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PHARMACOGNOSTIC AND PHYSICOCHEMICAL STANDARDIZATION OF COLA CARICIFOLIA (G.DON) K.SCHUM. LEAF AND STEM BARK

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ABSTRACT

Background: *Cola caricifolia* (G.Don) K.Schum. (Malvaceae) is a West African medicinal plant with documented ethnomedicinal uses, yet no pharmacognostic or physicochemical standards have been established for its leaf and stem bark, particularly from Nigerian accessions. **Methods:** Macroscopic and microscopic examinations were conducted using standard pharmacognostic protocols recommended by the World Health Organization. Physicochemical parameters including moisture content, total ash, acid-insoluble ash, water-soluble ash, sulphated ash, and alcohol- and water-soluble extractive values were determined. Qualitative and quantitative phytochemical analyses were performed on ethanolic extracts of both plant parts. **Results:** The leaf exhibited palmately lobed morphology with paracytic stomata, covering trichomes, glandular trichomes, spiral xylem vessels, palisade parenchyma, and rosette calcium oxalate crystals. The stem bark displayed peridermal architecture characterised by radially arranged cork cells, sclereids, stone cells, phloem parenchyma, and rosette calcium oxalate crystals. Physicochemical values for moisture content (leaves: 6.85 ± 0.21%; bark: 8.14 ± 0.18%), total ash (leaves: 8.91 ± 0.24%; bark: 10.63 ± 0.30%), and extractive values were within acceptable pharmacopoeial limits. Phytochemical screening revealed eight classes of secondary metabolites in the leaf and seven in the bark (saponins absent from bark), with differential quantitative distribution: leaves contained higher alkaloids, phenolics, flavonoids, and glycosides, while bark contained higher tannins, terpenoids, and steroids. **Conclusion:** This study provides the first comprehensive pharmacognostic monograph for *Cola caricifolia* leaf and stem bark, establishing baseline standards for botanical authentication, quality control, and inclusion in herbal pharmacopoeias.

KEYWORDS: Cola caricifolia; pharmacognosy; physicochemical standardization; phytochemistry; stem bark; Malvaceae.

1. INTRODUCTION

Medicinal plants remain a vital resource in global healthcare, particularly across sub-Saharan Africa where up to 80–90% of the population in rural communities relies on plant-based therapies as primary healthcare (World Health Organization, 2023). Among the strategies for integrating traditional herbal medicine into evidence-based practice, pharmacognostic standardization occupies a foundational role. The World Health Organization (2011) defines pharmacognostic monographs as essential tools for herbal drug authentication, quality control, and prevention of adulteration. Without standardized morphological and physicochemical benchmarks, the reproducibility and safety of herbal preparations cannot be guaranteed.

The genus *Cola* Schott and Endl. (Malvaceae, subfamily Sterculioideae), comprising approximately 125 species

indigenous to tropical African rainforests, is economically and culturally significant across West and Central Africa (Mbembo *et al.*, 2021). While *Cola nitida* and *Cola acuminata* are globally recognized for their caffeine-rich seeds, the lesser-known species *Cola caricifolia* (G.Don) K.Schum. — commonly referred to as monkey cola or fig-leaved kola — has received comparatively little scientific scrutiny despite widespread ethnomedicinal use across Côte d'Ivoire, Ghana, Nigeria, Cameroon, and Gabon (Sut *et al.*, 2020; Ekalu and Habila, 2020).

Ethnobotanical surveys document the use of *C. caricifolia* leaf decoctions for fever and malaria, bark infusions as analgesics and febrifuges, and root preparations for respiratory conditions (Evbuomwan *et al.*, 2023; Ajao *et al.*, 2022). Despite this traditional currency, only one peer-reviewed phytochemical study exists on its leaf chemistry — conducted on Ivorian material by Sut *et al.*, (2020) — and no published data exist on the stem bark. Furthermore, there are no pharmacognostic standards for any part of this plant, nor any data on Nigerian accessions, which may exhibit chemotypic differences due to soil, altitude, and climatic variation (Umoh *et al.*, 2022).

The absence of pharmacognostic standards for *Cola caricifolia* represents a critical gap. Standardization data, including macroscopic and microscopic characters, physicochemical parameters, and phytochemical profiles are prerequisites for reliable quality control, formulation of pharmacopoeial monographs, and cross-study comparability. This study therefore aimed to establish, for the first time, comprehensive pharmacognostic and physicochemical standards for the leaf and stem bark of *Cola caricifolia* from a Nigerian accession, and to determine the qualitative and quantitative phytochemical composition of ethanolic extracts from both plant parts.

2. MATERIALS AND METHODS

Ethical approval for the use of experimental animals in associated studies was obtained from the Ethics Committee for Animal Studies, Nnamdi Azikiwe University (NAU), Awka, Nigeria, in accordance with NAUREC policy.

2.1 Plant Collection and Authentication

Fresh samples of *Cola caricifolia* leaves and stem bark were collected from a local farm in Awka Community, Anambra State, Nigeria. Botanical authentication was performed at the Department of Pharmacognosy, Nnamdi Azikiwe University. Voucher specimens were deposited in the university herbarium. Fresh materials were air-dried under shade at room temperature, pulverized using a mechanical grinder, and stored in airtight containers until use.

2.2 Macroscopic Examination

Macroscopic examination of the leaf and stem bark was conducted using standard morphological protocols as recommended by the World Health Organization (2011) and Evans (2009). Physical attributes were observed with the naked eye and a hand lens. Dimensions were measured using a metric ruler and digital Vernier caliper. Parameters evaluated for the leaf included shape, apex, base, margin, venation, surface texture, and colour. For the stem bark, colour (outer and inner surfaces), odour, taste, texture, and exudate were assessed.

2.3 Microscopic Examination

Fresh leaf and powdered leaf microscopy were performed using a compound microscope (Olympus XSZ-107BN) at ×10 and ×40 magnifications following standard methods. Fresh leaf transverse sections were prepared with a sharp razor blade, mounted in glycerin, and stained with safranin O to reveal anatomical features including the epidermis, stomatal type, trichomes, palisade and spongy mesophyll, and vascular bundles. Powdered leaf was cleared with chloral hydrate, mounted in glycerin, and examined for epidermal fragments, trichomes, fibers, sclereids, calcium oxalate crystals, xylem vessels, and starch grains. Stem bark peridermal microscopy was conducted on powdered bark mounted with phloroglucinol-HCl (for lignified tissues) and iodine solution (for starch), enabling identification of cork cells, phloem parenchyma, sclereids, stone cells, fibers, and calcium oxalate crystals.

2.4 Physicochemical Analyses

All physicochemical determinations were performed in triplicate and expressed as percentage weight/weight (% w/w) mean ± SD. Moisture content (loss on drying) was determined by drying 3 g of powdered sample at 100–105°C to constant weight. Total ash, acid-insoluble ash, water-soluble ash, and sulphated ash were determined according to methods described by Evans (2009) and the World Health Organization (2011), using a muffle furnace at 450–800°C. Alcohol-soluble and water-soluble extractive values were determined by maceration of 5 g of powdered sample in 100 mL of 90% ethanol or distilled water respectively, followed by filtration, evaporation, and gravimetric quantification.

2.5 Phytochemical Screening

Qualitative phytochemical screening of the ethanolic leaf and stem bark extracts was carried out using standard methods described by Trease and Evans (2009) and Sofowora (2008) to detect alkaloids, tannins, phenolics, flavonoids, cardiac glycosides, terpenoids, saponins, and steroids. Quantitative phytochemical analyses were performed using established gravimetric and spectrophotometric methods: alkaloids by Harborne (1973), tannins by Van-Burden and Robinson (1981), phenolics by Singleton and Rossi (1965), flavonoids by the aluminium chloride colorimetric method, glycosides by Harborne (1973), terpenoids by Harborne (1973), saponins by Obadoni and Ochuko (2001), and steroids by Harborne (1973).

3. RESULTS

3.1 Macroscopic Characteristics

The macroscopic features of the *Cola caricifolia* leaf are summarized in Table 1. The primary defining characteristic of the leaf is its palmately lobed shape, deeply divided into 5–7 lobes with a corresponding palmate venation pattern. The leaf base is cordate, the apex acute to acuminate, and the margin entire to undulate along the lobes. The adaxial surface is smooth and bright green while the abaxial surface is slightly pubescent and paler in colour. The petiole is long and slender. Leaf texture is thin to slightly coriaceous.

Table 1: Morphological Characteristics of Cola caricifolia Leaf.

Feature	Description
Leaf Arrangement	Alternate
Leaf Type	Simple
Leaf Shape	Palmately lobed (deeply divided into 5–7 lobes, resembling a hand)
Leaf Apex	Acute to acuminate
Leaf Base	Cordate to truncate
Leaf Margin	Entire to undulate along the lobes
Venation	Palmate – veins radiate from a central point at the leaf base
Leaf Surface	Smooth (glabrous) upper surface; slight pubescence beneath
Leaf Texture	Thin, papery to slightly coriaceous
Petiole	Long and slender
Colour	Bright green adaxially; paler green abaxially

The organoleptic characteristics of the stem bark are presented in Table 2. The outer surface is brownish-grey while the inner surface reveals a reddish to orange-brown hue upon drying. Upon incision, the fresh bark oozes a reddish-brown sticky sap. The odour is characteristically woody with a faint spicy note, and the taste is bitter and mildly astringent. The dried bark texture is coarse, fibrous, and brittle.

Table 2: Organoleptic Characteristics of Cola caricifolia Stem Bark.

Parameter	Observation
Colour	Outer surface: brownish-grey; inner surface: reddish to orange-brown (when dried)
Odour	Characteristically woody with a faint spicy aroma
Taste	Bitter and mildly astringent
Texture	Coarse, fibrous, and brittle
Exudate	Oozes a reddish-brown, sticky sap upon incision

3.2 Microscopic Characteristics

3.2.1 Leaf Microscopy

Transverse section of the *Cola caricifolia* leaf (Fig. 1) revealed a dorsiventral organization bounded by a single-layered epidermis on both the adaxial and abaxial surfaces. The epidermis bore two distinct types of trichomes: covering trichomes (simple, non-secretory, unicellular to multicellular) and secretory glandular trichomes, both projecting from the epidermal surface. Paracytic stomata is characterized by two subsidiary cells aligned parallel to the guard cells, these were observed on the lower epidermis. Beneath the adaxial epidermis, a layer of vertically elongated, densely packed palisade parenchyma cells was present, responsible for the primary photosynthetic function. The mesophyll also contained loosely arranged spongy parenchyma with intercellular spaces facilitating gaseous exchange. Vascular bundles were collateral, containing xylem (with spiral/annular thickening) and phloem, embedded within the ground tissue. Calcium oxalate crystals of the rosette (druse) type were observed in the mesophyll parenchyma.

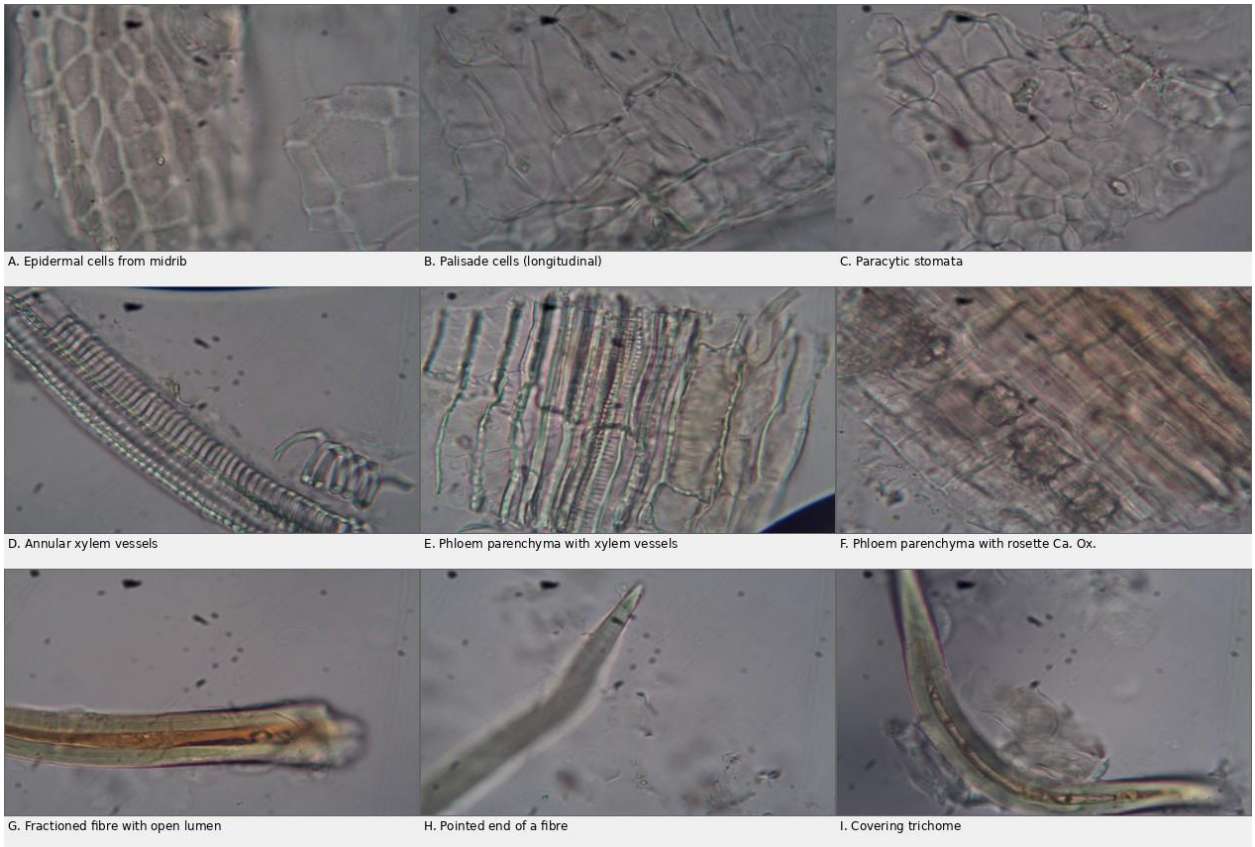

Fig. 1: Diagnostic microscopic features of Cola caricifolia leaf powder (x400). A: Epidermal cells from midrib; B: Longitudinal palisade cells; C: Paracytic stomata; D: Annular xylem vessels; E: Phloem parenchyma with xylem vessels; F: Phloem parenchyma with rosette calcium oxalate crystals; G: Fractioned fibre with open lumen; H: Pointed end of fibre; I: Covering trichome.

Powdered leaf microscopy confirmed the presence of epidermal cells with sinuous walls, paracytic stomata, covering trichomes with pointed ends, annular xylem vessels, phloem parenchyma cells associated with rosette calcium oxalate crystals, fibres with open lumen, and fragmented fibres. These diagnostic features collectively characterize the leaf powder of *Cola caricifolia* and serve as key identifiers in microscopic authentication.

3.2.2 Stem Bark (Peridermal) Microscopy

Peridermal microscopy of the *Cola caricifolia* stem bark (Fig. 2) demonstrated a well-developed periderm comprising cork cells arranged in radial rows. The cork cells were polygonal to rectangular in shape with suberized walls, forming a compact, continuous layer without intercellular spaces. Associated diagnostic elements included fragmented cork cells with rosette calcium oxalate crystals, bundles of phloem parenchyma cells, pairs of elongated fibres, thick fibres with visible lumen adjacent to calcium oxalate crystals, small stone cells, pairs of sclereids, fractured fibres associated with calcium oxalate crystals, and pairs of obliterated stone cells. The presence of rosette calcium oxalate crystals throughout both cork and phloem tissues is a distinctive feature of the bark powder.

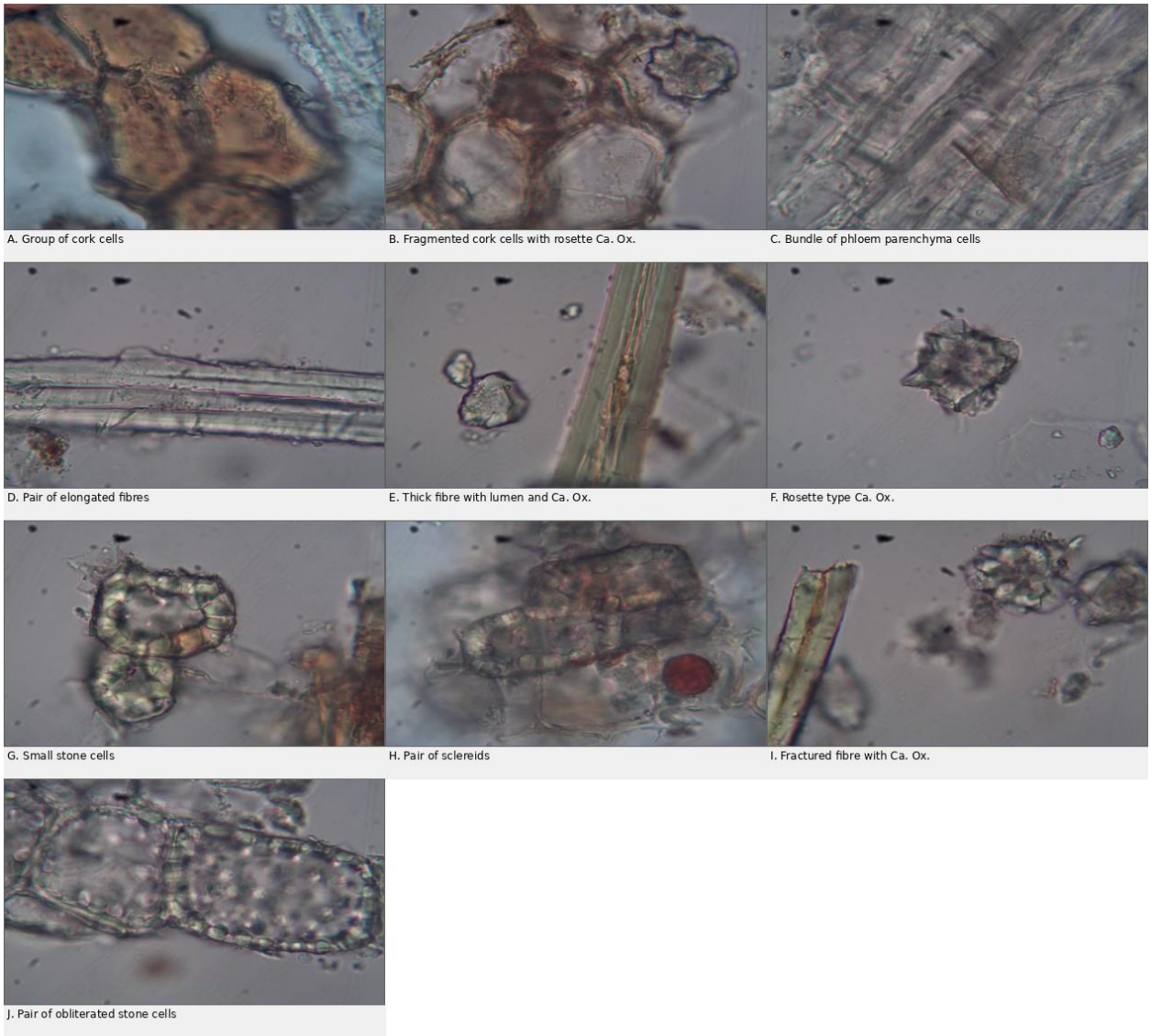

Fig. 2: Diagnostic microscopic features of Cola caricifolia stem bark powder (x400). A: Group of cork cells; B: Fragmented cork cells with rosette calcium oxalate; C: Bundle of phloem parenchyma cells; D: Pair of elongated fibres; E: Thick fibre with lumen and calcium oxalate; F: Rosette-type calcium oxalate crystals; G: Small stone cells; H: Pair of sclereids; I: Fractured fibre with calcium oxalate; J: Pair of obliterated stone cells.

3.3 Physicochemical Analysis

The physicochemical parameters of *Cola caricifolia* leaf and stem bark are presented in Table 3. Moisture content was lower in the leaf (6.85 ± 0.21%) compared to the stem bark (8.14 ± 0.18%), with both values within the WHO-recommended limit of ≤10% for dried herbal materials. Total ash was higher in the stem bark (10.63 ± 0.30%) than in the leaf (8.91 ± 0.24%), reflecting the greater inorganic mineral load characteristic of bark tissues. Acid-insoluble ash (leaf: 0.98 ± 0.07%; bark: 1.83 ± 0.10%) and water-soluble ash (leaf: 4.97 ± 0.14%; bark: 5.34 ± 0.12%) were both higher in the bark. Sulphated ash was also higher in the bark (11.22 ± 0.28%) than in the leaf (9.38 ± 0.19%). In contrast, both alcohol-soluble (leaf: 8.42 ± 0.20%; bark: 2.77 ± 0.25%) and water-soluble (leaf: 12.64 ± 0.26%; bark: 6.48 ± 0.22%) extractive values were substantially higher in the leaf, indicating a greater concentration of extractable polar bioactive constituents in the leaf tissue.

Table 3. Physicochemical Parameters of Cola caricifolia Leaf and Stem Bark (% w/w, mean ± SD)

S/N	Parameter	Leaves (% w/w)	Stem Bark (% w/w)
1	Moisture Content (Loss on Drying)	6.85 ± 0.21	8.14 ± 0.18
2	Total Ash	8.91 ± 0.24	10.63 ± 0.30
3	Acid-Insoluble Ash	0.98 ± 0.07	1.83 ± 0.10

4	Water-Soluble Ash	4.97 ± 0.14	5.34 ± 0.12
5	Alcohol-Soluble Extractive	8.42 ± 0.20	2.77 ± 0.25
6	Water-Soluble Extractive	12.64 ± 0.26	6.48 ± 0.22
7	Sulphated Ash	9.38 ± 0.19	11.22 ± 0.28

Preliminary qualitative phytochemical screening confirmed the presence of alkaloids, tannins, phenolics, flavonoids, glycosides, terpenoids, and steroids in both the leaf and stem bark, with saponins detected exclusively in the leaf. A detailed comparative qualitative and quantitative phytochemical analysis of the leaf and stem bark, including organ-specific secondary metabolite partitioning across a polarity-graded solvent extraction series, is reported separately (Comparative Phytochemical Profiling of Cola caricifolia Leaf and Stem Bark, companion manuscript).

4. DISCUSSION

This study presents the first pharmacognostic monograph for *Cola caricifolia* leaf and stem bark from a Nigerian accession, providing essential baseline data for botanical authentication and quality assurance. Extensive searches across academic databases confirm no prior published pharmacognostic investigation on this species, rendering these findings a novel and significant contribution to the pharmacognostic literature on the genus *Cola.*

The macroscopic features observed in this study are consistent with published morphological descriptions of the species, particularly the characteristic palmately lobed leaf shape, cordate leaf base, and distinctive organoleptic properties of the bark including its bitter astringent taste and reddish-brown sticky exudate (Cheek, 2022; Sut *et al.*, 2020). These sensory characteristics are diagnostically important in field identification and crude drug authentication. The bitter astringency of the bark correlates with its high tannin content (1.35%) and aligns with the traditional uses of bark decoctions as analgesics, consistent with the anti-inflammatory properties attributed to tannin-rich preparations across the Malvaceae family (Van Wyk and Wink, 2017).

The microscopic architecture of the leaf, particularly the presence of paracytic stomata, is a taxonomically informative diagnostic feature consistent with prior descriptions of *Cola caricifolia* (Cheek, 2022). Paracytic stomata are characteristic of several Malvaceae members and provide a reliable microscopic identity marker for this species. The observation of both covering and secretory glandular trichomes further enriches the diagnostic profile; glandular trichomes are associated with the production and storage of essential oils and other secondary metabolites, suggesting a potential role in the anti-biofilm and antimicrobial activities documented in associated investigations. The presence of calcium oxalate rosette crystals (druses) in both leaf mesophyll and bark tissues is noteworthy, as these structures are species-specific in their distribution and crystal habit and constitute key identity markers in both fresh and powdered drug examination (Evans, 2009).

The peridermal architecture of the stem bark, with its characteristic radially aligned cork cells, is consistent with the secondary protective function of the periderm and aligns with the structural description provided by Cheek (2022) for *C. caricifolia.* The abundance of sclereids and stone cells in the bark powder is a characteristic feature of many species in the family Malvaceae and provides additional microscopic authentication markers. These lignified elements also contribute to the mechanical strength and durability of the bark, properties that have been traditionally exploited in implement construction (Cheek, 2022).

The physicochemical parameters recorded in this study are broadly within pharmacopoeial limits prescribed for herbal materials. Both moisture content values (leaf: 6.85%; bark: 8.14%) satisfy the WHO guideline of ≤10%, indicating adequate drying that would limit microbial proliferation and enzymatic degradation during storage (World Health Organization, 2011). The higher total ash in the bark (10.63%) compared to the leaf (8.91%) is consistent with patterns reported for other Malvaceae species, where bark tissues generally accumulate greater mineral content due to silica deposition and mineral transport via the phloem (Evans, 2009). The acid-insoluble ash values (leaf: 0.98%; bark: 1.83%), reflecting silica and acid-insoluble mineral residues, remained at acceptable levels indicating the absence of significant soil contamination in the plant material.

The substantially higher extractive values in the leaf (alcohol-soluble: 8.42%; water-soluble: 12.64%) compared to the bark (2.77% and 6.48% respectively) indicate a greater yield of soluble bioactive constituents from leaf tissue. High water-soluble extractive values are associated with the presence of polar constituents such as glycosides, flavonoids, and phenolic acids, consistent with the quantitative phytochemical data showing higher concentrations of these classes in the leaf. These extractive values serve as important quality parameters in standardization, providing limits for adulteration detection and extract yield prediction.

The qualitative phytochemical screening results, particularly the exclusive detection of saponins in the leaf, are consistent with organ-specific secondary metabolite partitioning and lend further support to the

differential ethnomedicinal use of leaf and bark preparations within this species.

5. CONCLUSION

This study establishes the first comprehensive pharmacognostic and physicochemical monograph for the leaf and stem bark of *Cola caricifolia* (G.Don) K.Schum. from a Nigerian accession. The diagnostic microscopic features — including paracytic stomata, covering and glandular trichomes, annular xylem vessels, rosette calcium oxalate crystals, radially aligned cork cells, sclereids, and stone cells — provide reliable identity markers for botanical authentication of both crude and powdered drug materials. Physicochemical parameters fall within acceptable pharmacopoeial limits, confirming the quality and suitability of the plant material for further pharmacological investigation. The differential phytochemical distribution between leaf and bark, particularly the exclusive presence of saponins in the leaf and higher tannin and terpenoid content in the bark, provides a scientific basis for the organ-specific ethnomedicinal applications of this species. These findings provide the foundational standards necessary for quality control, adulteration detection, and eventual inclusion of *Cola caricifolia* in national and regional herbal pharmacopoeias, supporting the evidence-based integration of this underutilized West African species into modern therapeutic practice.

Declarations

Conflict of Interest

The authors declare no conflict of interest.

Authors' Contribution

M.I.O.: Conceptualization, methodology, data collection, and writing — original draft. C.H.N.: Supervision, writing — review and editing, and corresponding author. C.R.C.: Methodology, data collection, and formal analysis. I.C.I.: Resources, validation, and writing — review and editing.

Data Availability

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

Use of Artificial Intelligence/Large Language Models

Artificial intelligence-based tools were used for language editing and formatting of the manuscript only. No AI system was used for data generation, analysis or interpretation.

Use of Research Reporting Tool

The ARRIVE guidelines from the EQUATOR Network were consulted during the design and reporting of this animal study.

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