

Microscopic, Quantitative and Microchemical Characterization of the Leaves of *Phyllanthus niruri* L.

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Abstract

Phyllanthus niruri L. is a medicinal plant widely used in traditional medicine, making accurate authentication and quality evaluation of its crude leaf material important for pharmacognostic assessment. This study characterized the leaves of *P. niruri* using epidermal microscopy, powder microscopy, quantitative microscopy, and microchemical analysis. Leaves collected in October 2024 from Area BZ, Ahmadu Bello University, Zaria, Kaduna State, Nigeria, were authenticated at the Department of Botany, Ahmadu Bello University, Zaria, with a voucher number 'ABUH019007'. Ten fresh, healthy, mature leaves were examined for quantitative leaf characteristics, while additional leaf material was shade-dried, powdered, and subjected to microscopic and microchemical examination. Epidermal and powder preparations were examined using a light microscope, with representative photomicrographs obtained at $\times 100$ and $\times 400$ magnifications. The epidermal preparations showed irregular, thin-walled and wavy epidermal cells, numerous anisocytic stomata, numerous clusters of calcium oxalate crystals, and no observed trichomes. The available quantitative records indicated a stomatal number of 20/mm², stomatal index of 35.7%, and palisade ratio of 10.3. Quantitative measurements were based on ten sampled leaves, with three non-overlapping microscopic areas examined per leaf for stomatal counting. Powder microscopy revealed characteristic epidermal fragments, fibres, tracheids, calcium oxalate crystals, lignified tissues, starch-containing material, and tannin-containing tissues. Microchemical tests indicated the presence of cellulose, lignin, calcium oxalate, starch, and tannins in the examined leaf material. The findings provide a preliminary pharmacognostic profile of the examined *P. niruri* leaves and may support authentication and quality evaluation, although broader validation using adequately documented, geographically diverse samples and retained raw quantitative observations is required.

Keywords: *Phyllanthus niruri*, Epidermal Microscopy, Powder Microscopy, Quantitative Microscopy, Microchemical Analysis.

Introduction

Medicinal plants constitute important sources of crude drugs and therapeutic agents, but their safe and effective use depends on accurate authentication and quality control of the

plant material (Intharuksa et al., 2024). Misidentification, substitution, adulteration, and contamination can compromise the quality, consistency, and therapeutic reliability of herbal preparations (Muley et al., 2026). Pharmacognostic evaluation therefore provides important tools for establishing the identity and quality of crude drugs through systematic assessment of morphological, anatomical, microscopic, quantitative, physicochemical, and chemical characteristics (Alamgir, 2017). Microscopic examination is particularly valuable because anatomical and powder-microscopic features may remain useful for identification when the plant material has been fragmented or powdered.

Pharmacognostic characterization contributes to the establishment of diagnostic parameters that may help distinguish authentic plant materials from adulterants and closely related species. Features such as epidermal-cell pattern, stomatal type and distribution, trichome characteristics, calcium oxalate crystals, vascular tissues, and other microscopic structures can provide useful criteria for identification and quality assessment (Jayani et al., 2020; Sharma and Sheela, 2011). Quantitative leaf constants, including stomatal number, stomatal index, and palisade ratio, can provide additional information for pharmacognostic characterization, while microchemical tests provide complementary evidence concerning structural and cellular components such as cellulose, lignin, starch, calcium oxalate, and tannins. Consequently, combined pharmacognostic evaluation can contribute to the development of reference information for medicinal plant materials.

Phyllanthus niruri L. is a medicinal herb belonging to the family Phyllanthaceae and is widely used in traditional medicine in tropical and subtropical regions. It is commonly known as gale of the wind, stone-breaker, shatter-stone, or seed-under-leaf (Prabhuji et al., 2025). The plant is an annual herb that may grow up to approximately 30 cm in height, with slender, smooth stems and small, oblong to oblong-elliptic leaves. The flowers are minute, axillary, and unisexual, while the fruits and seeds possess characteristic morphological features that aid botanical identification (Saranya et al., 2026). Its traditional medicinal importance has been associated particularly with urinary and hepatobiliary conditions, including use in preparations for kidney and gallbladder disorders (Bhasme and Khan, 2022). These established medicinal uses make accurate identification and quality evaluation of *P. niruri* relevant to pharmacognostic and herbal-drug research.

Previous investigations of *P. niruri* have emphasized its phytochemical composition, physicochemical properties, pharmacological activities, and standardization. Jayani et al. (2020), for example, evaluated specific and non-specific quality parameters of *P. niruri* collected from three locations and demonstrated the importance of standardization in assessing medicinal plant materials. Microscopic studies of related *Phyllanthus* species have also shown that epidermal characters, stomatal types, and calcium oxalate crystals can provide useful diagnostic information for distinguishing closely related taxa (Sharma and Sheela, 2011). These studies provide an important comparative basis for interpreting the present anatomical findings.

Despite the medicinal importance of *P. niruri*, detailed and systematically documented information on its leaf epidermal characteristics, powder-microscopic features, quantitative leaf constants, and microchemical characteristics remains comparatively limited or fragmented. In particular, an integrated characterization combining epidermal microscopy, powder microscopy, quantitative microscopy, and microchemical analysis can provide a broader set of preliminary diagnostic observations for authentication and quality assessment. Documentation of these features is relevant because they may support identification of crude drug material and detection of possible adulteration or substitution, particularly where macroscopic characteristics are no longer readily discernible.

Therefore, this study aimed to characterize the leaves of *Phyllanthus niruri* L. using epidermal microscopy, powder microscopy, quantitative microscopy, and microchemical analysis, and to document diagnostic observations that may serve as preliminary pharmacognostic reference information for the identification and quality evaluation of the leaf drug.

Materials and Methods

Collection and Authentication

The leaves of *Phyllanthus niruri* L. were collected from Area BZ, Ahmadu Bello University, Zaria, Kaduna State, Nigeria, in October 2024. The plant material, with a voucher number 'ABUHo19007' was authenticated at the Department of Botany, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. Ten fresh, healthy, and mature leaves were retained for determination of leaf constants, while additional leaves were shade-dried, reduced to fine powder using a pestle and mortar, and stored for subsequent analysis.

Chemicals and Reagents

Water, iodine solution, dilute glycerol, concentrated HCl, dilute HCl, ferric chloride, phloroglucinol, tannic acid reagent, chloral hydrate, 20% sulfuric acid, and iodinated zinc chloride.

Epidermal Microscopy

The upper and lower epidermal surfaces, which could not be peeled, were cut, cleared, mounted, and observed under a light microscope. The observed anatomical features were recorded (Jayani et al., 2020).

Powder Microscopy

The powdered leaf material was cleared and mounted in glycerol for microscopic examination. The preparation was examined under a light microscope for characteristic cellular and tissue structures, including epidermal fragments, fibres, vascular elements, crystals, and other diagnostic structures (Jayani et al., 2020).

Quantitative Microscopy

Quantitative microscopy was used to assess selected leaf constants of *Phyllanthus niruri* leaves.

(i) Stomatal number: A leaf preparation was cleared in chloral hydrate to improve transparency. A square-millimetre field was delineated using a stage micrometer, and the number of stomata within the defined area was counted microscopically. For each of the ten sampled leaves, three non-overlapping microscopic areas were examined, giving three stomatal counts per leaf. The three counts obtained from each leaf were averaged to obtain a leaf-level stomatal number, and the ten leaf-level observations were subsequently averaged to obtain the reported mean stomatal number per square millimetre.

The individual raw stomatal counts underlying the reported value were not retained in the available study records; therefore, the original observations could not be independently recalculated or used to estimate variability.

(ii) Stomatal index: Stomatal index was calculated as the percentage of stomata relative to the total number of stomata and epidermal cells within the assessed area using the formula: Stomatal index (S.I.) = [Number of stomata per unit area / (Number of epidermal cells + Number of stomata per unit area)] × 100.

(iii) Palisade ratio: small leaf sections were treated with chloral hydrate and examined under a microscope with a focused camera lucida. Palisade cells within each group were counted, including cells more than half covered by the epidermal cells.

Microchemical Analysis

- i. Cellulose test: The sample was treated with iodinated zinc chloride followed by sulfuric acid.
- ii. Lignin test: Phloroglucinol and hydrochloric acid were added to the sample.
- iii. Calcium oxalate test: The cleared sample was mounted under a cover slip and examined microscopically. Two drops of concentrated hydrochloric acid were then added and the preparation was re-examined.
- iv. Tannin test: A drop of ferric chloride was added to the cleared sample, covered, and examined microscopically.
- v. Starch test: Two drops of N/50 iodine solution were added to the powdered leaf sample, covered, and examined microscopically.

Photomicrography

Photomicrographs of the prepared leaf specimens and powdered samples were obtained using a laboratory light microscope at ×100 and ×400 magnifications. Images were captured by positioning a smartphone camera over the microscope eyepiece. The figures presented in the manuscript should be submitted together with the corresponding original digital image files to permit editorial verification of image provenance and reproduction quality.

Data Presentation and Statistical Considerations

The findings from the macroscopic and microscopic examinations were recorded and presented descriptively. Representative photomicrographs were included to illustrate observed diagnostic features. Microscopic and microchemical observations were reported descriptively, while quantitative microscopy results were reported using the available recorded values. Because the individual raw stomatal and epidermal-cell observations were unavailable, measures of variability such as range and standard deviation could not be calculated retrospectively. No inferential statistical analysis was performed.

Results and Discussion

A. Epidermal Microscopy



Figure 1A: Surface view of the upper epidermis of *Phyllanthus niruri* viewed under compound microscope ($\times 400$).

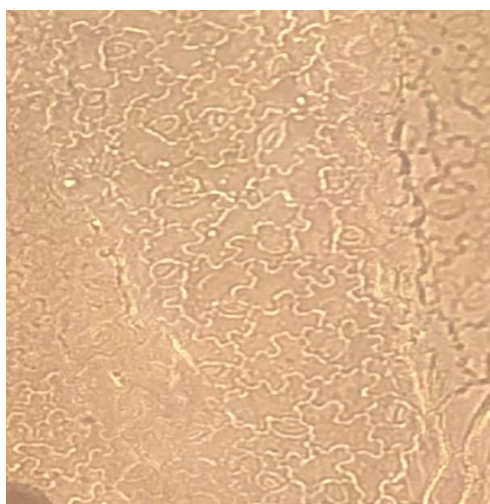


Figure 1B: Surface view of the lower epidermis of *Phyllanthus niruri* viewed under compound microscope ($\times 100$).

Figure 1: Upper and lower epidermis of *Phyllanthus niruri*.

Source: Authors' photomicrograph, 2024.

Phyllanthus niruri leaves exhibited irregular, wavy, thin-walled epidermal cells, numerous anisocytic stomata, numerous medium-sized clusters of calcium oxalate crystals, and no observed trichomes. The wavy epidermal-cell walls and occurrence of anisocytic stomata are broadly consistent with microscopic observations reported for related Phyllanthus species. Sharma and Sheela (2011) reported wavy epidermal walls in *P. amarus* and anisocytic stomata among the diagnostic characters observed in several Phyllanthus species. Jayani et al. (2020) likewise demonstrated the value of microscopic characteristics in the standardization of *P. niruri*. The present findings therefore support the usefulness of epidermal characters as part of a combined pharmacognostic profile rather than as isolated species-defining criteria.

The occurrence of calcium oxalate crystals is also consistent with observations reported in related Phyllanthus species, although crystal distribution and form may vary among taxa. Published comparative work has documented calcium oxalate crystals in several Phyllanthus species and demonstrated that epidermal and crystal characters can assist in distinguishing closely related materials (Sharma and Sheela, 2011). Differences between studies may reflect species identity, geographical origin, developmental stage, environmental conditions, and methodological differences in specimen preparation and microscopy. The absence of observed trichomes in the present preparations should similarly be interpreted cautiously because the absence of a structure in the examined field does not necessarily establish its universal absence from the species. Collectively, the observed epidermal features provide useful preliminary anatomical information for authentication and quality assessment of the examined material.

B. Powder Microscopy



Figure 2A: Fragment of fibre of *Phyllanthus niruri* leaf viewed under compound microscope (×100).



Figure 2B: Fragment of tracheid of *Phyllanthus niruri* leaf viewed under compound microscope ($\times 100$).



Figure 2C: Fragment of calcium oxalate crystals of *Phyllanthus niruri* leaf viewed under compound microscope ($\times 100$).

Source: Authors' photomicrograph, 2024.

Powder microscopy is an important component of pharmacognostic evaluation because herbal materials are frequently marketed and used in powdered form, where gross morphological features may no longer be available for identification. The characteristic fragments observed in the powdered leaves, including calcium oxalate crystals, epidermal-cell fragments, fibres, tracheids, lignified tissues, starch-containing material, and tannin-containing tissues, provide supplementary microscopic evidence that may support authentication of the crude drug. Comparable microscopic diagnostic structures, including

epidermal fragments, crystals, fibres, and vascular elements, have been reported in *Phyllanthus* materials (Sharma and Sheela, 2011). These characters may therefore assist routine quality control, particularly when combined with anatomical and other pharmacognostic observations.

C. Quantitative Microscopy

Table 1. Quantitative microscopic observations of fresh leaves of *P. niruri*

Parameter	Reported value
Stomatal number	20/mm ²
Stomatal index	35.7%
Palisade ratio	10.3

Source: Available study records, 2024.

The available quantitative records indicated a stomatal number of 20/mm², stomatal index of 35.7%, and palisade ratio of 10.3. These values are presented as preliminary observations from the examined material. The original individual stomatal and epidermal-cell counts used to derive the stomatal number and stomatal index were not available at the time of manuscript revision. Consequently, independent recalculation, range, and standard deviation could not be performed. The values should therefore not be interpreted as validated species-wide standards.

Quantitative leaf constants can contribute to pharmacognostic characterization, but their values may vary with geographical origin, plant maturity, environmental conditions, sampling design, and methodological procedures. Stomatal number and stomatal index may be particularly useful when comparative measurements are obtained using standardized procedures. The present study did not determine vein-islet number or vein-termination number because these parameters were not included among the quantitative measurements undertaken during the original examination. They were therefore not estimated retrospectively. Future studies should retain individual observations for every sampled leaf and include additional quantitative parameters where appropriate.

D. Microchemical Analysis

Table 2: Results of microchemical analysis of powdered leaves of *P. niruri*

Tests	Inference
Starch	Present
Calcium oxalate	Present
Lignin	Present
Cellulose	Present
Tannins	Present

Source: Authors' observations, 2024.

The positive microchemical reactions obtained in the present study indicate the presence of cellulose, lignified tissues, calcium oxalate, starch, and tannin-containing tissues in the examined leaves. These findings are broadly consistent with previous pharmacognostic and phytochemical observations in species of the genus *Phyllanthus*. Calcium oxalate crystals, for example, have been reported microscopically in *P. amarus*, *P. maderaspatensis*, *P. simplex*, and *P. urinaria*, although they were reported to be absent in *P. fraternus* in a comparative study (Sharma and Sheela, 2011). Starch-containing tissues have also been documented in *P. amarus* and *P. fraternus*, supporting the occurrence of starch as a common storage material in some *Phyllanthus* species (Sen et al., 2011). Similarly, tannins have been reported in *Phyllanthus* species (Balkrishna et al., 2025). The occurrence of these constituents in the present *P. niruri* material therefore agrees generally with observations reported for related *Phyllanthus* species.

The microchemical results should not, however, be interpreted as a comprehensive phytochemical profile. Microchemical analysis primarily demonstrates the presence or localization of selected constituents within plant tissues, whereas phytochemical profiling requires appropriate extraction and analytical procedures. The present findings are therefore best considered supportive evidence for the pharmacognostic characterization of the examined *P. niruri* leaf material.

Pharmacognostic Significance

The combined findings from epidermal microscopy, powder microscopy, quantitative microscopy, and microchemical analysis provide a preliminary pharmacognostic profile of the leaves of *Phyllanthus niruri*. The observed epidermal features, including anisocytic stomata, irregular and wavy epidermal cells, calcium oxalate crystals, and absence of observed trichomes, contribute to the microscopic characterization of the examined material. The powder-microscopic features provide additional diagnostic information that may be useful when the crude drug is encountered in powdered form. The quantitative observations, including stomatal number, stomatal index, and palisade ratio, may contribute to pharmacognostic characterization; however, the underlying raw observations were unavailable for independent verification, and individual measures of variability could therefore not be calculated. In addition, vein-islet and vein-termination numbers were not determined in the present study. Consequently, these quantitative findings should be regarded as preliminary observations rather than validated species-wide standards.

The positive microchemical reactions for cellulose, lignin, calcium oxalate, starch, and tannins provide supplementary evidence supporting the anatomical characterization of the examined leaf material. Overall, the findings may contribute to preliminary authentication and quality evaluation of *P. niruri*. Establishment of comprehensive pharmacognostic standards would require further studies using authenticated voucher specimens, adequately documented samples from multiple populations, retained raw quantitative

observations, appropriate measures of variability, and comparative material from related or potentially adulterating species.

Conclusion

The leaves of *Phyllanthus niruri* L. exhibited characteristic epidermal, powder-microscopic, quantitative, and microchemical features, including anisocytic stomata, wavy epidermal cells, calcium oxalate crystals, absence of observed trichomes, and characteristic powdered-leaf structures. The available stomatal number, stomatal index, and palisade ratio provide preliminary quantitative observations, while the microchemical findings demonstrate the presence of cellulose, lignin, calcium oxalate, starch, and tannins in the examined material. Collectively, these findings contribute preliminary pharmacognostic information that may support authentication and quality evaluation of *P. niruri* leaves. Because the original individual quantitative observations were unavailable for verification and variability estimation, the reported quantitative values should not be regarded as validated species-wide standards. Broader validation using authenticated voucher specimens, larger and geographically diverse samples, retained raw observations, and comparative material from related species is recommended.

Declaration

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Conflict of Interest: The authors declare no conflict of interest.

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