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Original Research Article

PHARMACOGNOSTIC STANDARDIZATION AND MOLECULAR AUTHENTICATION OF *Parquetina nigrescens* (AFZEL.) BULLOCK ROOTS

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ABSTRACT

Quality assurance of herbal medicines remains a critical challenge, particularly for plant materials sold in powdered form where traditional identification methods become ineffective. *Parquetina nigrescens* (Afzel.) Bullock (Apocynaceae) features prominently in African traditional healthcare systems, yet its root material lacks established quality control parameters. This investigation aimed to develop pharmacognostic and molecular markers for authenticating and standardizing powdered P. nigrescens roots. Authentication procedures encompassed powder microscopic examination, histochemical staining, physicochemical profiling, and phytochemical characterization. Chemical fingerprinting was accomplished through thin-layer chromatography (TLC), while molecular identification employed rbcL gene amplification and sequencing. Microscopic assessment revealed diagnostically valuable features including lignified fibres, parenchymatous cells, cork cells, and starch grains. Histochemical analysis confirmed the presence of cellulose, lignin, suberin, calcium oxalate crystals, mucilage, tannins, starch, aleurone grains, and inulin. Physicochemical constants (moisture: 4.53%; total ash: 32.20%; acid-insoluble ash: 2.50%) fell within acceptable ranges, though total ash showed notable variability. Phytochemical screening detected carbohydrate, flavonoids, tannins, saponins and steroids/triterpenes, with phenolic compounds being the most abundant (1.10 ± 0.02 mg GAE/g). TLC analysis produced reproducible characteristic bands (Rf 0.3–0.95). The rbcL amplicon yielded a 540 bp sequence (GenBank PV611177) demonstrating 98–100% homology with Apocynaceae members. These findings establish integrated quality control benchmarks for P. nigrescens roots, facilitating their safe utilization and reducing adulteration risks. The deposited rbcL sequence contributes to expanding DNA reference libraries for African medicinal flora.

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INTRODUCTION

The therapeutic importance of medicinal plants continues to grow, particularly in resource-limited settings where traditional remedies constitute primary healthcare options for significant portions of the population [1]. This increasing reliance on plant-based medicines, however, has intensified concerns regarding product quality, authenticity and safety. Adulteration and species misidentification represent persistent threats to herbal medicine integrity. These quality challenges pose problems when dealing with powdered plant materials, as the loss of recognizable morphological features renders visual identification virtually impossible. Establishing reliable pharmacognostic standards has therefore become valuable for verifying identity and ensuring consistent quality of herbal products. Such standardization typically incorporates microscopic evaluation, physicochemical testing, and phytochemical screening to establish diagnostic parameters [2,3]. Microscopic powder analysis evaluation offers characteristic markers including

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fibres, vessels, starch grains, and calcium oxalate crystals that facilitate species identification and adulterant detection. Physicochemical indices such as ash and extractive values provide additional benchmarks for assessing purity [4]. Chromatographic techniques, particularly TLC, generate chemical fingerprints that enable batch-to-batch consistency verification. More recently, DNA barcoding has emerged as a powerful molecular authentication tool, especially valuable for processed or powdered materials where morphological identification is precluded. The *rbcL* region has demonstrated particular utility as a standard barcode for plant identification and species discrimination [5,6]. A perennial climber from the Apocynaceae family, *Parquetina nigrescens* is widely used across African traditional medicine systems. Its roots are especially prized therapeutically and are typically consumed as a fine powder. Yet, despite this widespread ethnobotanical importance, there's a notable gap in the pharmacognostic and molecular data needed to ensure the quality control of its powdered root form. To address this, our study aimed to develop a set of integrated standards for authenticating powdered *P. nigrescens* roots. We approached this by combining microscopic evaluation, physicochemical tests, phytochemical screening, TLC profiling, and DNA barcoding.

MATERIALS AND METHODS

Plant Collection and Authentication

Fresh root samples were collected in March 2023 from the Birnin Gwari Local Government Area in Kaduna State, Nigeria. For taxonomic identification, we consulted Mr. Namadi Sanusi at the Herbarium Unit of Ahmadu Bello University's Botany Department in Zaria. A voucher specimen, numbered ABUH03431, was subsequently deposited there for future reference.

Sample Preparation

Back in the lab, we started by washing the collected roots thoroughly under running tap water. They were then shade-dried at room temperature—hovering around 25 to 30°C—for a full two weeks. Once completely dry, the roots were ground into a fine powder using a mechanical grinder. This powdered material was kept in airtight containers at ambient temperature until we were ready to run our analyses.

Powder Microscopy

Microscopic examination of the powdered root followed established protocols [7]. A small quantity powdered samples were cleared with chloral hydrate and mounted on glass slides using glycerin. Observations were performed under an Olympus CX21 light microscope (Tokyo, Japan) at ×400 magnification. Digital images were captured, and scale bars (50 µm) were incorporated into photomicrographs.

Histochemical Analysis

Cell wall and cell content characterization employed standard histochemical staining procedures according to WHO guidelines [8]. Reagents included phloroglucinol-HCl for lignin detection, iodine solution for starch, zinc chloride-iodine for cellulose, Sudan III for suberin/cutin, ferric chloride for tannins, ruthenium red for mucilage, Million's reagent for aleurone grains, and acetic acid for calcium oxalate crystals [9].

Physicochemical Determinations

Water-soluble ash, total ash, acid-insoluble ash, alcohol-soluble extractive, water-soluble extractive, and moisture content (loss on drying) were determined in triplicate following WHO protocols [8].

Moisture content: Approximately 3 g of powder was heated in a pre-dried crucible at 105°C for an hour, cooled in a desiccator, and weighed until a constant weight was obtained. Moisture percentage was calculated as

$$Moisture\ content = \frac{initial\ weight\ -\ dry\ weight}{initial\ weight} \times 100$$

Total ash: Two grams of air-dried powder were ignited at 600°C until white ash formation.

$$Total\ ash\ percentage\ = \frac{ash\ weight}{sample\ weight} \times 100$$

Acid-insoluble ash: The total ash was boiled with 25 mL dilute HCl (10% v/v) for 5 minutes. The insoluble residue was then filtered, washed, ignited, and weighed.

$$\text{Acid} - \text{insoluble ash percentage} = \frac{acid-insoluble\ ash\ weight}{sample\ weight} \times 100$$

Water-soluble ash: The total ash was boiled with 25 mL distilled water for about 5 minutes, filtered, and the insoluble residue ignited.

$$Water - soluble\ ash = \frac{total\ ash\ weight - acid{-}insoluble\ ash\ weight}{sample\ weight}\ X\ 100$$

Alcohol-soluble extractive: Five grams of powdered plant material was macerated with 100 mL of 70% ethanol for 6 hours with intermittent shaking, then allowed to stand for 18 hours. After filtration, 20 mL of filtrate was taken evaporated to dryness on a water bath and weighed.

$$Extracive\ percentage = \frac{residue\ weight}{sample\ weight} \times 100$$

Water-soluble extractive: The procedure described for **Alcohol-soluble extractive** was repeated using distilled water instead of ethanol.

Extract Preparation

Powdered roots (500 g) underwent cold maceration with 2.5 L of 70% ethanol for 72 hours at room temperature with occasional agitation. It was then filtered through muslin cloth, and the residue re-extracted four times until the extract became colourless. The combined filtrates were concentrated under reduced pressure using a rotavapour at 40°C and dried in a desiccator.

Qualitative Phytochemical Screening

The ethanol root extract was screened for the presence of carbohydrates, saponins, tannins, flavonoids, steroids/triterpenes, cardiac glycosides, anthraquinones, cyanogenic glycosides, and alkaloids using standard protocols [2,9,11,12].

Quantitative Phytochemical Screening

Total phenolic content (TPC): Determined by Folin-Ciocalteu method using gallic acid standard [13]. Absorbance was taken at 765 nm, with results expressed as mg gram gallic acid equivalent per gram extract (mg GAE/g).

Total flavonoid content (TFC): Assessed via aluminium chloride colorimetric method using quercetin standard [14]. Absorbance was taken at 510 nm, with results expressed as mg quercetin equivalent per gram (mg QE/g).

Tannin content: Quantified by Folin-Ciocalteu method [15] with gallic acid standard, taking absorbance at 725 nm. Results expressed as mg GAE/g.

Saponin content: Determined using vanillin-sulphuric acid method with diosgenin standard [16]. Absorbance was taken at 544 nm, results expressed as mg diosgenin equivalent per gram (mg DE/g).

Thin-Layer Chromatography (TLC)

TLC was performed on silica gel 60 F_{254} plates (Merck). The ethanol root extract was applied as spots and developed in ethyl acetate : methanol (9:1). After development, the TLC plates were observed under UV light (254 and 366 nm) and sprayed with various detection reagents: p-anisaldehyde-sulphuric acid (general detection), ferric chloride (phenolics), Liebermann-Burchard reagent (steroids/terpenoids), Bontrager's reagent (anthraquinones), aluminium chloride (flavonoids), and Dragendorff's reagent (alkaloids). R_f values were calculated [17].

DNA Barcoding

DNA extraction: Total genomic DNA was isolated from 200 mg of powdered root using the EasyPrep™ Genomic DNA Miniprep Kit (Bioland Scientific, USA) following manufacturer's instructions [18].

PCR amplification: The *rbcL* gene was amplified using universal primers:
rbcLa-F: 5'-ATGTCACCACAAACAGAGACTAAAGC-3'
rbcLa-R: 5'-GTAAAATCAAGTCCACCRCG-3'

PCR reactions (25 µL) contained: 1× PCR buffer, 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.4 µM each primer, 1 U Taq DNA polymerase, and 50 ng template DNA. The PCR conditions comprised initial denaturation at 94°C for 5 minutes; 35 cycles of 94°C for 30 seconds, 54°C for 30 seconds, 72°C for 1 minute; final extension at

72°C for 5 minutes. Negative controls (no template) were included.

Gel electrophoresis: PCR products (5 µL) were separated on 1.5% agarose gel stained with ethidium bromide, visualized under UV light, and photographed using a 100 bp DNA ladder as size marker.

Sequencing and phylogenetic analysis: Amplicons were purified and bidirectionally sequenced (ABI Prism 310 Genetic Analyzer). The sequences obtained were analyzed using BLASTn against NCBI GenBank and phylogenetic trees constructed using Neighbor-Joining method with 1000 bootstrap replicates.

RESULTS

Powder Microscopy

Microscopic examination revealed characteristic tissue elements including parenchymatous cells, cork cells, simple round to oval starch grains, elongated fibres, and thick-walled lignified fibres (Figure 1, Figure 2).

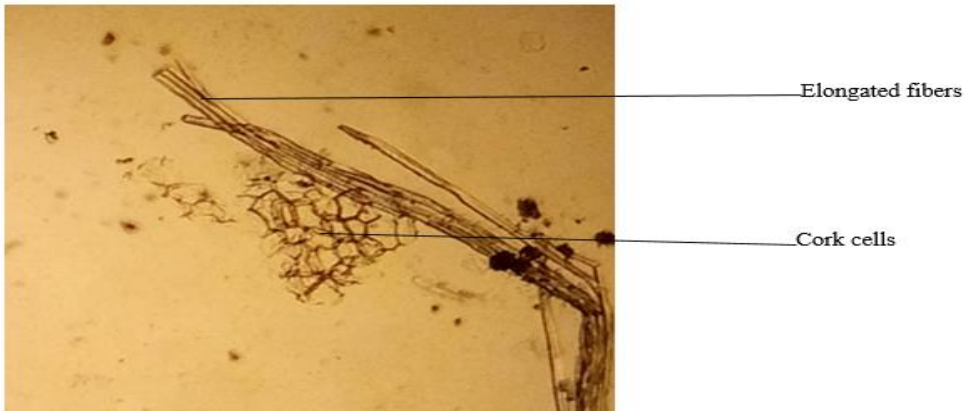


Figure 1: Photomicrograph of *P. nigrescens* root powder showing cork cells and elongated fibres (×400).

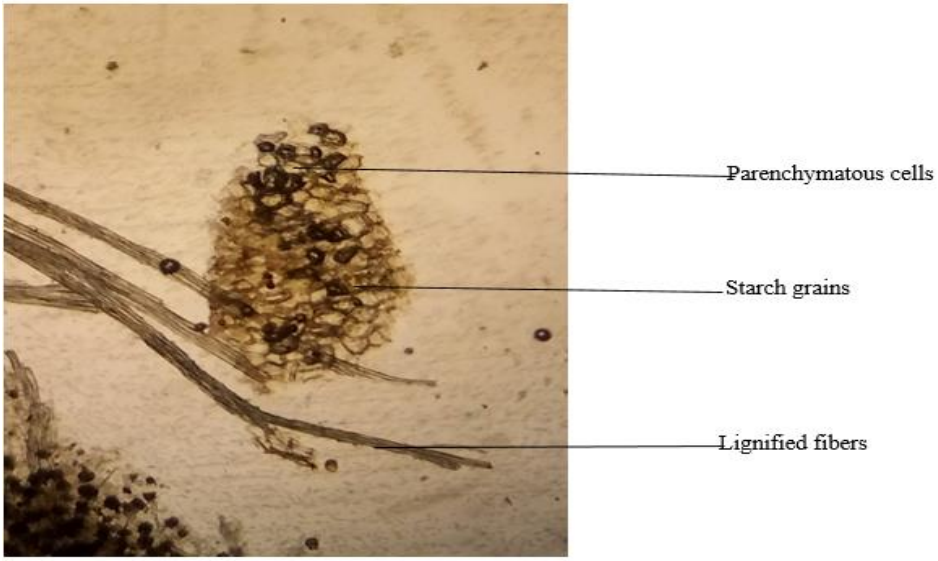


Figure 2: Photomicrograph showing lignified fibres, starch grains, and Parenchymatous cells (×400).

Histochemical Findings

Staining reactions confirmed the presence of cellulose, lignin, suberin/cutin, gum/mucilage, calcium oxalate crystals, starch, tannins, aleurone grains, and inulin. Calcium carbonate was notably absent (Table 1).

Physicochemical Parameters

Physicochemical evaluation results are summarized in Table 2. Moisture content (4.53%) was substantially below the WHO recommended limit (<14%). Total ash value (32.20%) exhibited considerable variability between replicates (SD ≈ 1.50), potentially indicating mineral heterogeneity or contamination. Acid-insoluble ash (2.50%) was low, suggesting minimal siliceous contamination.

Table 1: Chemomicroscopic (histochemical) features of *Parquetina nigrescens* root powder

Constituents Tested	Observation	Inference
Cell wall Material		
Cellulose	Blue to blue-violet	Present
Lignin	Pink stained	Present
Suberin/Cutin	Orange red	Present
Cell Inclusion		
Gum and Mucilage	Transparent, spherical dilated fragment on a black background	Present
Calcium Carbonate	No effervescence	Absent
Calcium Oxalate Crystal	Dissolution of shiny Crystal without effervescence	Present
Starch Grains	Reddish blue colour	Present
Tannins	Greenish black	Present
Aleurone Grains	Yellowish brown	Present
Inulin	Spherical aggregation of crystals inulin turned brownish red and dissolved	Present

Table 2: Physicochemical parameters of *P. nigrescens* root powder

Parameter	Value obtained (%w/w) ± SEM
Moisture content	4.53 ± 0.25
Total ash value	32.20 ± 1.50
Water soluble ash	18.00 ± 0.00
Acid insoluble ash	2.50 ± 1.12
Alcohol extractive value	0.27 ± 0.17
Water extractive value	4.00 ± 3.26

Key: SEM = Standard Error of Mean, n = 3

Extraction Yield

Ethanol (70%) extraction of *P. nigrescens* roots yielded 8.4% (w/w) dark brown residue.

Phytochemical Constituents

Qualitative screening detected carbohydrates, saponins, tannins, flavonoids, steroids/triterpenes, cardiac glycosides, and anthraquinones in the ethanol root extract. Alkaloids and cyanogenic glycosides were absent (Table 3).

Quantitative Phytochemical Results

Quantitative analysis (Table 4) revealed total phenolics as the most abundant class (1.10 ± 0.02 mg GAE/g), followed by tannins (0.94 ± 0.36 mg GAE/g), saponins (0.85 ± 0.22 mg DE/g), and flavonoids (0.63 ± 0.04 mg QE/g).

TLC Profiling

TLC of the ethanol root extract developed in ethyl acetate : methanol (9:1) produced characteristic bands with Rf values as detailed in Table 5, establishing a reproducible chemical fingerprint.

Table 3: Qualitative phytochemical constituents of *P. nigrescens* ethanol root extract

Constituent	Inference
Carbohydrates	Present
Saponins	Present
Anthraquinones	Present
Cardiac glycosides	Present
Cyanogenic glycosides	Absent
Flavonoids	Present
Tannins	Present
Steroids/Triterpenes	Present
Alkaloids	Absent

Table 4: Quantified secondary metabolites in *P. nigrescens* ethanol root extract

Secondary metabolite	Quantity (mg/g extract) ± SD (n=3)
Total phenolics	1.10 ± 0.02 (GAE)
Tannins	0.94 ± 0.36 (GAE)
Saponins	0.85 ± 0.22 (DE)
Flavonoids	0.63 ± 0.04 (QE)

GAE = gallic acid equivalent; DE = diosgenin equivalent; QE = quercetin equivalent.

Table 5: TLC profile of *P. nigrescens* ethanol root extract

Detecting reagent	Solvent system	No. of spots	Colour of spots	R_f values	Inference
p-Anisaldehyde-H_2SO_4	EA : M (9:1)	6	Dark green, dark green, dark green, pink, pink, orange	0.30, 0.40, 0.64, 0.83, 0.86, 0.95	General detection
Ferric chloride	EA : M (9:1)	4	Black, black, black, black	0.60, 0.74, 0.82, 0.91	Phenolics present
Liebermann–Burchard	EA : M (9:1)	6	Pink, dark green, green, pink, pink, pink	0.30, 0.40, 0.60, 0.72, 0.81, 0.88	Steroids/terpenoids present

Detecting reagent	Solvent system	No. of spots	Colour of spots	R_f values	Inference
Bontrager's	EA : M (9:1)	1	Yellow	0.89	Anthraquinones present
Aluminium chloride	EA : M (9:1)	1	Yellow (fluorescent)	0.94	Flavonoids present
Dragendorff's	EA : M (9:1)	No spots	–	–	Alkaloids absent

EA = ethyl acetate; M = methanol.

Molecular Authentication

Successful genomic DNA extraction from *P. nigrescens* roots enabled PCR amplification of the *rbcL* gene, producing a single band of approximately 540 bp (Figure 3). Negative controls showed no amplification. Sequencing generated a high-quality 540 bp fragment (GenBank accession PV611177). The chromatogram displayed clear, distinct peaks (Figure 4), with the nucleotide sequence presented in Table 6. BLASTn analysis against NCBI GenBank revealed 98–100% sequence similarity with Apocynaceae members (Table 7).

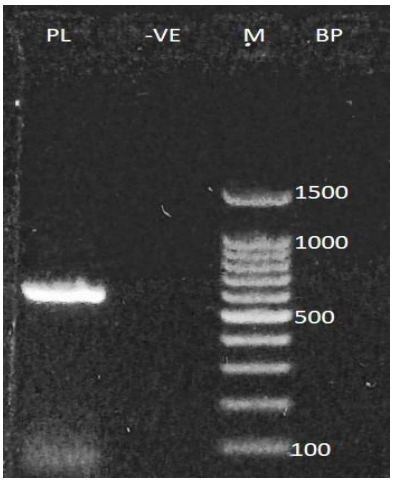


Figure 3: Agarose gel electrophoresis of *rbcL* PCR products. Lane M: 100 bp DNA ladder; Lane PL: *P. nigrescens* (540 bp); Lane -VE: negative control.



Figure 4: Chromatogram of *rbcL* sequence (ABI Prism 310).

Table 6: *rbcL* nucleotide sequence (540 bp) of *P. nigrescens*

AGATAAATTGAACAAATATGGTCGTCCCCTGTTGGGATGTACTATTAAACCAAAATTGGGGTTATCAGCTAAAAAC TACGGTAGGGCGGT
TTATGAATGTCTTCGTGGTGGACTTGATTTTACCAAAGATGATGAAAACGTGAACTCCCAACCGTTTATGCGTTG GAGAGATCGTTTCTT
GTTTTGTGCCGAAGCTCTTTTTAAAGCACAGGCTGAAACCGGCGAAATCAAAGGGCATTACTTGAATGCTACTGC AGGTACATGCGAAGA
AATGTACAAAAGAGCTATATTTGCTAGAGAATTGGGAGCTCCTATCGTAATGCATGACTACTTAACAGGGGGATT CACTGCAAATACGAG
CTTGGCTCATTATTGCCGAGATAATGGCCTACTTCTTCACATCCACCGTGCAATGCACGCAGTTATTGATAGACA GAAGAATCATGGTAT
GCACTTCCGCGTACTAGCTAAAGCGTTACGTATGTCTGGTGGAGATCATATTCACGCGGGTACCGTAGTAGGTA AACTTGAAGGGGAAAG

(A = adenine, C = cytosine, G = guanine, T = thymine).

Table 7: Top BLASTn results for *rbcL* sequence of *P. nigrescens*

S/N	Plant name	Family	Similarity (%)	Accession number
1	*Toxocarpus wightianus*	Apocynaceae	100.00	EU196292.1
2	*Cryptolepis sinensis*	Apocynaceae	100.00	OP326279.1
3	*Streptocaulon juventas*	Apocynaceae	99.81	KX527180.1
4	*Pentopetia* spp.	Apocynaceae	99.07	AJ419755.1
5	*Hemidesmus indicus*	Apocynaceae	98.89	EU196277.1

Phylogenetic Placement

The Neighbor-Joining phylogenetic tree (Figure 2) positioned *P. nigrescens* within a well-supported clade containing other Apocynaceae members, clustering most closely with *Toxocarpus wightianus* and *Cryptolepis sinensis* (bootstrap value 98%).

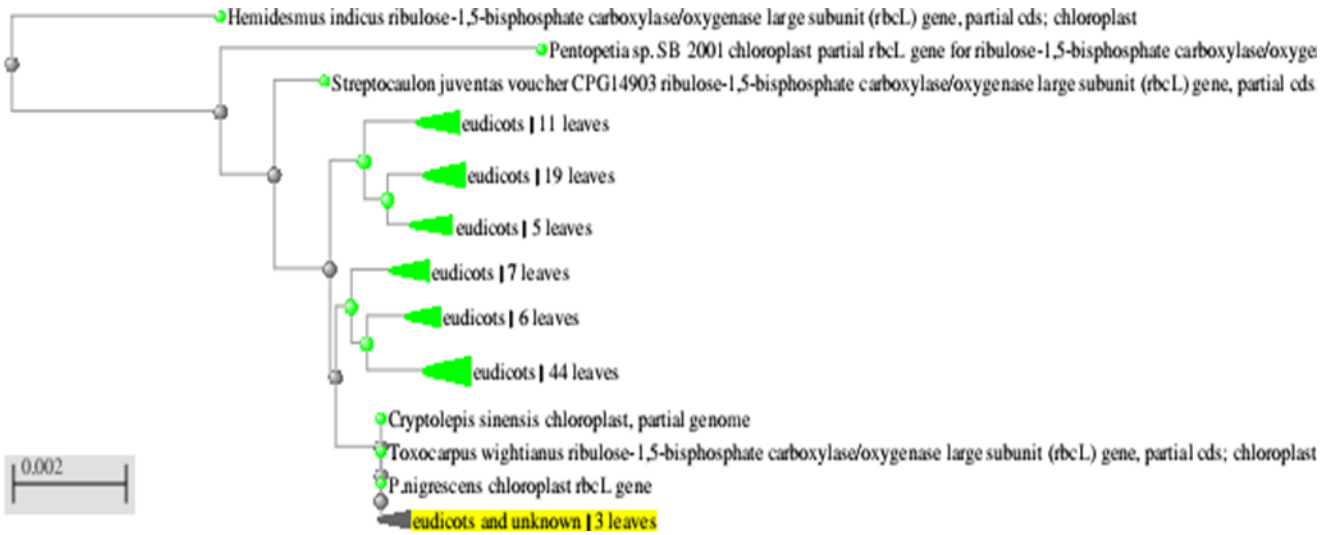


Figure 5: Phylogenetic tree based on *rbcL* sequences showing relationships of *Parquetina nigrescens* within Apocynaceae (Neighbor-Joining method, 1000 bootstrap replicates).

DISCUSSION

This study was able to established a pharmacognostic and molecular framework for *Parquetina nigrescens* root standardization by integrating multiple analytical approaches. Such systematic evaluation is essential for reliable identification, authentication, and quality control for the safe and effective use of this plant in traditional medicine.

Microscopic and histochemical assessment of the powdered plant revealed some anatomical features which could be diagnostic such parenchymatous cells, cork cells, starch grains, lignified fibres, and various cell inclusions (cellulose, lignin, suberin, calcium oxalate crystals, mucilage, tannins, starch, aleurone grains, and inulin). These characters can serve as markers for adulterant detection and align with established pharmacognostic principles emphasizing microscopy's fundamental role in herbal authentication [8]. The presence of lignified fibres and calcium oxalate crystals is consistent with Apocynaceae family root anatomy which facilitates species identification [20].

Physicochemical parameters provided are important in determining quality of the plant material. Moisture content (4.53%) was below the WHO limit of 14%, indicating minimal risk of microbial contamination and enzymatic degradation during storage [8]. While total ash (32.20%) was notably elevated, the low acid-insoluble ash (2.50%) suggested minimal silica/sand contamination. This high total ash likely reflects the plant's natural mineral composition or edaphic factors in the collection area. Water-soluble ash (18.00%) indicated substantial water-soluble inorganic constituents. The higher water extractive value (4.00%) compared to alcohol extractive (0.27%) suggested predominance of polar constituents, consistent with the presence of phenolic compounds and saponins. These parameters establish valuable benchmarks for purity assessment and adulteration detection [21].

Phytochemical analysis confirmed the presence of tannins, saponins, flavonoids, steroids, triterpenes, and cardiac glycosides, while alkaloids and cyanogenic glycosides were absent. This profile corroborates previous reports on *P. nigrescens* [22], supporting reproducibility. Quantitative analysis revealed phenolics as the most abundant class (1.10 mg GAE/g), followed by tannins (0.94 mg GAE/g), saponins (0.85 mg DE/g), and flavonoids (0.63 mg QE/g). High Phenolic content suggests significant antioxidant potential, potentially contributing to therapeutic applications in oxidative stress-related conditions [23]. Flavonoids, recognized for their anti-inflammatory and neuroprotective activities, may support traditional use in neurological disorders [24]. Tannins and saponins, associated with antimicrobial,

immunomodulatory, and anti-inflammatory effects, provide phyochemical rationale for the ethnomedicinal relevance of *P. nigrescens* [25,26,27].

TLC fingerprinting generated distinct, reproducible spots with characteristic R_f values, establishing a practical chemical profile for routine quality assessment. Such fingerprints are valuable for identity verification, adulterant detection, and batch-to-batch consistency monitoring [28,29].

Molecular authentication using the *rbcL* gene successfully validated *P. nigrescens* identity and confirmed its phylogenetic placement within Apocynaceae. The 540 bp amplicon size was consistent with the expected size for angiosperm fragment length. High sequence similarity (98–100%) with related taxa, particularly *Toxocarpus wightianus* and *Cryptolepis sinensis*, demonstrated this barcode's reliability for family-level identification. The phylogenetic tree positioned *P. nigrescens* within a well-supported clade (bootstrap 98%) with these species, consistent with morphological and phytochemical parallels. Despite being a conserved locus, *rbcL* demonstrated sufficient discriminatory power in this context, making it a useful marker for routine authentication. Nevertheless, incorporating additional loci such as *matK* or ITS is recommended for enhanced species-level resolution and genetic diversity studies [30,31,32]. The deposition of this sequence (PV611177) contributes to expanding DNA barcode databases for African medicinal plants.

Collectively, these findings demonstrate that integrating pharmacognostic, phytochemical, and molecular approaches provide a robust framework for the reliable identification and quality control of *P. nigrescens* roots, while establishing a valuable reference for future pharmacognostic, taxonomic, and drug development research.

CONCLUSION

This study has established a reliable quality control method to authenticate *Parquetina nigrescens* roots, ensuring purity and safety while preventing adulteration. Furthermore, the phytochemical quantification of active compounds like phenolics, tannins, saponins, and flavonoids provides scientific backing for the plant's traditional medicinal uses.

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Author SAA was responsible was conceptual framework and research design. Author UFS was responsible for data analysis and drafting of the manuscript while author KYF proof read the manuscript and also assisted in research design.

AUTHORS' CONTRIBUTION

Author SAA was responsible was conceptual framework and research design. Author UFS was responsible for data analysis and drafting of the manuscript while author KYF proof read the manuscript and also assisted in research design.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

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