Original Research Article

PHYTOPHARMACOGNOSTIC ST AND ARDIZATION OF THE LEAVES OF *BUCHHOLZIA CORIACEA* ENGLER. (CAPPARIDACEAE)

Comment [M1]:

ABSTRACT

Buchholzia coriacea Engl. is a medicinal plant belonging to the family Capparidaceae. It has antimicrobial, anthelmintic, antidiabetic and antihypercholesterol activities. The aim of this study was to employ the quality control parameters in the evaluation of the leaf of *B. coriacea*. The plant leaves were collected, air dried, pulverized and stored in a clean glass container. Standard procedures were carried out to obtain the microscopic features of the fresh and powdered samples, micromeritic, chemomicroscopy, fluorescence properties, soluble extractive values, moisture contents, ash values. Results of the microscopic study using fresh and powdered leaf samples revealed the presence of paracytic stomata on the abaxial surface (hypostomatic) with mean length of 31.8 ± 0.62 cm. The cell shape was polygonal and straight anticlinal cell wall pattern. Results of the micromeritic properties of the powdered sample showed bulk volume of 31.00± 0.70cm, tapped volume of 25.1± 0.20 cm, bulk density of 0.32 ± 0.01g/m, tapped density of 0.39± 0.00g/ml, flow rate of 2.2 ± 0.08g/s, angle of repose of 26.1 ± 1.3 degree, carr's index of 18.8± 1.34 % , Hausner's ratio of 1.2 ± 0.02, pH of 8.0 when cold and 8.2 when hot for the powdered sample. Chemomicroscopy study revealed the presence of lignin, mucilage, calcium oxalate crystals, oil, calcium carbonate and starch was absent. Results for the ethanol-soluble extractive value was 7 ± 0.00% $^{w}/_{w}$, water-soluble extractive value was 14 ± 0.00% $^{w}/_{w}$ and methanol-soluble extractive value was 3 ± 0.00% $^{w}/_{w}$ for the powdered samples. Results for the moisture content was 10.3 ± 0.00% $^{w}/_{w}$, total ash values was 6.3± 0.00% $^{w}/_{w}$, acid-insoluble ash value was 1 ± 0.00% $^{w}/_{w}$, water-soluble ash value was 3±0.00% $^{w}/_{w}$ and sulfated ash values was 7.5 ± 0.00% $^{w}/_{w}$. In conclusion, the above evaluation methods and parameters there in could be used to identify and authenticate both the fresh and powdered crude drug product of *Buchholzia coriacea*.

Comment [M2]: Carr's

Keywords: Antihypercholesterol, *Buchholzia coriacea*, Chemomicroscopy, Micromeritic, Pharmacognostic, Standardization, Therapeutic .

Comment [M3]: Rearrange keywords

INTRODUCTION

Herbal medicine is recognized as the most common form of alternative medicine [1]. The use of herbs in the treatment of illness has been very successful over the years and its historical usage has been valuable in drug discovery and development. The popularity and availability of the traditional remedies have generated concerns regarding the safety, efficacy and responsibility of practitioners using traditional remedies [2].

The world health organization (WHO) estimates that 80% of the World's population relies on the alternative plant-based medicine as their primary medicinal intervention especially in the

developing countries, even in developed countries [3,4]. Herbal remedies are considered safer and less damaging to the human body than synthetic drugs [5].

However, the lack of standardization has been a major concern regarding the use of herbal medicines [6]. Therapeutic efficacy of medicinal plants depends upon the quality and quantity of chemical constituents. Standardization of herbal formulations is essential in order to assess the quality of drugs, based on the concentration of their active principles. It becomes extremely important to make an effort towards standardization of the plant materials as medicine [7]. The process of standardization can be achieved by stepwise pharmacognostic studies [8].

The plant *Buchholzia coriacea* is a shrub or medium-sized tree, evergreen, with a dense crown, large glossy leathery leaves arranged spirally and clustered at the ends of the branches, and conspicuous cream-white flowers in racemes at the end of the branches. The bark of the plant is smooth, blackish-brown or dark-green. Slashes are deep red turning dark brown [9, 10]. The leaves of the plant are large, petiolate, oblanceolate to elliptic, shortly acuminate or acute at apex, cuneate at base, 15-30 x 5-11cm, thinly coriaceous, glabrous, midrib very prominent below, about 10 lateral nerves, each running directly into the one above and forming distinct loops close to the margin, prominent below, stalk 10-15 cm long, swollen for about 1cm at both ends, pale green. It is widely distributed in African countries, from Guinea and Sierra Leone to Cameroun, Nigeria, Congo, Angola, Ghana, Liberia and Gabon. The seeds contain essential trace metals cations and phosphorus. The plant also contains amino acids, fatty acids, minerals such as cation (Calcium, magnesium, sodium and potassium) there is absence of non-essential trace metals such as lead and chromium. [11]. It also contains alkaloids, saponins, tannins, flavonoids, oxalates, phytates, cardiac glycosides, steroids, resins, carbohydrate, anthraquinone and glycosides [12].

Several plant parts of *Buchholzia coriacea* are commonly used in traditional medicine in West and Central Africa. Bark extracts are applied as an enema to treat back pain, leaf decoctions are taken to treat sterility in women, leaf infusions are applied to the eyes against filarial nematodes, and powdered or pulped leaves are used to treat fever, rheumatism, ulcers, boils and haemorrhoids. Pharmacologically, the seeds are used in the management of diabetes and also possess antioxidant property [8, 13].

Comment [M4]: Caps

Comment [M5]: ??

Figure 1 *Buchholzia coriacea* Engl in its natural habitat
A: With Flowers B: With Fruits

Phylogeny of *Buchholzia coriacea* Engl (Scientific Classification) According to Angiosperm Phylogeny Group (APG 2016) System [14].
Kingdom – Plantae

Clade – Tracheophytes
Clade – Angiosperms
Clade – Eudicots
Order – Brassicales
Family – Capparidaceae
Genus – *Buchholzia*
Species – *Buchholzia coriacea* Engler.
Common name: Wonderful Kola
Local name: Igbo – uke, Yoruba – Uworo

MATERIALS AND METHOD

Collection and Identification of Plant

The leaves of the plant were collected from medicinal plants farm, faculty of pharmacy, University of Uyo, along Ikpa road, Uyo Local Government Area, Akwa Ibom State, Nigeria in August 2019. The plant was identified and authenticated in Faculty of Pharmacy, University of Uyo herbarium with the identification number: *Buchholzia coriacea* - UUPH No. 17(b)

Preparation of the Collected Plant Leaves

The fresh plant leaves were collected air-dried, pulverized and packed in dry container, well labelled and used when needed.

Determination of Microscopical Features

Qualitative microscopy of leaf procedure
Matured fresh leaves of the plant were cut between the base and the apex. Epidemical peels of both the adaxial and abaxial surfaces were made by placing the leaf on a clean glass slide. The sample surface was irrigated with water holding the sample from the end. The epidermis above the desired surface was scrapped off carefully with a sharp razor blade. Loose cells were washed away from the epidermal peel with water until the desired epidermis was reached the epidermal peels were further cleared with sodium hypochlorite and rinsed thoroughly with water. The epidermal peels were stained with aqueous solution of safranin-O for five (5) minutes and mounted in 10% glycerol and Olympus C X 21 binocular microscope. Photographs of the microscopical features such as stomatal, morphological prepared slides were taken with an amscope. Also, the powder, Transverse section of the leaf of the sample were also prepared, mounted and viewed with the microscope and photographs taken [15].

Quantitative Leaf Microscopy
Quantitative microscopy parameters such as leaf constant studies which include stomatal length and width, guard cell length and width, stomatal number, stomatal index, epidermal cell length and width, epidermal cell number, epidermal cell thickness were carried out using standard procedures.

All measurements were made using a calibrated ocular micrometer and ten (10) microscopic fields chosen at random were used and data presented as mean ± SEM.

The stomatal index (S.I) was determined according to Metcalfe and Chalk [16] using the formula:
Stomatal Index (SI) = S/E +S x 100

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

Micromeritics

The flow property was determined using standard methods [17] which constitutes;

Bulk Density and Tapped Density

The weight of 10 g of dried powdered leaf was weighed into 100 mL measuring cylinder and the volume occupied was noted as the bulk volume (Vb). The cylinder was gently tapped repeatedly to obtain a constant volume noted as the tapped volume (Vt). Bulk density was calculated using the formula below;

$$B\rho = \frac{M}{Vb}$$

Where;

$$T\rho = \frac{M}{Vt}$$

Where $B\rho$= Bulk density
M = Mass of powder
Vb= Bulk volume of powder
$T\rho$ = Tapped density
Vt= tapped volume

Interparticulate porosity is calculated using the formula below;

IP = $\frac{\rho T - \rho B}{\rho T + \rho B}$

Hausner's ratio and Carr's index

Hausner's ratio a function of interparticle friction is calculated using the formula

$$Hausner's\ ratio = \frac{T\rho}{B\rho}$$

While Carr's Index is measured as

$$Carr's\ index = \frac{T\rho - B\rho}{T\rho} \times 100$$

Where; $T\rho$ = Tapped density
$B\rho$ = Bulk density.

Angle of repose

$$\theta = Tan^{-1}\left(\frac{Heap\ height\ of\ powder}{Radius\ of\ heap\ base}\right)$$

pH

A pH meter (Jenway, Stafford Shire, UK) was used to determine the pH of both cold and hot extract of the leaf.

Chemomicroscopic Analysis of Leaf Powder

Powdered leaf was examined for its chemomicroscopy properties using standard procedures [18].

Fluorescence Analysis of Leaf Powder

The fluorescent analysis of dried leaf powder was carried out using standard method [19].

Physico-chemical Evaluation of Leaf Powder

The physicochemical parameters such as moisture content, ash values (total ash, acid insoluble ash, water soluble ash, sulphated ash), soluble extractive values viz. ethanol, methanol and water were performed according to the official method [20].

Comment [M6]: Chemical test?

RESULTS

Table 1: Epidermal, Stomatal Characteristics of *Buchholzia Coriacea* leaf

Leaf Surface	Abaxial Surface	Adaxial Surface
Epidermal cell shape	Polygonal	Polygonal
Epidermal cell wall pattern	straight anticlinal wall	straight anticlinal wall
Epidermal Cell length(µm)	54.50±0.8	57.82±0.8
Epidermal cell width(µm)	24.07±0.6	25.27±0.4
Stomatal Length (µm)	31.84±0.6	Absent
Stomatal Width (µm)	18.64±0.4	Absent
Stomatal Number	44.4±0.3	Absent
Stomatal Index	9.41%	Absent
Type of stomata	Paracytic	Absent

Results presented as Mean±SEM of Ten (10) Replicates

Table 2: Micromeritic evaluation of *Buchholzia Coriacea* Powdered Leaf

Micromeritic Parameters	Leaf Powder
Bulk Volume (mL)	31.00±0.70

Tapped Volume (mL)	25.16±0.20
Bulk Density (g/mL)	0.32±0.01
Tapped Density (g/mL)	0.39±0.01
Hausner Ratio	1.23±0.02
Carr's Index (%)	18.88±1.34
Flow Rate (g/sec)	2.27±0.08
Angle of Repose (^{0})	26.15±1.35
Ph Cold Hot	 8.06 8.22

Results presented as Mean±SEM of Three (3) Replicate

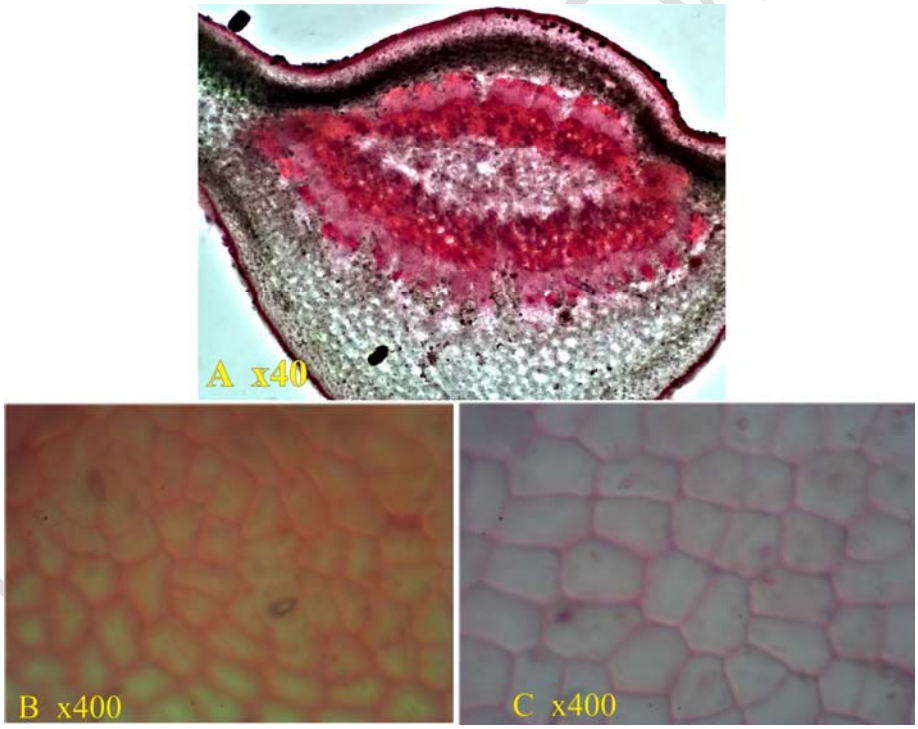

Figure 2: Microscopic Features of Fresh Leaf of *B. coriacea* – (A) Midrib of the leaf showing vascular bundles, (B) paracytic stomata (C) epidermal cell (polygonal in shape and straight anticlinal cell wall pattern)

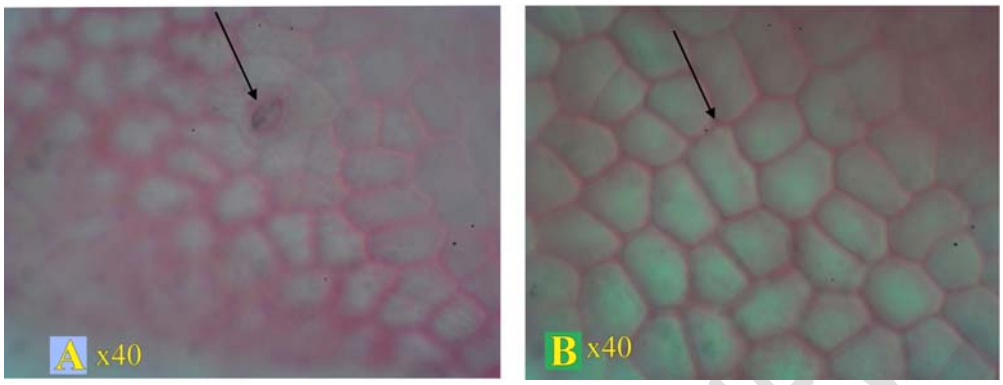

Figure 3: Microscopic Features of Powdered leaf of *B. coriacea* – (a) paracytic stomata (b) Epidermal cell (polygonal in shape and straight anticlinal cell wall pattern)

Table 3 Showing Chemomicroscopic Evaluation of Leaf of *B. coriacea* Powder

Parameters	**Leaf**
Lignin	+
Starch	–
Oils	+
Calcium Carbonate	+
Calcium Oxalate crystal	+
Mucilage	+

+ = Present, - = Absent

Table 4: Results for Fluorescence Properties of *B. coriacea*

Extract	Sample	Physical observation Colour	UV-365 Nm Colour	UV-253 Nm Colour
Water	Leaf	Brown	Dark brown	Dark grey
Methanol	Leaf	Light green	Orange	Grey
Ethanol	Leaf	Light green	Orange	Light grey
DCM	Leaf	Green	Dark red	Lemon
n-hexane	Leaf	Colourless	Green	Lemon
Ethylacelate	Leaf	Light yellow	Maroon	Lemon

Table 5:Result of Moisture Content, Total Ash Value, Acid-Insoluble ash Value, Water-Soluble Ash Value, Sulfated Ash Value, Water-Extractive Value, Methanol Extractive Value and Ethanol Extractive Value of the *Buchholzia coriacea* powdered leaf.

PARAMETERS	Weight (g)	Percentage (% $^{w}/_{w}$)
Moisture Content	0.31	10.3
Total Ash value	0.19	6.3
Acid-insoluble ash value	0.03	1.0
Water-soluble ash value	0.09	3.0
Sulfated ash value	0.15	7.5
Water-soluble extraction value	0.56	14
Methanol-soluble extraction value	0.12	3
Ethanol-soluble extraction value	0.28	7

Results presented as Mean±SEM of Three (3) Replicates

Discussion

Plants have been seen as a source of medicinal agents for thousands of years since the origin of man [21]. The first step towards ensuring quality of any medicinal product is to ensure quality of its starting materials by proper authentication. However, there has been an increase in consciousness of need for standardization of medicinal plants with potential use [22]. Pharmacognostic studies is more reliable [23], and according to WHO, the qualitative and quantitative microscopic features would prove useful for laying down pharmacopoeial standards. Morphology as well as various pharmacognostic aspects of leaf of the plant was studied. The qualitative microscopic studies of the epidermal layers of the research plant, revealed the presence of Paracytic type of stomata, present in their abaxial surface of the leaf and absent in the adaxial surface (hypostomatic) and absence of trichomes on both surfaces. The epidermal cell wall layers of the researched plant showed mean stomatal index of 9.41, mean stomatal length of

31.8μm, mean stomatal width of 18.6μm and mean stomatal number of 44.4 (per area) on the abaxial surface. Other microscopic features are presented in Table 1. The Transverse section of the midrib of the leaf reveals the presence of vascular bundles.

The micrometric studies showed angle of repose of 26.1 degrees, Hausner's ratio of 1.2 and Carr's index of 18.8% for the leaf powders. Micrometric is an important consideration in the development of solid dosage formulations, which is mostly used for physical, mechanical and chemical processes [24]. It influences a number of process parameters in manufacturing pharmaceutical formulations. The knowledge and effect of particle size distribution of active pharmaceutical ingredients, as well as excipients, will be useful to solve the difficulties in critical process parameters. In particular regards to tablet and capsule, controlling the particle size and particle size distribution is mainly important because they have direct impact on the flowability, tableting, content uniformity, weight variation and dissolution rate which ultimately affect the bioavailability of drug. Good flow properties of powders are essential for uniform filling into dies of tableting machines and for easy movement of materials around a production facility. Factors that affect the flow properties of powder include: moisture content, temperature, particles size, particle shape (texture), and time of storage. The angle of repose is considered to be the most classical technique used for characterizing the flow properties of powders. Angle of repose is a characteristic related to inter-particulate friction or resistance to movement between particles [25] . An alternative test is to determine the Carr's index which relates the bulk density of the material to the tapped density and from the results obtained the powder was said to have an excellent to fair flow characteristics.

Chemomicroscopy study revealed the presence of mucilage, lignin, calcium oxalate, calcium carbohydrate, and oil, but absence of starch. The water-soluble extract value was 14% $^{w}/_{w}$, methanol-soluble extractive value was 3% $^{w}/_{w}$, ethanol-soluble extractive value was 7% $^{w}/_{w}$ for the leaf powder as shown in table 5, extractive values determine the amount of the active constituent in a given plant material when extracted with a particular solvent. The extractive values of a solvent with a crude drug are useful for their evaluation and also for the estimation of specific constituents soluble in that particular solvent used for extraction. The extractive values are useful for the estimation of the degree of exhaustion of the plant's powdered sample and they give the idea about the nature of the chemical constituents present in the crude drug.

The moisture content obtained was 10.3% $^{w}/_{w}$ for the leaf powder which is within the stated limit as shown in table 5. The African pharmacopoeia limit of moisture content for vegetable drug ranges from 8% $^{w}/_{w}$ to 14% $^{w}/_{w}$ with few exceptions (e.g. digitalis leaf, 6% $^{w}/_{w}$). High moisture content is uneconomical, and in the presence of suitable temperature could lead to enzymatic activation and hydrolytic reactions as well as proliferation of microbial growth which may ultimately lead to degradation of active constituents. Excess moisture in a sample suggests that the drug has been incorrectly prepared or inappropriately stored. This is especially important for materials that absorb moisture easily or deteriorate quickly in the presence of water. Thus, moisture content limits are generally specified in pharmacopoeias, as indicated above.

The total ash value was 6.3%$^{w}/_{w}$, acid-insoluble ash value was 1%$^{w}/_{w}$ (this shows low content of earthly materials), water-soluble ash value was 3%$^{w}/_{w}$ and sulfated ash value was 7.5% $^{w}/_{w}$ for leaf powder as shown in table 5. The Ash values are used to determine the quality and purity of crude drug. Generally, the amount of ash contained in a crude vegetable must be low. It indicates

to some extent the amount of care taken in the preparation of the drug. It indicates also the presence of various impurities like carbonates, oxalates and silicates. Acid-insoluble ash value indicates contamination with earthly material. Thus, high acid-insoluble ash value indicates soil contamination in the drug. Water-soluble ash value is used to estimate the amount of inorganic compound present in crude vegetable drugs. The sulfated ash is the residual substance not volatilized when the sample is incinerated with concentrated sulphuric acid. This method gives results which are more precise since inorganic substances contained in the plant sample remain as sulphates, and is thus to determine the amount of inorganic substances, but occasionally for determining the amount of inorganic substances, or the amount of impurities contained in a heat volatile inorganic substance.

Conclusion

The data generated from this study would be of help in the authentication of various parts of *Buchholzia coriacea*, as an important constituent of various herbal drug formulations as well as to establish the purity and adulteration of the drug preparation.

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Comment [M7]: allignment

Comment [M8]: allignment