Pharmacognostic and Taxonomic Studies of *Cola parchycarpa* K. Schum. (Malvaceae)

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**Authors’ contributions**

This work was carried out in collaboration between both authors. Author MEB supervised the author IIJ who carried out the bench work and wrote the first draft of the manuscript. Author MEB perfected the final manuscript. Both authors read and approved the final manuscript.

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**ABSTRACT**

*C. pachycarpa* is a lesser-known member of the genus *Cola* in the family Malvaceae. In Nigeria its fruits are edible but with non-edible seeds as the general *Cola* ‘Kolanut’. This study is carried out to evaluate the taxonomic and pharmacognostic characters of *Cola pachycarpa* K. Schum. for its identification, authentication and standardization. The pharmacognostic and taxonomic characters were determined from macroscopy, microscopy, petiole anatomy, powder microscopy, chemomicroscopy, micromeritic properties, ash values, extractive values, fluorescence analysis and phytochemical screening using standard methods. The leaves of *C. pachycarpa* were alternate, petiolate, compound and trifoliate. Petioles were within 45-50 cm long, leaflets 20-38 cm long, 10-19 cm wide, the middle leaflet were often longer than others. Leaflets were short petiolulate to subsessile, leaflet shape was elliptic, apex acuminate, margin entire and texture hairy on the abaxial surface of the leaflet with brown caducuous hairs on the abaxial surface only. Stem was erect, woody and scabrid about 4-10 cm in diameter. The fruit was 15 cm long and 7 cm wide with about 3-5 seeds occurring in a pod. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoid, saponsins, tannins and cardiac glycosides but anthraquinone was...
absent for the leaf while all were present except alkaloid, cardiac glycoside and anthraquinone in
the stem. Epidermal cells were polygonal with straight anticlinal walls. Drusiferous crystals were
observed on the petioles. Leaflets were hypostomatus with anisocytic stomata and stellate
trichomes on the abaxial surface. The chemomicroscopic study revealed the presence of lignin,
starch, cellulose, oils, calcium oxalate crystals, mucilage and protein for both leaf and stem. The
fluorescence characteristics showed the presence of different colours supporting the presence
of various phytococonstituents for both leaf and stem. The flow properties for both leaf and stem
were fair and passable with the angle of repose of 35° and 45° respectively. The findings of the
research will help in the identification and authentication of the plant as well as establishing
standards for quality, purity, safety, efficacy and reproducibility in phytomedicine.

Keywords: Cola pachycarpa; macroscopic; physico-chemical; fluorescence; chemotaxonomic;
stomata; drusiferous.

1. INTRODUCTION

Focus on plant research has increased in the world today and a large body of evidence has
shown vast potential of medicinal plants used in numerous traditional systems. Approximately
80% of the worldwide population turns to plant derived medicines as their leading line of
defense for maintaining health and fighting diseases [1].

Mostly, all medicines whether synthetic or non-synthetic of plant origin, should fulfill the basic
requirements of quality, safety and efficacy [2,3]. Reproducibility of quality, safety and efficacy are
achieved by comprehensive processes and procedures of standardization. Thus proper identification becomes paramount as the World Health Organization stated that the macroscopic
and microscopic description of medicinal plants is the first step towards establishing the identity
and the degree of purity of such material and should be carried out before any test is undertaken [4].

Cola pachycarpa K. Schum. (Malvaceae) is one of many species of Cola referred to as Monkey
Kola. The genus Cola comprises of about 140 species [5,6] Unlike C. nitida and Cola acuminata
C. pachycarpa possesses non-edible seeds enclosed in an edible mesocarp. Primates (monkey) and humans relish the fruit [7] which is found in the lowland rainforest and forest
outliers. The mature trees can attain a height of 20-60 ft (6-18 m), girth up to 0.04-0.1 m. Leaves
are crowded at the top of stem. The plant fruits between July-November [7]. Traditionally, in the
eastern part of Nigeria, the Igbo uses the seeds as stimulants [8].

Although in 2017 [7] chemical evaluation of amino acids have been done on the plant, no
pharmacognostic and taxonomic work has been carried out on it. The present investigation used
different pharmacognostic and taxonomic parameters to supplement the identification and
standardization information.

Classification of Cola pachycarpa:

Kingdom: Plantae
Clade: Angiosperms
Clade: Eudicots
Clade: Rosids
Order: Malvales
Family: Malvaceae
Subfamily: Sterculioidae
Genus: Cola
Species: C. pachycarpa K. Schum.

Local Names: Efik/Ibibio; Afi A Ndiya, Hausa, Goro mbiy, Yoruba; Obi edun, Igbo; Achicha

Source: Angiosperm Phylogeny Group [9].

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Material

The fresh samples of Cola pachycarpa were collected on 17th September, 2018 from Osomba
Hills in Osomba, Akamkpa Local Government Area, Cross River State with the GPS reading as
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± 61 m 5° 27’37.28N, 008° 39’39.4E and preserved in FAA (Formalin Acetic Acid). The plant was authenticated by a taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Uyo and voucher specimen have been deposited in the herbarium. The collected leaves and stems were washed under running tap water, rinsed with distilled water, chopped into pieces, dried under shade at room temperature. The dried leaves and stems were powdered using electric blender, sift through 350 microns sieve size and stored in airtight bottles to avoid moisture and humidity prior to use.

2.2 Macro-Morphological Evaluation of Leaf and Stem

2.2.1 Organoleptic (sensory) parameters

Organoleptic (sensory) parameters of fresh leaf and stem as well as their powders such as colour, odour, and taste were evaluated by the sense organs and documented [10].

2.2.2 Morphological characteristics

Morphological and related taxonomic observation were made on the stem, leaves (apex, base, margin, hairiness) petioles, stipules and fruits and the characters were described using standard methods [10].

2.3 Microscopic Evaluation of Leaf

2.3.1 Qualitative microscopy

For the purpose of anatomical studies, the standard median portion of the well expanded matured leaf was obtained. Epidermal peels of both abaxial and adaxial surfaces were made by placing the leaf on a clean glass slide with the surface to be studied facing down. The specimens were irrigated with water holding it downward from one end and then the epidermis above the desired surface was scrapped off carefully with sharp razor blade. The loose cells were then washed off with water and the epidermis was stained in 1% aqueous solution of safranin-O for 4-8 minutes and washed again in water to remove excess stain and mounted in 10% glycerol on a glass slide and covered with a glass cover slip before viewing with an Olympus CX21 binocular microscope. Photomicrographs were taken from good preparations using the Olympus CX21 binocular microscope fitted with an MD500 amscope microscope eyepiece camera. Measurements were done at ×10 while ×40 for photomicrographs [11].

2.3.2 Quantitative microscopy

Quantitative microscopy parameters such as leaf constant studies viz. stomatal length and width, stomatal pore length and width, guard cell length and width, stomatal number, stomatal index, epidermal cell length and width, epidermal cell number, epidermal cell thickness, vein islet number, vein termination number, areole length and width and trichome length and width was carried out using standard procedures [11].

All measurements were made using a calibrated ocular micrometer and thirty (30) microscopic fields chosen at random were used and data presented as mean ± Standard Error of Mean (SEM).

The stomata index (S.I) was determined according to Killedar et al. [11] using the formula:

\[
\text{Stomatal Index (SI)} = \frac{S}{E} + S \times 100
\]

Where: S = number of stomata per unit area
E = number of epidermal cells in the same area.

2.4 Microscopic Evaluation of Petiole Section

Transverse sections of the petioles were prepared by embedding the portion in pawpaw tissue after which free-hand sectioning was made with a sharp razor blade. The sections were cleared in 20% sodium hypochlorite (NaOCl) for 3-5 minutes and thereafter thoroughly rinsed five times in distilled water. Sections were stained in safranin-O solution for a period of 2-3 minutes then rinsed carefully with distilled water to remove excess stain. Dehydration followed and then sections were mounted in 10% glycerol solution and viewed with an Olympus CX21 binocular microscope. Photomicrographs were taken with an MD500 amscope camera [12].

2.5 Evaluation of Powders

Chemomicroscopic studies of the coarse powders of the leaf and stem was carried out to study the microscopical characters as well as their chemomicroscopic properties viz. cellulose, mucilage, lignin, starch, protein, oils and calcium oxalate crystals [13,14].

Preliminary phytochemical screening was carried out on the powdered leaf and stem after extracting with 60% ethanol and allowed to stand for 72 hours with occasional shaking. It was then
filtered using cotton wool then the filtrate concentrated to dryness on a water-bath at 45°C. The extracts were then subjected to phytochemical screening using standard methods [15].

The fluorescent analysis of *C. pachycarpa* dried leaf and stem powders was carried out using the standard method [16].

The physicochemical parameters such as moisture content, ash values (total ash, acid insoluble ash, water soluble ash), soluble extractive values viz. ethanol, ethyl acetate, methanol and water were performed according to the WHO prescribed guidelines on quality control methods for medicinal plant materials [17].

The micromeritic characteristics of leaf and stem powder viz. bulk density, tap density, angle of repose, Hausner’s ratio, Carr’s index and pH were determined according to standard methods [18].

### 2.6 Statistical Analysis

All experiments were repeated at least three (3) times except for the quantitative microscopy where thirty (30) determinations were done. Results were reported as Mean ± SEM (Standard Error of the Mean).

### 3. RESULTS

#### 3.1 Macromorphological and Organoleptic Evaluation of Leaf and Stem of *C. pachycarpa*

The results of the macro-morphological, micro-morphological and organoleptic evaluation of leaf and stem of *C. pachycarpa* are summarized in Table 1 and Fig. 1.

#### 3.2 Microscopic Evaluation of Leaf

The microscopic evaluation of the leaf and petiole of *C. pachycarpa* are summarized in Fig. 2.

The thickness of the adaxial and abaxial epidermal cell walls were 7.57±0.29 µm and 2.08±0.07 µm respectively (Table 2).

The length and width of the epidermal cells for both abaxial and adaxial surfaces were 65.48±2.65 µm and 39.35±1.61 µm and 77.01±3.43 µm and 33.53±0.67 µm respectively. The number of epidermal cells on the adaxial surface was 408.53±3.02 and abaxial surface was 126.00±1.68 (Table 2).

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**Fig. 1.** A-Abaxial surface: B-Adaxial surface: C-whole fruit: D-fruit showing white mesocarp: E-greenish-brown leaf powder: F-light-brown stem powder
Table 1. Macromorphological, micro-morphological and organoleptic evaluation of leaf, stem and fruit of *C. pachycarpa*

| Parameters | Macromorphological Characters | Micromorphological Characters | Organoleptic Characters |
|-----------|-------------------------------|-------------------------------|-------------------------|
| **Leaf**  |                               |                               |                         |
| Type      | Alternate, petiolate, compound and trifoliate |                               |                         |
| Leaf length | 20-38 cm                       |                               |                         |
| Leaf width | 10-19 cm                       |                               |                         |
| Petiole length | 45-50 cm                     |                               |                         |
| Leaf apex  | Short acuminate               |                               |                         |
| Margin    | Entire to wavy                |                               |                         |
| Texture   | Adaxial surface: Glabrous     | Abaxial surface: Pubescence   |                         |
| Leaf shape | Elliptic                      |                               |                         |
| **Stem**  |                               |                               |                         |
| Type      | Erect                         |                               |                         |
| Shape     | Cylindrical                   |                               |                         |
| Size      | 4-10 cm in diameter           |                               |                         |
| Indumentum | Pubescence                    |                               |                         |
| **Fruit** |                               |                               |                         |
| Colour    | Epicarp-brown                 | Mesocarp-white                |                         |
| Length    | 11-15 cm                      |                               |                         |
| Width     | 5-7 cm                        |                               |                         |
| **Seeds** | 3-5 occurring in pod          | Adaxial                       | Abaxial                 |
| Epidermal cell |                               | Adaxial surface: green Cubeaxial surface: grey Leaf powder: greenish-brown Stem powder: light brown |
| Cell shape | Polygonal                     | Polygonal                     |                         |
| Anticlinal wall pattern | Straight                      | Straight                     |                         |
| Stomata type | -                            | Anisocytic                   |                         |
| Trichome   | -                             | Stellate                      |                         |
| Colour     | Adaxial surface: green Cubeaxial surface: grey Leaf powder: greenish-brown Stem powder: light brown |                         |                         |
| Odour      | No characteristic taste       |                               |                         |
| Taste      | No characteristic taste       |                               |                         |

*C. pachycarpa* leaves were hypostomatic (stomata on abaxial surface only). The mature stomatal complex type was anisocytic (Fig. 2B) as seen in Table 2 the stomatal length (49.01±0.67 µm), stomatal width (36.09±0.92 µm), stomatal pore length (26.82±0.59 µm), stomatal pore width (9.11±0.21 µm), stomatal number (17.87±0.34), stomatal index (12.41%), guard cell length (46.31±0.73 µm) and guard cell width (19.28±0.19 µm) respectively were obtained from the abaxial surface only.

Stellate trichomes were observed on the abaxial surface with the length and width as 264.26±9.37 µm and 12.99±0.73 µm respectively (Table 2). Lower values were obtained for areole length (232.15±10.85 µm) areole width (175.11±8.35) and vein termination number (1.67±0.12) for the abaxial epidermis (Table 2). While higher values for areole length (285.78±7.35 µm), areole width (208.19±4.46 µm) and vein termination number (3.63± 0.11) were obtained for the adaxial epidermis. On the other hand, the vein islet numbers were higher (9.26±9.38) for the abaxial epidermis than for the adaxial epidermis (3.63±0.11).

The preliminary phytochemical screening results are summarized in Table 3.
Fig. 2. (A): Polygonal epidermal cells and straight ACWP adaxial surface × 40; (B): Anisocytic stomata (AnS) and polygonal epidermal cell wall (PoEc) abaxial × 40; (C): Stellate trichome (ST) in the abaxial epidermis × 40; (D): Vein termination (VT) and areole (ARL) adaxial surface × 40; (E): Petiole showing Cuticle (C); Epidermia (E); Collenchyma (Col); Parenchyma (Par); Endodermis (Endo); Stele (S); × 40; (Ei): Vascular tissues (VasT); Calcium oxalate crystals (Coc) petiole × 40

Table 2. Qualitative and quantitative micro-morphological characters of *C. pachycarpa* (Mean ±S.E)

| S/N | Parameters                           | Leaf surface |
|-----|-------------------------------------|--------------|
|     |                                     | Adaxial      | Abaxial      |
| 1.  | Cell shape ×40                      | Polygonal    | Polygonal    |
|     | pattern ×40                         | Straight     | Straight     |
|     | Cell number ×10                     | 408.53±3.02  | 126.00±1.68  |
|     | Cell length(μm) ±SEM ×10            | 77.01±3.43   | 65.48±2.65   |
|     | Cell width(μm)±SEM ×10              | 33.53±0.67   | 39.35±1.61   |
|     | Cell length/width ratio             | 2:1          | 1:1          |
|     | Cell wall thickness (μm)±SEM ×10    | 7.57±0.29    | 2.08±0.07    |
| 2.  | Vein architecture                    |              |              |
|     | Areole length(μm)±SEM ×10           | 285.78±7.35  | 232.15±10.85 |
|     | Areole width(μm)±SEM ×10            | 208.19±4.46  | 175.11±8.35  |
|     | Termination number ×10              | 3.63±0.11    | 1.67±0.12    |
|     | Vein islet number ×10               | 3.63±0.11    | 9.26±9.38    |
| 3.  | Stomatal characters                  |              |              |
|     | Length                              | 49.01±0.67   |              |
|     | Width                               | 36.09±0.92   |              |
|     | Guard cell length(μm)±SEM ×10       | 46.31±0.73   |              |
|     | Guard cell width ×10                | 19.28±0.19   |              |
|     | Stomatal number ×10                 | 17.87±0.34   |              |
|     | Stomatal type ×40                   | Anisocytic   |              |
|     | Pore length(μm)±SEM ×10             | 26.82±0.59   |              |
|     | Pore width(μm)±SEM ×10              | 9.11±0.21    |              |
|     | Stomatal index %                     | 12.41        |              |
| 4.  | Trichome                            | 264.26±9.37  |              |
|     | Length(μm)±SEM ×10                  |              | 12.99±0.73   |
|     | Width(μm)±SEM ×10                   |              |              |

*Data was presented in Mean±SEM (Mean±Standard Error of Mean of 30 determinations).*
3.3 Physicochemical Evaluation

Dried powders of the leaf and stem were used for the quantitative evaluation of different physicochemical determination. The moisture contents of the dried leaf and stem powders of 10.58 %/w/w and 13.12% w/w were determined by loss on drying method and is presented in Table 4. These results showed that the moisture contents were not high and could not encourage fungal and bacterial growth. The analytical results of the total ash were found to be 8.24% w/w and 11.15% w/w. The total ash for both leaf and stem were amorphous and greyish white in colour. The water soluble ash of 3.71% w/w and 3.20% w/w, acid-insoluble ash of 0.99% w/w and 4.33% w/w were obtained respectively for the leaf and stem.

For the extractive values, the water-soluble extractive values for the leaf and stem were found to be higher in water soluble fractions with values of 12.50±0.10% w/w and 9.40±0.03% w/w compared to those of ethanol, ethyl acetate and methanol respectively as seen in Table 4.

3.4 Powder Microscopy

The results for the powder microscopy is as shown in Fig. 3.

Table 3. Preliminary phytochemical screening result for leaf and stem of C. pachycarpa

| Test                | C. pachycarpa |
|---------------------|---------------|
|                     | Leaf | Stem |
| Alkaloids           | +    | -    |
| Flavonoids          | +    | +    |
| Saponins            | +    | +    |
| Tannins             | +    | +    |
| Cardiac glycosides  | +    | -    |
| Anthraquinone       | -    | -    |

Key: + = Present, - = Absent

3.5 Micromeric Evaluation of the Leaf and Stem of Cola pachycarpa

The micromeric evaluation for the leaf and stem powders for C. pachycarpa are summarized in Table 5. The leaf and stem powders had Hausner’s ratios of 1.33±0.01 and 1.37±0.02, compressibility index of 25.00±0.67 and 26.80±0.88% and angle of repose 35° and 45° respectively. The pH of 7.56 and 7.57 for the leaf and stem respectively when cold was neutral but acidic when hot with 3.66 and 4.84 respectively.

Table 4. Physicochemical constants of leaf and stem of Cola pachycarpa

| Parameter               | Leaf (%/w/w) | Stem (%/w/w) |
|-------------------------|--------------|--------------|
| Moisture content        | 10.58±0.02   | 13.12±0.04   |
| Total ash               | 8.24±0.01    | 11.15±0.00   |
| Water-soluble ash       | 3.71±0.00    | 3.20±0.00    |
| Acid-insoluble ash      | 0.99±0.00    | 4.33±0.00    |

Extractive value (%/w/w)

| Extractive value         | Leaf (%/w/w) | Stem (%/w/w) |
|--------------------------|--------------|--------------|
| Water-soluble            | 12.50±0.10   | 9.40±0.30    |
| Ethanol                  | 9.10±0.10    | 2.90±0.70    |
| Ethyl acetate            | 2.00±0.20    | 0.50±0.40    |
| Methanol                 | 8.00±0.30    | 2.30±0.30    |

Data are represented as average of 3 determinations in mean ± SEM (Standard Error of Mean)

Fig. 3. Powder microscopic characters of C. pachycarpa
(A): Polygonal epidermal cell shape and straight ACWP × 10; (B): Stellate trichome ×4; (C): Anisocytic stomata, polygonal cell shape and straight/curved ACWP × 40
Table 5. Micromeritic evaluation of powdered leaf and stem of *C. pachycarpa*

| Micromeritic Parameters | *C. pachycarpa* leaf | *C. pachycarpa* stem |
|-------------------------|----------------------|----------------------|
| Bulk Volume (mL)        | 42.33±0.44           | 50.83±0.17           |
| Tapped Volume (mL)      | 31.17±0.16           | 36.83±0.44           |
| Bulk Density (g/mL)     | 0.24±0.00            | 0.20±0.00            |
| Tapped Density (g/mL)   | 0.32±0.00            | 0.27±0.00            |
| Hausner Ratio           | 1.33±0.01            | 1.37±0.02            |
| Carr’s Index (%)        | 25.00±0.67           | 26.80±0.88           |
| Diameter of Heap (cm)   | 7.44±0.03            | 7.39±0.04            |
| Height of Heap (cm)     | 2.56±0.09            | 3.70±0.15            |
| Flow Time (sec)         | 7.00±0.00            | 30.00±1.00           |
| Flow Rate (g/sec)       | 1.42                 | 0.33                 |
| Angle of Repose (°)     | 35                   | 45                   |
| pH                      | 7.56±0.00            | 7.57±0.00            |
| Cold                    | 3.66±0.00            | 4.84±0.00            |
| Hot                     | 3.66±0.00            | 4.84±0.00            |

Results presented as Mean±SEM (Standard Error of Mean) of Three (3) Determinations

3.6 Chemomicroscopic Evaluation

The chemomicroscopic examination of the leaf and stem revealed the presence of lignin, starch, mucilage, calcium oxalate crystals, cellulose, fatty oil and protein as summarized in Table 6.

3.7 Fluorescence Analysis of Leaf and Stem Powder

The characteristic colour behavior of the dried leaf and stem powdered drug dissolved in organic solvents was observed both under visible and Ultra-violet (UV) light. The reactions of the drugs emitted fluorescence light as summarized in Table 7. The powdered drug solutions had exhibited a wide range of fluorescence colours under the UV and visible light.

Table 6. Chemomicroscopic evaluation of the leaf and stem of *C. pachycarpa*

| Test                  | *C. pachycarpa* Leaf | *C. pachycarpa* Stem |
|-----------------------|----------------------|----------------------|
| Lignin                | +                    | +                    |
| Starch                | +                    | +                    |
| Cellulose             | +                    | +                    |
| Oils                  | +                    | +                    |
| Calcium oxalate crystals | +                  | +                    |
| Mucilage              | +                    | +                    |
| Protein               | +                    | +                    |

Key: + = present; - = absent

4. DISCUSSION

Traditionally the seeds are used as stimulant and the fruits as source of nutrients by the locals [8]. Due to the various medicinal properties of *C. pachycarpa* plant, researchers are encouraged to explore more information on this drug. Adulteration due to substitution with a close relative and misidentification of crude drugs can cause serious health problems on administration of the drug therefore caution need be taken to safeguard the consumers. Establishing the identity and degree of purity becomes paramount before any test is undertaken.

So in the present study important diagnostic characters determining the authenticity and purity of the medicinal plant parts were observed and recorded.

Organoleptic evaluations were carried out by means of the sense organs thereby defining some diagnostic characteristics of the plant material which can be measured as a first step to the establishment of degree of purity and identity. These principles were sensitive criteria based on individual’s perception [19].

Macroscopic characteristics revealed that the leaves were green on the adaxial surface but grey on the abaxial surface (Fig. 1), odourless and tasteless. Leaf is alternate, petiolate, compound trifoliate. Petiole up to 50 cm long, leaflets 20-38 cm long, 10-19 cm wide, the middle leaflet often longer than others. Leaflets is subsessile to short petiolulate. Leaflet shape is elliptic, apex is short acuminate, margin entire to wavy and texture is hairy on both surfaces of the leaf with brown caducuous hairs on the lower surface. Stem erect, woody, about 4-10 cm in diameter. Fruits have edible white mesocarp which is sweet and tasty. Fruit epicarp is velvety and fruits have within 3-5 seeds (white) occur in clusters of 4-5 on the stem [20].
Table 7. Fluorescence analysis of *C. pachycarpa* leaf and stem powder

| Extract                        | Sample  | Visible light | Under UV light | Under UV light |
|--------------------------------|---------|---------------|----------------|---------------|
|                                |         |               | Short Wave     | Long Wave     |
|                                |         |               | length (253.7nm)| length (365nm)|               |
| Picric acid(2,4,6-trinitrophenol)| Leaf    | Brown         | Black          | Dark black    |
|                                |         |               |                |               |
|                                |         | Light brown   | Brown          | Black         |
|                                |         |               |                |               |
|                                | Stem    | Light brown   | Brown          | Maroon        |
|                                |         |               |                |               |
| Dichloromethane                | Leaf    | Green         | Brown          | Pink          |
|                                |         |               |                |               |
|                                |         | Light green   | Orange         | Pink          |
|                                |         |               |                |               |
|                                | Stem    | Light green   | Orange         | Pink          |
|                                |         |               |                |               |
| Ethyl acetate                  | Leaf    | Light green   | Orange         | Pink          |
|                                |         |               |                |               |
|                                |         | Maroon        | Red            | Pink          |
|                                |         |               |                |               |
|                                | Stem    | Maroon        | Red            | Pink          |
|                                |         |               |                |               |
| Methanol                       | Leaf    | Green         | Red            | Maroon        |
|                                |         |               |                |               |
|                                |         | Brown         | Light pink     | Brown         |
|                                |         |               |                |               |
|                                | Stem    | Brown         | Light pink     | Brown         |
|                                |         |               |                |               |
| Water                          | Leaf    | Green         | Orange         | Brown         |
|                                |         |               |                |               |
|                                |         | Grey          | Light ash      | Light brown   |
|                                |         |               |                |               |
|                                | Stem    | Grey          | Light ash      | Light brown   |
|                                |         |               |                |               |
| Ferric chloride                | Leaf    | Black green   | Light pink     | Pink          |
|                                |         |               |                |               |
|                                |         | Light green   | Brown          | Light brown   |
|                                |         |               |                |               |
|                                | Stem    | Light green   | Brown          | Light brown   |
|                                |         |               |                |               |
| Acetic acid                    | Leaf    | Forest green  | Red            | Brown         |
|                                |         |               |                |               |
|                                |         | Light brown   | Brown          | Red           |
|                                |         |               |                |               |
|                                | Stem    | Light brown   | Brown          | Red           |
|                                |         |               |                |               |
| Iodine in water (1%)           | Leaf    | Green         | Black          | Light Brown   |
|                                |         |               |                |               |
|                                |         | Black         | Black          | Brown         |
|                                |         |               |                |               |
|                                | Stem    | Black         | Black          | Brown         |
|                                |         |               |                |               |

Leaf epidermal studies as stated by Carlquist [21] provides a variety of features that could be useful for taxonomic purposes and most researchers have applied leaf anatomy for solving taxonomic problems in species of plants for example Adedeji [22] on *Emilia*, Illoh [23] on genus *Celosia*, Bassey and Sunday on varieties of *Lasianthera africana* [24] and Adedeji and Illoh [25] on *Hibiscus*. Therefore, the various parameters studied in this research were, stomatal pore width and length, guard cell width and length, stomatal number, epidermal cell number, epidermal cell width and length, epidermal cell wall thickness, venation (vein islet, vein termination, areole) and powder analysis. The values of these parameters are useful for detecting adulterants [26].

The quality control of crude drugs and herbal formulation is of paramount importance in justifying their acceptability and safety assurance in modern system of medicine. But one of the major problems faced by the herbal drug industry is non-availability of rigid quality control profile for herbal material and their formulations. Standardization is an essential measurement for ensuring the quality control of the herbal drugs and also encompasses the entire field of study from birth of a plant to its clinical application [27].

The shape of the epidermal cells of *C. pachycarpa* studied was polygonal on the adaxial and abaxial surfaces respectively as shown in Fig. 3A and 3B but with straight anticinal cell wall pattern and slightly curved anticinal cell wall pattern for both adaxial and abaxial surfaces respectively. Aworinde [28] reported on the irregular epidermal cell shape and also slightly curved anticinal cell wall pattern for *C. millenii* and *C. hispida*. These are distinctive delimiting feature with regards to the epidermal cell shape and anticinal cell wall pattern (ACWP). These finding were in agreement with Stace [29] who suggested that environmental conditions such as humidity play a significant role in determining the pattern of anticinal cell walls.

*C. pachycarpa* leaves were hypostomatic (stomata on the abaxial surface only) which is a distinctive feature for *C. pachycarpa*. Aworinde [28] reported same for *C. millenii* and *C. hispida* with the absence of stomata on their adaxial surfaces but present on their abaxial surfaces. Metcalfe and Chalk, [10] distinguished *Waltheria indica* from other species using the presence of paracytic and anisocytic stomata which was diagnostic. Anisocytic stomata were observed on the abaxial surface as Pan and Jacobs [30] reported that majority of the Monkey Kola had brachyparacytic and paracytic stomatal complex but hexacytic was not common. The occurrence of more stomata on the abaxial surface is an adaptation to water loss [31] as this signifies a coping strategy to survive drought as was seen in *C. pachycarpa* with stomatal number of (17.87±0.34). The stomatal index (12.41%), stomatal length (49.01±0.67 µm), stomatal width (36.09±0.92 µm), stomatal pore length (26.82±0.59 µm), stomatal pore width (9.11±0.21 µm) (Table 3) were recorded also. Goji and
Ayodele [32] used same parameters in delimitation of some Cola species. Bassey and Sunday [23] were able to differentiate the forest and riverine variety of Lasianthera africana using stomatal size, cell wall pattern and length of trichome. Mbagwu et al. [31] reported that an adaptation to water loss was due to the large density of stomata on the abaxial surface and this appears to be a coping strategy to survive drought. Also, in agreement with Metcalfe and Chalk [10] and Mbagwu and Edeoga [33] who observed that stomata are usually more on the lower epidermis in species of Amaranthus and Vigna respectively.

Measurements of stomatal pore length and width may also support its identification. Gill and Nyawuame [34] have used similar characters in the systematics and phylogeny of the members of Bicarpellatae. However, care should be taken as these measurements can be influenced by the physiological activities of the guard cells especially photosynthesize (any compound that is a product of photosynthesis) accumulation.

Foliar venation has been used by Levin [35] to provide insight into relationship within the subfamily Phyllathiodeae and also in the tribe Euphobiaceae by Seghal and Paliwal [36]. For the abaxial and adaxial surfaces; vein termination number (1.67±3.00 µm and 3.63±0.11), vein islet number (11.26±9.38 µm and 3.63±0.11), areole length (232.15±10.85 µm and 285.78±3.35) and width (175.11±8.35 µm and 208.19±4.46) for C. pachycarpa were recorded respectively. The venation was pinnate. The areole shape was quadrangular with various sizes while the veinlet endings was linear to curved and biforked. For this study the venation characters viz; number of vein termination number, vein islet number, average areole length and width may be useful for the identification of this Monkey Kola.

However, investigation of the morphology of calcium oxalate crystal in Monkey Kola has been very limited [26]. Drusiferous crystals was recorded in the petiole of C. pachycarpa (Fig. 2Ei) which may be used in the identification and delimitation of the taxa as Graciamo-Ribeiro et al [37] reported same for the species of Manihot.

Plants are the major contributors of natural products and are usually rich in medicinal and nutritional properties. Many natural products are biologically active and have been used for thousands of years as traditional medicines. Chemical constituents in plants can be helpful in discovering new medicinal plants and solving taxonomical problems [38,39,40]. Alkaloids, flavonoids, saponins, tannins and glycosides were found present in the leaf extract while all mentioned except alkaloids and glycosides were found absent for the stem extract. However, anthraquinone was absent for both leaf and stem. Oyemitan et al. [41] reported the presence of same for the leaf extract for C. pachycarpa.

4.1 Powder Evaluation

The moisture content for leaf and stem were calculated through loss on drying method and were found to be 10.58%w/w and 13.12%w/w respectively (Table 4) which were within the recommended range of 8-14%w/w for vegetable drug and this is an indication that the plant can be stored for a long period of time with less probability of microbial attack [42]. Water soluble extractive value were highest for both leaf and stem, which were found to be 12.50±0.10%w/w and 9.40±0.30%w/w respectively compared to other extractive values of the present study (Table 4). This may be due to the presence of high amount of water soluble compounds in the leaves and stems of C. pachycarpa. The water permeates the cells and thus, a better extractant for C. pachycarpa leaf and stem.

Mbah et al., [18] studied the pharmaceutical characterization of Bindeia ferruginea Benth (Euphorbiaceae) using the flow properties. Leaf and stem powder were greenish brown and brown respectively (Fig. 2), with no specific odour or taste. They leaf and stem powders had fair and passable flow properties with angle of repose 35° and 45° respectively as Umoh et al [43] reported a poor flow for the leaf of Culcasia scandens with an angle of repose of 38°. Bassey et al [44] reported the relatedness between Nephrolepis biserrata, Nephrolepis exaltata and Nephrolepis undulata as having a fair flow property with the angle of repose of 38°-36° respectively which is a is a property related to inter-particle friction of resistance to movement between particles [45] Diagnostic microscopic powder features include polygonal epidermal cell shape and straight anticinal cell wall pattern, stellate trichome and anisocytic stomatal complex type (Fig. 3; A, B and C) respectively.

The micromeritic properties like bulk density, tap density, angle of repose, Hausner’s ratio and Carr’s index indicates the flow properties as well as interparticulate resistance between powders.
This information predicts the stability and solubility of crude drug. Increase in bulk density reduces paste thickness which is important in the preparation of drugs.

The ratio of tapped density to the bulk density of the powder is called Hausner’s ratio. This ratio is a useful measure of cohesion reflecting particle friction. Hausner’s ratio higher than 1.4, the powder is considered a cohesive difficult to fluidize powder. It is free flowing powder when ratio is lower than 1.25. Carr’s compressibility index is good if the value ranges between 5%-15% [19]. For C. pachycarpa leaf and stem had 25.00±0.67 and 26.80±0.88 (Table 5) signifying a poor flow. The angle of repose is considered to be the most classical technique used for characterizing the flow properties of powders. It is a characteristic related to interparticulate friction or resistance to movement between particles [46, 47]. If the angle of repose is more than 50°, the powder will not flow satisfactorily and if it is near 25°, the powder will flow easily. The angles of repose for C. pachycarpa were 34.5° and 45° for the leaf and stem respectively which showed a poor flow due to the fiber-like nature of the powder. The micromeritic properties help to characterize and standardize the pre-formulation properties of the herbal powder, in other to determine its suitability for formulation into solid dosage forms [18]. This result may be used in identification and authentication of C. pachycarpa.

The pH values for cold infusion for both leaf and stem ranges between 7.57 – 7.57 while hot infusion were within 3.66 – 4.84 (Table 5). Specifically, the cold infusion for C. pachycarpa is basic while the hot infusions were acidic. Consumption of acidic beverages (as seen with herbal teas) can give a sour taste, even resulting in irritation of the oral mucosa. Phelan and Rees [48] have reported similar observation on their work adding that consumption of acidic infusion and decoction of herbal drugs has the potential of wearing off the teeth enamel leading to dental problems. From the results, the most acidic determines the one that impacts on the dental enamel by causing demineralization of the tooth [49].

Chemomicroscopy analysis of the leaf and stem of the plant recorded the presence of lignin, cellulose, mucilage, calcium oxalate, starch, oil and protein as shown in Table 6. In fluorescence analysis the powdered treated with different chemical reagents were observed under visible and short UV light. The colour change for the leaf and stem powders were distinctive and reproducible revealing the solvent properties to the phytoconstituents Table 7.

5. CONCLUSION

The results of the study can serve as a valuable source of information and provide suitable standards for identification of this plant material in future investigations and applications.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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