

PHARMACOGNOSTIC EVALUATION OF THE LEAVES OF *Coccinia barteri* HOOK F (CUCURBITACEAE)

ABSTRACT

Background: In general, *Coccinia barteri* is offered and sold in its raw form. This natural form could be adulterated, substituted, or contaminated. Therefore, the purpose of this study is to use pharmacognostic analyses to verify the authenticity of the leaves of the *Coccinia barteri* plant.

Methods: The organoleptic studies were carried out through sensory organs. Histological analyses were conducted by microscopic examination of the specimen mounted on hand slides. Phytochemical screening and chemomicroscopic evaluation were also carried out using various standard methods.

Results: The leaves of *Coccinia barteri* come in a range of forms and sizes. They are lustrous, bright, and dark green. They are also tiny and feature opposite imparipinnate compound leaves. The organoleptic evaluation showed a leafy odour and an astringent taste. Microscopic evaluation showed glandular trichomes, wavy epidermal cells, and anomocytic stomata. The leaf had anomocytic stomata and hypostomatic. with an average stomatal number, index, density, length, width and size of $13.75 \pm 0.40\%$, $15.44 \pm 0.42 \text{ mm}^{-2}$, $80.88 \pm 2.82 \text{ mm}^{-2}$, $29.26 \mu\text{m}$, $26.17 \pm 0.4 \mu\text{m}$ and $766.38 \pm 39.49 \mu\text{m}$, respectively.

The phytochemical screening revealed the presence of phenols, terpenoids, saponins, alkaloids, proteins, glycosides, and flavonoids, while tannins and fixed oils were not detected. Chemotaxonomic studies showed the presence of starch grains, lignified tissues, calcium oxalates, gum, and the absence of protein and oil globules.

Analytical evaluation of the leaves of *Coccinia barteri* produced 7.00% total ash, 0.93% acid insoluble ash, 2.96% water soluble ash, 8.40% moisture content, 29.67% alcohol extractive value, 40.17% water extractive value, and 21.00% ethyl acetate extractive value.

Conclusion: This study provides information on the morphology, microscopic, and phytochemical profile of the leaves of *Coccinia barteri*.

Keywords: *Coccinia barteri*, pharmacognostic evaluation, phytochemicals, flavonoids

Introduction

Organoleptic, botanical, physical, chemical, biological, and pharmacological aspects are included in the multidisciplinary area of pharmacognosy, which is used to investigate and assess unprocessed pharmaceuticals derived from natural sources. (1). Typically, raw and natural forms of crude plant medications are exchanged, distributed, and sold in the marketplaces. These crude drugs are used globally for their claimed health benefits. The majority of people in developing countries rely heavily on crude drugs, either fresh or dried, to meet their primary health care needs. However, their growing market has resulted in a corresponding rise in accidental or intentional contamination and adulteration (2,3). Adulterated and contaminated crude drugs may result from a lack of correct authentication due to their close similarities with other materials. Adulterated crude drugs may also result from an intentional act for economic benefit. Lack of adequate regulation and quality control of crude drugs has also contributed to this problem. Thus, the pharmacognostic (organoleptic and macroscopic) and botanical characterizations of these materials are necessary to confirm their purity, safety, and efficacy.

The fundamental role of a natural product scientist is to ensure that the required standards prescribed in the pharmacopoeias for natural crude materials are maintained by passing through strict standardization procedures. Therefore, the authentication of a crude drug is a quality assurance process that ensures the correct raw plant materials are used to prepare herbal products. In the markets, crude plant medicines are typically traded, disseminated, and sold in their raw and natural forms (4).

Standardization of crude drugs is accomplished through step-by-step pharmacognostic investigations, which also include organoleptic, morphological, and anatomical investigations, qualitative and quantitative phytochemical screening, and quantitative microscopic determinations like stomata number, index, vein islet number, veinlet termination number, and palisade ratio. Accurate identification, authentication, and quality assurance of raw herbal ingredients are required for phytomedicine to be of reproducible quality and for herbal products to be secure and efficient. (5).

New drugs have been discovered with the help of phytochemical screening, and new affordable homoeopathic materials are now available for the treatment of serious ailments. (6). Phytochemicals are bioactive components of plants, as they exhibit different physiological actions on the human body. Thus, they are beneficial to human health by treating various diseases.

Barteriiis is a perennial climbing plant of the order Cucurbitales that belongs to the family Cucurbitaceae, generally referred to as the gourd, melon, and pumpkin family of flowering plants. (7). It is well-known for producing fruit each year and is common in Asia and Africa. *Coccinia barteri* occurs in evergreen forests. The fruits of *C. barteri* have historically been used as both food and medicine. The juice of the roots and leaves was used to cure diabetes, gonorrhoea, and constipation in ancient times, which is when it first became known to have therapeutic uses. (8). The herb's leaves, stems, and roots are among the portions that have historically been used to cure a variety of ailments, including bronchitis, jaundice, burns, skin eruptions, fever, insect bites, allergies, eye infections, gonorrhoea, and syphilis. (9). *C. barteri* is a

powerful herb with established biological activities, as evidenced by the finding of components with aldose reductase inhibitors and antioxidant actions. (7). In accordance with findings, *C. barteri* leaves have high concentrations of phenolic and flavonoid components and possess antioxidant and antidiabetic properties (1). Researchers have looked at the anti-glycation and insulinotropic characteristics of *C. barteri* fruit. (11). The fruits of the *C. barteri* tree, hitherto only used for culinary purposes, now serve as a clean source of herbal medicine. The plant's cold infusion is utilized in some parts of West Cameroon and Nigeria to cure venereal diseases. (12).

This study's main objective was to assess the pharmacognostic and phytochemical characteristics of *Coccinia barteri*'s leaf in order to provide details for its authentication.



Fig. 1: *Coccinia barteri* (Hook f) plant

Materials

Methanol (99.8%), ethyl acetate, chloroform, ferric chloride, million reagent, alpha-naphthanol, dragendorff reagent, Benedict's solution, diluted hydrochloric acid, sodium hydroxide, ammonia, sulfuric acid, and detecting reagents were acquired from the research laboratory of the Pharmacognosy Department at Enugu State University of Science and Technology and are of analytical grade. The *Coccinia barteri* leaves were harvested in November 2021 from Nru in the Nsukka, LGA of Enugu State, Nigeria, and validated by Mr. Felix, a taxonomist with the Pharmacognosy Department at the University of Nigeria, Nsukka. The morphological parameters, such as height, tree size, phyllotaxes, kind, etc., were noted at the time of collection in their native habitat. The plant specimen was taken while it was in bloom, air dried, mounted on a herbarium sheet, given the voucher specimen number ESUTPCG 2020, and deposited in the herbarium.

Methods

The macroscopical study was done by organoleptically observing the shape, colour, size, odour, taste, surface, surface fracture, texture, apex, type, venation, and leaf margin.

Fresh Leaf Microscopy

The clearing approach was used to prepare the foliar epidermis on the adaxial (upper) and abaxial (lower) surfaces of the leaves. By immersing the leaf samples in commercial bleach "Hypo" containing 3.5% sodium hypochlorite for 18 hours, the samples were cleaned. The leaf sample epidermal strips were then carefully scraped with a pair of forceps and placed on a clean slide. Safranin solution was used to stain the slide, which was then covered with a cover slip. (13). A light phase contrast microscope (Motic B3, Motic Carlsbad, CA, USA) was used to magnify the slides by 40x, 100x, and 400x. The Moticom 2.0 image system with software (Motic Carlsbad, CA, USA) attached to the microscope was used to take the photomicrographs. The following parameters were observed and assessed:

- Epidermal cells were counted and documented, along with their type and quantity.
- Stomata type: Complex stomatal kinds were seen and recorded using terminologies that have been reported. (14)
- With 10 fields of view for each sample, the length and width of the stomata were measured using the Motic microscope software.
- Stomatal density: The number of stomata per square millimeter was used to calculate the stomatal density.
- Stomatal index: The stomatal index was determined as follows:

$$SI = \frac{S}{S + E} \times 100$$

S + E

Where: S = number of stomata in a field of view

E = number of epidermal cells in the same field of view

- Trichome parameters: The same methods used to determine stomata's types, sizes, densities, and indices were used to determine the trichomes' attributes.
- Vein islet number: Palisade ratio and vein islet termination number were calculated.

All parameters were observed on both the adaxial and abaxial surfaces of the leaves (13).

Using a Reichert sledge microtome, the leaf's transverse section (TS) was created. The sections were selected with the use of a camel hair brush from the tip of the microtome knife into separate Petri dishes containing 70% pure alcohol and labeled properly. The sections were microtomed at 10 to 15 uni microns. As biological stains for distinguishing lignified tissues, safranin and Fast green were used.

ANALYTICAL DETERMINATION

DETERMINATION OF EXTRACTIVE YIELDS

1) Water Extractive Value

A 2g sample was macerated for 24 hours in 100 ml of chloroform water before being filtered. In order to achieve a constant dry weight that could be cooled in desiccators and weighed, the filtrate was dried in an oven at 105°C.

2) Alcohol Extractive Value

A 24-hour maceration in 100 ml of 100% ethanol with 2g of the sample was followed by filtering. To get a constant dry weight extract, the filtrate was dried in an oven at 105°C. The extract was then chilled in a desiccator and weighed. (15).

3) Ethylacetate Extractive Value

A 2g sample was macerated for 24 hours in 100 ml of ethylacetate and then filtered. To achieve a constant weight, the filtrate was dried in an oven at 105°C. After the extract had cooled in a desiccator, its weight was measured.

DETERMINATION OF ASH VALUES

1. **a) Total Ash Values:** A porcelain crucible was heated for approximately 15 minutes at 35 °C in a muffle furnace, cooled in a desiccator for one hour, and weighed (W1). 2g of the sample were precisely weighed and then reweighed (W2) into the heated porcelain crucible. For roughly 6 hours, the sample was burned to ash in a muffle furnace at 650 °C until it turned gray (white ash). Using a crucible tong, the crucible was withdrawn, desiccated to chill it, and then reweighed (W3). $W2-W1/W3-W1$ expressed as a percentage was used to calculate the ash content.
2. **b) Water-Soluble Ash Value:** A porcelain crucible was placed in a muffle furnace, ignited to a constant weight at 35 °C, and cooled and weighed (W1). 2g of the powdered drug was placed in the crucible and reweighed (W2). The crucible containing the drug was incinerated to burn off the carbon content at 650 °C. The crucible was cooled in a desiccator, reweighed, and the contents transferred into a small beaker. About 5 ml of water was added to the content, which was boiled for 5 minutes, filtered with an ashless filter paper, and dried in the oven. The filter paper containing the residue was compressed into the crucible and subjected to heat at 650 °C until the ashless filter paper was eliminated and the crucible was reweighed. The percentage was then calculated and recorded.
1. **c) Acid Insoluble Ash Value:** The total ash obtained from incinerating the powdered leaf at 650 °C was transferred into a beaker containing 25 ml of dilute hydrochloric acid, boiled in a water bath for about 5 minutes, and filtered with an ashless filter paper. The beaker and crucible were washed repeatedly through the filter paper with hot water until they were free from acid (i.e., neutral to litmus paper). The insoluble matter and the ashless filter paper were dried in the oven and ignited in the muffle furnace at 650 °C to a constant weight, and the amount of acid insoluble ash per gram of the powdered drug was calculated.

CHEMOMICROSCOPY

The leaves were crushed using a traditional mortar and pestle after being shade-dried. A chemomicroscopic study was done on the powders to see if there were any lignified vessels, calcium oxalate crystals, or starch present. A small amount of the sample was carefully put onto a glass slide. A single drop of chloral hydrate was added, and it was continually heated on a Bunsen burner until bubbles appeared, indicating that the tissues had been successfully cleared.

Test for Starch

On a glass slide, a tiny amount of the cleaned leaf powder was mixed with a drop of iodine. A drop of glycerin was added, and the mixture was then examined at 400x magnification using a light phase contrast microscope (Motic B3, Motic Carlsbad, CA, USA).

Test for Lignin

Phloroglucinol and concentrated HCl were added in a 1:1 ratio to a small amount of the cleaned powder on a glass slide. Using a light phase contrast microscope (Motic B3, Motic Carlsbad, CA, USA) set at x100 magnification, a drop of glycerin was applied.

Test for Cellulose

Phloroglucinol and concentrated HCl were added in a 1:1 ratio to a small amount of the cleaned powder on a glass slide. Using a light phase contrast microscope (Motic B3, Motic Carlsbad, CA, USA) set at x100 magnification, a drop of glycerin was applied.

Test for Tannins

To check for tannin, 1% lead acetate was diluted into around 2 ml of the drug's aqueous extract. The presence of tannins was revealed by the appearance of a yellowish precipitate.

Test for Calcium oxalate crystals

A small amount of the cleared leaf powder was placed on a glass slide, and a drop of strong acetic acid was added. A light phase contrast microscope (Motic B3, Motic Carlsbad, CA, USA) at x400 magnification was used to monitor the addition of a drop of glycerin.

Test for Gums and Mucilage

A tiny amount of the cleaned powder sample was mixed with one drop of Ruthenium red. The presence of gums and mucilage was suggested by the emergence of pink coloration.

PHYTOCHEMICAL TESTS

To a small amount of the cleaned powder sample, Ruthenium red was applied in a drop. Gums and mucilage were present, as evidenced by the development of pink coloration. (12,16).

RESULTS

Macroscopic and microscopic characterization

The organoleptic evaluation of *Coccinia barteri*'s leaf revealed a smooth, dark green leaf with a fresh leafy scent. The leaf has a hypostomatic and anomocytic type of stomata and the epidermal cells were irregularly shaped. The results of the microscopic evaluation of the leaf are shown in Figs 2 -5.

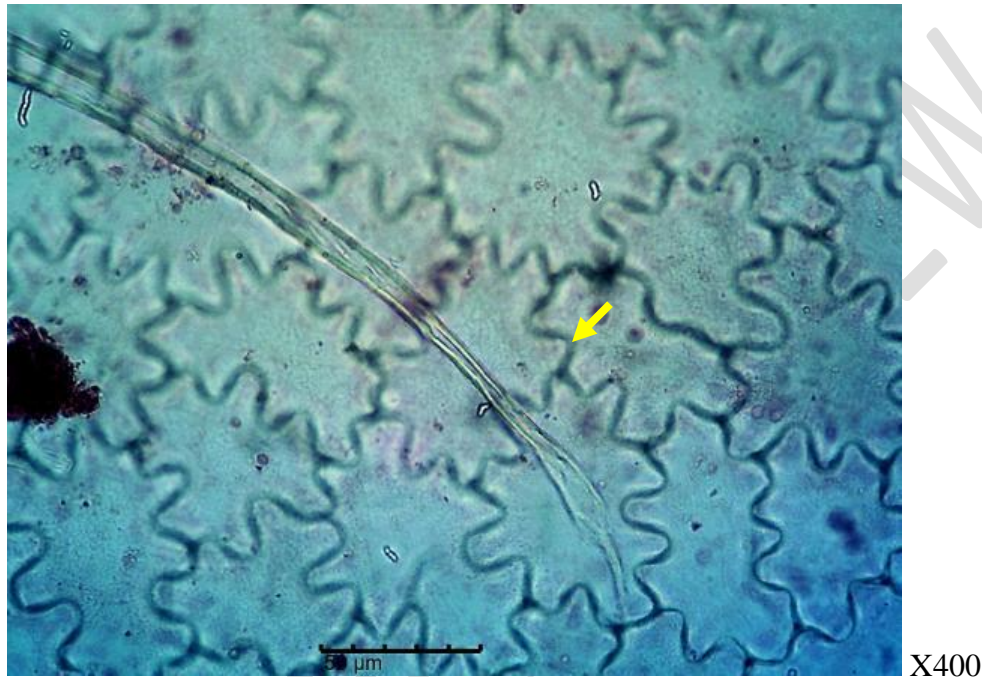


Fig 2: Adaxial surface of the leaf of *C. barteri* showing wavy epidermal cells (yellow arrow)

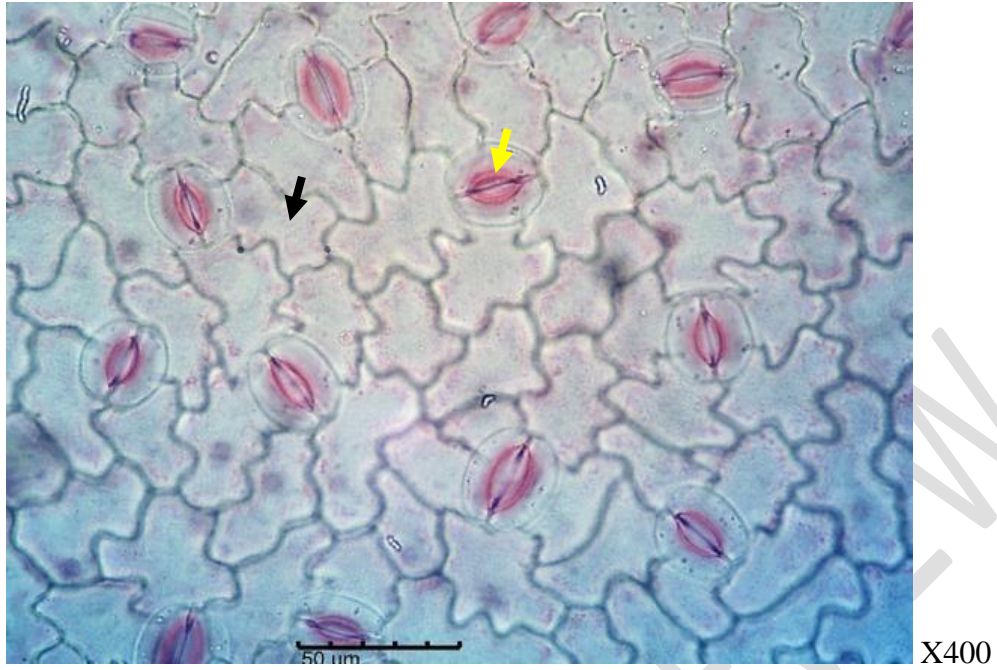


Fig 3: Abaxial surface of the leaf of *C. barteria* showing anomocytic type of stomata (yellow arrow) and wavy epidermal cell (black arrow)

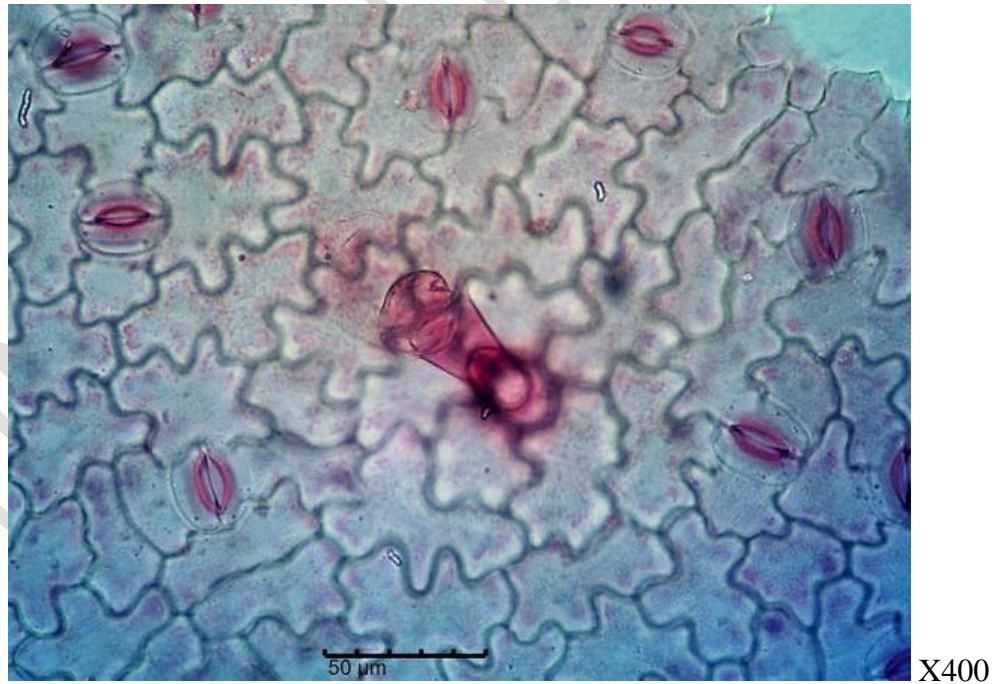


Fig 4: Leaf epidermal surface of *C. barteri* showing glandular trichome

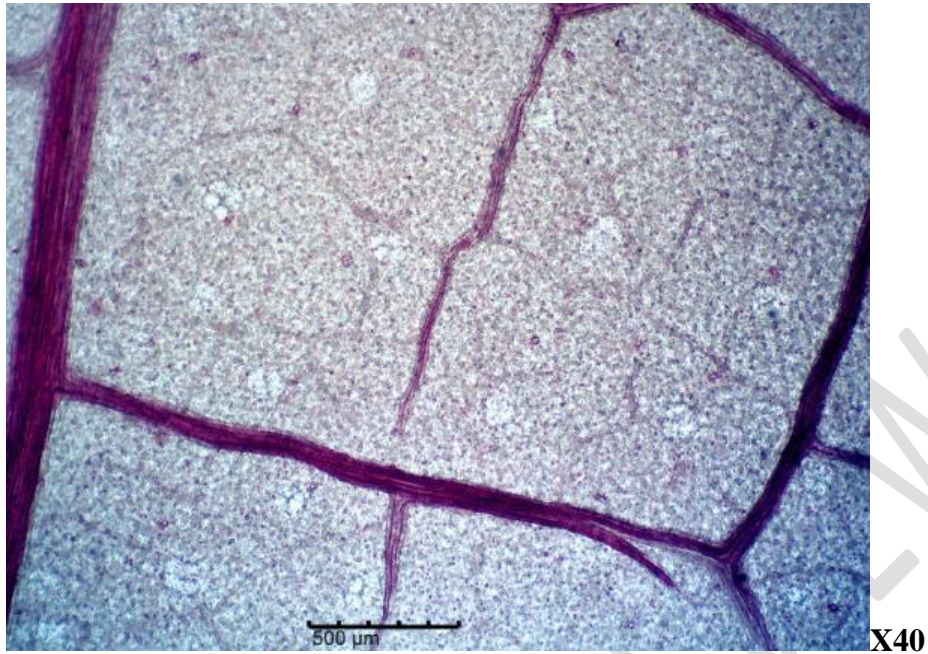


Fig 5: Venation pattern of *Coccinia barteri*

The transverse section of a leaf, as depicted in Figure 6, reveals a leaf with a single-layered epidermis. Palisade mesophyll cells in the shape of columns and spongy mesophylls follow. The central pith and concentric vascular bundle, which includes the phloem, cambium, and xylem, were visible. Trichomes were not seen. The petiole's transverse section revealed a similar pattern but differed in that it had trichomes. (Fig 7).

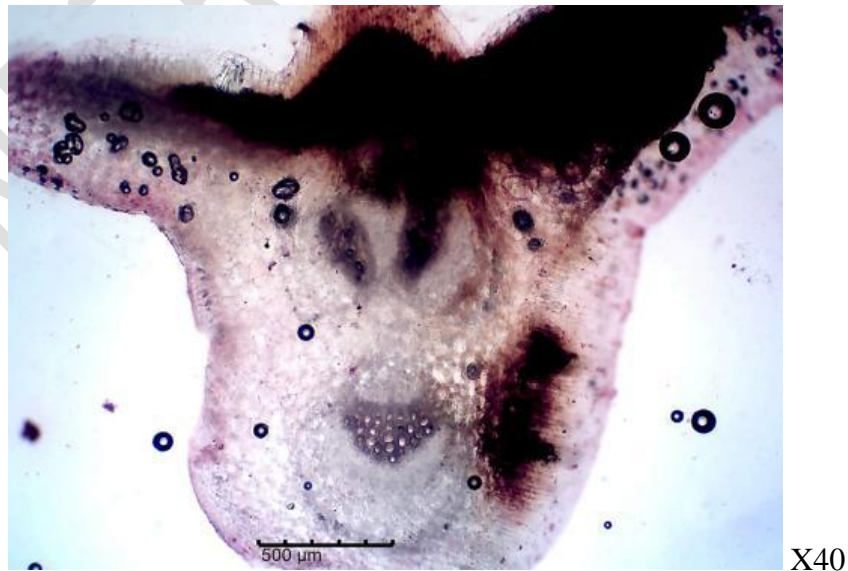


Fig 6: Transverse section of the leaf of *C. barteri*



X40

Fig 7: Transverse section of the petiole of *C. barteri*

Table 1 presents the findings of the macroscopic and microscopic analyses of *Coccinia barteri*'s leaf..

Table 1: Macroscopic and microscopic characterization of *Coccinia barteri*'s Leaf.

PARAMETERS	FEATURES
Colour	Dark green
Texture	Smooth
Odour	Fresh leafy scent
Venation	Reticulate
Epidermal cell	The upper and bottom sides of epidermal cells have wavy anticlinal cell walls that give them an irregular shape.
Stomata type	The leaf is anomocytic (lacks subsidiary cells with epidermal cells directly linked with the guard cells) and hypostomatic (stomata only exist on the lower surface).
Trichome	Present – Glandular type on the lamina and unicellular covering type on the petiole
Stomata number	13.75 ± 0.40 per field of view
Stomata density (mm ⁻²)	80.88 ± 2.82
Stomata length (μm)	29.26 ± 1.25

Stomata width (μm)	26.17 ± 0.40
Stomata size (μm^2)	766.38 ± 39.49
Stomata index (%)	15.44 ± 0.42

Analytical evaluation of the powdered leaf sample

Table 2 displays the findings of the analytical assessment of the powdered leaf sample of *Coccinia barteri*.

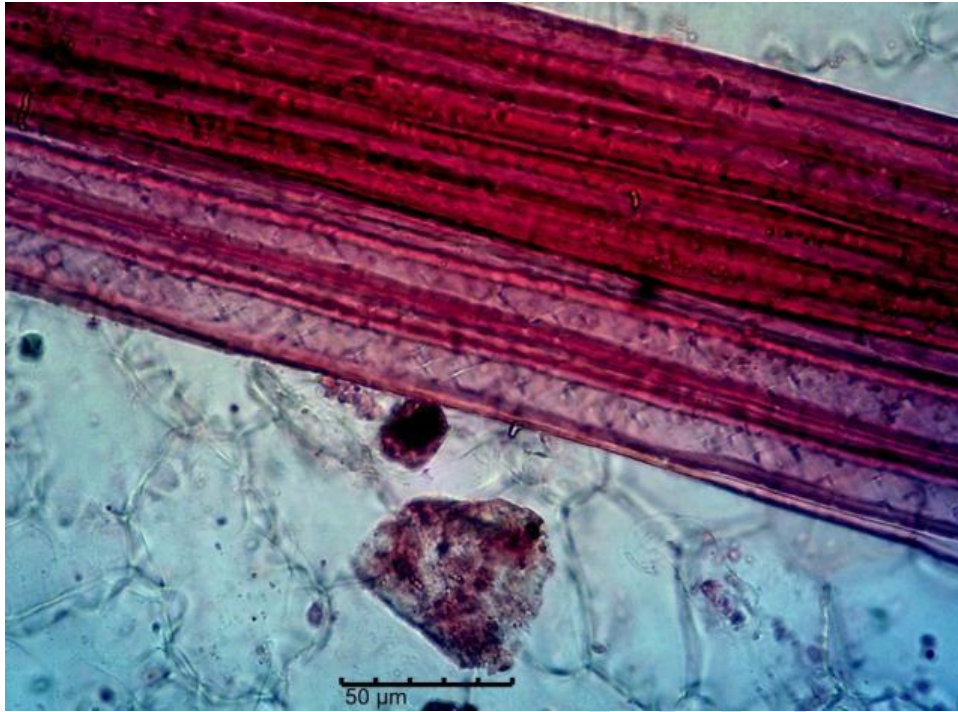
Table 2: The results of the analytical evaluation of the powdered leaf sample

PARAMETER	MEAN WEIGHT (g)	WEIGHT (%)
Moisture content	0.17	8.40 ± 1.10
Total ash	0.14	7.00 ± 0.46
Water soluble ash	0.044	2.96 ± 0.57
Acid insoluble ash	0.014	0.93 ± 0.19
Ethylacetate soluble extractive value	0.42	21.00 ± 5.07
Ethanol extractive value	0.60	29.67 ± 1.76

Values expressed in mean \pm Standard Deviation, n= 3.

Chemomicroscopy of the leaf of *C. barteri*

The chemomicroscopic analysis of the leaf of *C. barteri* revealed the presences of starch, lignin, calcium oxalates, tannin, cellulose and mucilage while protein and oil globules were absent (as shown in Table 3). Fig 8 shows the photomicrograph of the leaf fragment with a prism-shape calcium oxalate crystal and bundle of fiber and vessel elements. The photomicrographs of the leaf powder in Figs. 9 and 10 display an isolated fiber and vessel element.



4100

Fig 8: Photomicrograph of the leaf fragment showing a prism-shape calcium oxalate crystal and bundle of fiber and vessel elements



X400

Fig 9: Photomicrograph of the leaf powder showing an isolated fiber element



Fig 10: Photomicrograph of the leaf powder showing an isolated pitted vessel element

Table 3: Chemomicroscopy of the leaf of *C. barteri*

Parameter	Reagents	Result
Starch grains	Iodine solution	Present
Lignified tissues	Conc. HCl + Phloroglucinol	Present
Calcium oxalates	Iodine solution Conc. Sulphuric acid	Present; Prism-shaped
Tannin	Ferric chloride	Present
Cellulose	Zinc chloride, Conc. Sulphuric acid	Present
Gum/Mucilage	Ruthenium red	Present
Protein	Biuret reagent, Nihydrin	Absent
Oil globules	Sudan III reagent	Absent

Phytochemical constituents of *Coccinia barteri*

The *Coccinia barteri* leaf underwent a phytochemical examination, which revealed the presence of flavonoids, tannins, saponin, alkaloids, glycosides, and steroids. There was no evidence of protein or decreasing sugar in any of the extract.

Table 4: The phytochemical constituents of *Coccinia barteri*

Constituent	Methanol Extract	Ethyl Acetate	Chloroform	Aqueous Methanol
Flavonoid	+	+	+	+
Protein	-	-	-	-
Tannins	+	+	+	+
Reducing sugar	-	-	-	-
Saponins	+	+	+	+
Alkaloid	+	+	+	+
Glycosides	+	-	+	+
Steroids	+	-	+	+

Key: positive = + and negative = -

DISCUSSION

The macroscopic, microscopic, physiochemical, and phytochemical investigations of the *Coccinia barteri* Hook leaf as part of the pharmacognostic assessment of the plant were evaluated. At the time of collection, on-site morphological observations of the 15 m tall climber perennial plant's leaf were made. The leaf has a green, astringent flavor and smell. The fundamental elements in standardizing and recognizing crude pharmaceuticals are the evaluation of their macroscopic and organoleptic (sensory) qualities. It is a straightforward procedure that does not require the use of technical equipment. Physical, microscopic, and morphological analyses provide quick and useful information for identifying and assessing the purity and quality of raw pharmaceuticals. (17–19). The macroscopic examination reveals epidermal cells with undulated anticlinal cell walls on both the top and lower surfaces, which are unevenly formed. To prevent adulteration, these criteria might be employed in the qualitative assessment of the plant during collection.

The chemical components of the crude medication are measured by the extractive yield using various solvents. A higher aqueous methanol extraction yield suggests the existence of active ingredients in the crude medicine that are soluble in aqueous methanol. A drug's moisture content should be as low as possible to prevent the microbiological or chemical deterioration of unprocessed medications. The moisture content of 8.40% is within the ideal range for minimum microbial activity (20). Ash value is a criterion to judge the identity or purity of crude drugs.

The major classes of secondary metabolites present were identified using qualitative phytochemical screening of different extracts of the formulation using color responses or alterations. The results showed that *Coccinia barteri* leaf extracts of methanol, chloroform, and aqueous methanol all had the same flavonoids, tannins, saponins, alkaloids, glycosides, and steroids. However, neither steroids nor glycosides were found in the ethyl acetate extract. All the extracts lacked proteins and reducing sugar. These results are in agreement with earlier reports. The biological actions of the plant are caused by the presence of these secondary metabolites. These compounds work together or separately to increase the leaf's pharmacological actions.

Conclusion

Coccinia barteri is a perennial climber about 15 m tall with opposite imparipinnate compound leaves, a branched stem, and a small greyish-yellow flower. The assurance of the legitimacy, safety, and efficacy of the leaf will be made easier with the aid of its pharmacognostic characterization as *Coccinia barteri*. This study encourages the usage of pure *Coccinia barteri* leaf by providing the profile of the authenticated drug as a standard for its identification.

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