limit

Qualitative Proximate composition of *P. niruri*

The proximate composition of *P. niruri* leaf extract was investigated. The result showed that *P. niruri* has high content of Moisture Content (39.33± 0.48 %), Carbohydrate (31.47 ± 0.58%) and Crude Fibre (15.26 ± 0.37%). The result also revealed low total ash content, recording 8.6 ± 0.28%. crude protein and moisture, crude fat were found to be 2.35 ± 0.17% and 6.6 ± 0.57 % respectively (Table 3).

Table 3: Proximate Analysis of *P. niruri* ethanol extract

Parameters	Percentage Yield (%)
Carbohydrate	31.47 ± 0.58
Crude Fat	6.60 ± 0.57
Crude Fibre	15.26 ± 0.37
Crude Protein	2.35 ± 0.17
Moisture Content	39.33± 0.48
Total Ash Content	8.60 ± 0.28

Discussion

The global resurgence of interest in plant-based therapeutics underscores the importance of medicinal plants as valuable sources of bioactive compounds. According to the World Health Organization (2019), over 80% of the global population relies on herbal medicines for primary healthcare, emphasizing the need to explore traditional plants such as *P. niruri* for novel, safer therapeutic alternatives.

In this study, comprehensive phytochemical screening and proximate analysis of ethanol extracts from *P. niruri* leaves were conducted to elucidate its biochemical profile and support its ethnopharmacological relevance. Phytochemical analysis revealed a diverse array of secondary metabolites, including flavonoids, saponins, tannins, alkaloids, phenols, terpenoids, cardiac glycosides, anthraquinones, and phlobatannins, while steroids and quinones were absent. This broad phytochemical spectrum reinforces the medicinal versatility of *P. niruri*, which has been historically used to treat hepatitis, urolithiasis, diabetes, and other metabolic disorders. The selective absence of certain metabolite classes, such as steroids, may indicate a targeted biosynthetic allocation toward antioxidant and anti-inflammatory compounds, warranting further biochemical investigation.

The presence of polyphenols, particularly flavonoids and tannins, is noteworthy given their well-documented antioxidant and free-radical scavenging capacities (Nisar *et al.,* 2018; Harikrishnan *et al*., 2020; Thuy *et al*., 2025). These compounds neutralize reactive oxygen species by donating electrons or hydrogen atoms, thereby preventing lipid peroxidation and oxidative damage (Mavi *et al*., 2004). Such antioxidant mechanisms are critical in mitigating oxidative stress implicated in chronic diseases, including cancer, diabetes, and cardiovascular disorders (Simeon *et al*., 2018). Moreover, the antioxidative potential of *P. niruri* supports its traditional use in managing inflammatory and infectious diseases (Omoregie and Oikeh, 2015).

Quantitative profiling revealed that total phenolics (120.5 ± 5.1 mg/g), flavonoids (85.2 ± 3.9 mg/g), and tannins (45.8 ± 2.5 mg/g) were the most abundant phytochemicals. This polyphenolic richness aligns with previous metabolomic studies on *Phyllanthus species* (Bagalkotkar *et al.,* 2006; Patel *et al*., 2011), further supporting its potent antioxidant capacity and possible therapeutic role in oxidative stress-related pathologies. Polyphenols and flavonoids are known to act as effective free-radical scavengers and

metal chelators, while also modulating inflammatory signalling pathways such as NF-κB and MAPK (Tungmunnithum *et al*., 2018; Williams *et al*., 2004). Moderate concentrations of saponins (32.1 ± 1.8 mg/g), terpenoids (22.7 ± 1.5 mg/g), and alkaloids (18.9 ± 1.1 mg/g) were also observed. These compounds are pharmacologically relevant due to their established anti-inflammatory, antimicrobial, and hepatoprotective activities (Kumar and Kuttan, 2005; Harish and Shivanandappa, 2006). Terpenoids possess membrane-stabilizing and enzyme-inhibitory properties, while alkaloids are often associated with neuroactive and antimicrobial functions (Wink, 2015). Cardiac glycosides (4.1 ± 0.2 mg/g) and phlobatannins (6.5 ± 0.4 mg/g) were detected in lower quantities, and the absence of steroids and quinones suggests a specific metabolic adaptation within *P. niruri*.

The elevated levels of polyphenols and flavonoids corroborate prior findings that attribute the hepatoprotective and nephroprotective effects of *Phyllanthus* extracts to their antioxidative and anti-inflammatory mechanisms (Wu *et al*., 2015). This biochemical profile supports the ethnomedicinal use of *P. niruri* in managing hepatic dysfunction, urolithiasis, and metabolic disorders such as diabetes (Valletta *et al*., 2023).

The proximate analysis further complements these findings. The high crude fibre content (15.26 ± 0.37%) suggests dietary benefits, as fibre supports gastrointestinal health and aids in metabolic regulation, including glycaemic control and weight management (Agostoni *et al*., 2012; Ojo *et al*., 2025). The substantial moisture content (39.33 ± 0.48%) reflects the need for optimal drying and storage conditions to prevent microbial contamination and maintain extract stability (Khadka, 2021). Additionally, the carbohydrate content (31.47 ± 0.58%) indicates a significant energy contribution, while moderate crude fat (6.6 ± 0.57 %) and total ash (8.6 ± 0.28 %) values suggest the presence of essential lipids and minerals vital for physiological processes (Nelson and Cox, 2008; Mahmood *et al*., 2024). Collectively, the phytochemical and nutritional profiles of *P. niruri* affirm its ethnopharmacological value. Its rich polyphenolic and alkaloid content provides a biochemical basis for the reported antioxidant, anti-inflammatory, hepatoprotective and antimicrobial activities (Kumara *et al*., 2023; Upadhyay and Tiwari, 2023). Furthermore, emerging evidence suggests that *P. niruri* exhibits anticancer and antiviral properties by modulating oxidative stress pathways and inhibiting viral replication (Khan *et al*., 2012; Al-Gbouri and Hamzah, 2018; Shrivastava and Dwivedi, 2020).

CONCLUSION

This study demonstrated that *P. niruri* leaves are a rich source of bioactive and nutritional compounds with potential therapeutic significance. The high levels of polyphenols, flavonoids, and tannins provide strong biochemical support for the plant's traditional medicinal uses. Moreover, its favourable proximate composition, characterized by high carbohydrate and fibre contents, suggests additional nutraceutical potential. Overall, these findings substantiate the ethnopharmacological relevance of *P. niruri* and provide a scientific foundation for its continued exploration as a candidate for drug discovery and functional food development.

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