Phytopharmacognostic Standardization of the Leaves of *Buchholzia coriacea* Engler. (Capparidaceae)

Romanus A. Umoh¹*, Uwemedimo F. Umoh¹, Imoh I. Johnny¹, Anwanabasi E. Udo², Victor U. Anah³, Omodot T. Umoh⁴, Akwaowoh A. Elijah⁵ and Ndifreke S. Essienutin¹

¹Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Uyo Akwa Ibom State, Nigeria.
²Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, Uyo Akwa Ibom State, Nigeria.
³Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmacy, University of Uyo, Uyo, Akwa Ibom state, Nigeria.
⁴Department of Botany and Ecological Studies, Faculty of Sciences, University of Uyo, Uyo Akwa Ibom State, Nigeria.
⁵Department of Clinical Pharmacy and Biopharmacy, Faculty of Pharmacy, University of Uyo, Uyo Akwa Ibom State, Nigeria.

**Author’s contribution**

This work was carried out in collaboration among all authors. Authors RAU and IIJ designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors UFU, AEU, VUA and AAE managed the analysis of the study. Authors OTU and NSE managed the literature searches. All authors read and approved the final manuscript.

**Article Information**

DOI: 10.9734/JOCAMR/2020/v11i230183

*Editor(s):* (1) Dr. Arun Singh, Bareilly International University, India.

*Reviewers:* (1) Saurabha Bhimrao Zimare Naoroji Godrej, Centre For Plant Research (NGCPR), India.
(2) Manish A. Kamble, Rashtrasant Tukadoji Maharaj Nagpur University, India.

Complete Peer review History: [http://www.sdiarticle4.com/review-history/61554](http://www.sdiarticle4.com/review-history/61554)

**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...
1. 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 [2]. The popularity and availability of the traditional remedies have generated concerns regarding the safety, efficacy and responsibility of practitioners using traditional remedies [3,4].

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 [4,5,6]. Herbal remedies are considered safer and less damaging to the human body than synthetic drugs [7].

However, lack of standardization has been a major concern regarding the use of herbal medicines [8]. 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 [9,10]. The process of standardization can be achieved by stepwise pharmacognostic studies [11].

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 [12]. The bark of the plant is smooth, blackish-brown or dark-green [13]. Slashes are deep red turning dark brown [13,14]. The leaves of the plant are large, petiolate, ob lanceolate 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 Cameroon, 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 [15]. It also contains alkaloids, saponins, tannins, flavonoids, oxalates, phytates, cardiac glycosides, steroids, resins,
carbohydrate, anthraquinone and glycosides [16,17].

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 [18]. Pharmacologically, the seeds are used in the management of diabetes and also possess antioxidant property [11,19].

Phylogeny of *Buchholzia coriacea* Engl (Scientific Classification) According to Angiosperm Phylogeny Group (APG 2016) System [20].

Kingdom – Plantae
Clade – Tracheophytes
Clade – Angiosperms
Clade – Eudicots
Order – Brassicales
Family – Capparidaceae

2. MATERIALS AND METHODS

2.1 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).

2.2 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.

Fig. 1. *Buchholzia coriacea* Engl in its natural habitat
A: With Flowers B: With Fruits
2.3 Determination of Microscopical Features

2.3.1 Qualitative microscopy of leaf procedure

Matured fresh leaves of the plant were cut between the base and the apex. Epidermal 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 [21].

2.3.2 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 [22] using the formula:

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

Where,

\( S \) = number of stomata per unit area

\( E \) = number of epidermal cells in the same area.

2.4 Micromeritics

The flow property was determined using standard methods [23] which constitutes.

2.5 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 \((V_b)\). The cylinder was gently tapped repeatedly to obtain a constant volume noted as the tapped volume \((V_t)\). Bulk density was calculated using the formula below;

\[
B\rho = \frac{M}{V_b}
\]

Where;

\[
T\rho = \frac{M}{V_t}
\]

Where,

\( B\rho \) = Bulk density

\( M \) = Mass of powder

\( V_b \) = Bulk volume of powder

\( T\rho \) = Tapped density

\( V_t \) = tapped volume

Interparticulate porosity is calculated using the formula below;

\[
IP = \frac{\rho_t - \rho_B}{\rho_t} \times 100
\]

2.6 Hausner’s Ratio and Carr’s Index

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

\[
\text{Hausner’s ratio} = \frac{T\rho}{B\rho}
\]

While Carr’s Index is measured as

\[
\text{Carr’s index} = \frac{T\rho - B\rho}{T\rho} \times 100
\]

Where;

\( T\rho \) = Tapped density

\( B\rho \) = Bulk density.

2.7 Angle of Repose

\[
\theta = \tan^{-1}\left(\frac{\text{Height of powder}}{\text{Radius of heap base}}\right)
\]
Table 1. Epidermal, Stomatal Characteristics of *Buchholzia coriacea* leaf

<table>
<thead>
<tr>
<th>Leaf Surface</th>
<th>Abaxial Surface</th>
<th>Adaxial Surface</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epidermal cell shape</td>
<td>Polygonal</td>
<td>Polygonal</td>
</tr>
<tr>
<td>Epidermal cell wall pattern</td>
<td>straight anticlinal wall</td>
<td>straight anticlinal wall</td>
</tr>
<tr>
<td>Epidermal cell length (µm)</td>
<td>54.50±0.9</td>
<td>57.83±0.9</td>
</tr>
<tr>
<td>Epidermal cell width (µm)</td>
<td>24.07±0.6</td>
<td>25.28±0.5</td>
</tr>
<tr>
<td>Stomatal Length (µm)</td>
<td>31.84±0.6</td>
<td>Absent</td>
</tr>
<tr>
<td>Stomatal Width (µm)</td>
<td>18.64±0.5</td>
<td>Absent</td>
</tr>
<tr>
<td>Stomatal Number</td>
<td>44.4±0.3</td>
<td>Absent</td>
</tr>
<tr>
<td>Stomatal Index</td>
<td>9.41%</td>
<td>Absent</td>
</tr>
<tr>
<td>Type of stomata</td>
<td>Anomocytic</td>
<td>Absent</td>
</tr>
</tbody>
</table>

Results presented as Mean±SEM of ten replicates (n=10)

2.8 pH

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

2.9 Chemomicroscopic Analysis of Leaf Powder

Powdered leaf was examined for its chemomicroscopic properties using standard procedures [24].

2.10 Fluorescence Analysis of Leaf Powder

The fluorescent analysis of dried leaf powder was carried out using standard method [25].

2.11 Physico-Chemical Evaluation of Leaf Powder

The physicochemical parameters such as moisture content, ash values (total ash, acid insoluble ash, water soluble ash, sulfated ash), soluble extractive values viz. ethanol, methanol and water were performed according to the official methods [26].

3. RESULTS

The results of the epidermal and stomatal characteristics of *B. coriacea* are summarized in Table 1 and Figures 2 and 3.

3.1 Micromeritic Evaluation of the Leaf of *B. coriacea*

The micromeritic evaluation for the leaves of *B. coriacea* are summarized in Table 2. The leaf powder had Hausner’s ratio of 1.23±0.02, compressibility index of 18.88±1.34 and angle of repose 26.1±1.30. The pH of liquid extract of 8.0 when cold and 8.2 when hot.

Table 2. Micromeritic evaluation of *Buchholzia coriacea* Powdered Leaf

<table>
<thead>
<tr>
<th>Micromeritic Parameters</th>
<th>Leaf Powder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulk Volume (mL)</td>
<td>31.00±0.70</td>
</tr>
<tr>
<td>Tapped Volume (mL)</td>
<td>25.1±0.20</td>
</tr>
<tr>
<td>Bulk Density (g/mL)</td>
<td>0.32±0.01</td>
</tr>
<tr>
<td>Tapped Density (g/mL)</td>
<td>0.39±0.01</td>
</tr>
<tr>
<td>Hausner Ratio</td>
<td>1.23±0.02</td>
</tr>
<tr>
<td>Carr’s Index (%)</td>
<td>18.9±1.35</td>
</tr>
<tr>
<td>Flow Rate (g/sec)</td>
<td>2.2±0.08</td>
</tr>
<tr>
<td>Angle of Repose (°)</td>
<td>26.1±1.3</td>
</tr>
<tr>
<td>Ph</td>
<td>Cold 8.0</td>
</tr>
<tr>
<td></td>
<td>Hot 8.2</td>
</tr>
</tbody>
</table>

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

3.2 Chemomicroscopic Evaluation

The chemomicroscopic evaluation of the powdered leaf revealed the presence of Lignin, oils, calcium oxalate crystals and mucilage while starch was absent as summarized in Table 3.

Table 3. Showing Chemomicroscopic evaluation of leaf of *B. coriacea* Powder

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lignin</td>
<td>+</td>
</tr>
<tr>
<td>Starch</td>
<td>-</td>
</tr>
<tr>
<td>Oils</td>
<td>+</td>
</tr>
<tr>
<td>Calcium Carbonate</td>
<td>+</td>
</tr>
<tr>
<td>Calcium Oxalate crystal</td>
<td>+</td>
</tr>
<tr>
<td>Mucilage</td>
<td>+</td>
</tr>
</tbody>
</table>

+= Present, - = Absent
Fig. 2. Microscopic Features of Fresh Leaf of *B. coriacea* – (A) Midrib of the leaf showing vascular bundles, (B) anomocytic stomata (C) epidermal cell (polygonal in shape and straight anticlinal wall pattern)

Fig. 3. Microscopic Features of Powdered leaf of *B. coriacea* – (a) anomocytic stomata (b) Epidermal cell (polygonal in shape and straight anticlinal cell wall pattern)
3.3 Fluorescence Analysis

The results of the fluorescence analysis exhibited a wide range of fluorescence colours under the ultraviolet and visible light as summarized in Table 4.

3.4 Physicochemical Constants of Leaf of *B. coriacea*

The results of the physicochemical evaluation of the powdered leaf of *B. coriacea* is as summarized in Table 5.

4. DISCUSSION

Plants have been seen as a source of medicinal agents for thousands of years since the origin of man [27]. 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 [28]. Pharmacognostic studies is more reliable [29], and according to WHO, the qualitative and quantitative microscopic features would prove useful for laying down pharmacopoeial standards.

The preliminary phytochemical screening results of *B. coriacea* revealed the presence of alkaloids, saponins, phlobatannins, flavonoids, tannins and cardiac glycosides while anthraquinone was absent as reported by Ejikeugwu et al [30].

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 anomocytic 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 of31.8±0.5 μm, mean stomatal width of 18.6 μm and mean stomatal number of 44.4±0.3 (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 micromeric studies showed angle of repose of 26.1± 1.3 degrees, Hausner’s ratio of 1.23±0.02 and Carr’s index of 18.9 ±1.35% for the leaf powder. Micromeric is an important consideration in the development of solid dosage formulations, which is mostly used for physical, mechanical and chemical processes [31].

<table>
<thead>
<tr>
<th>Extract</th>
<th>Sample</th>
<th>Physical observation colour</th>
<th>UV-365 Nm colour</th>
<th>UV-253 Nm colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>Leaf</td>
<td>Brown</td>
<td>Dark brown</td>
<td>Dark grey</td>
</tr>
<tr>
<td>Methanol</td>
<td>Leaf</td>
<td>Light green</td>
<td>Orange</td>
<td>Grey</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Leaf</td>
<td>Light green</td>
<td>Orange</td>
<td>Light grey</td>
</tr>
<tr>
<td>DCM</td>
<td>Leaf</td>
<td>Green</td>
<td>Dark red</td>
<td>Lemon</td>
</tr>
<tr>
<td>n-hexane</td>
<td>Leaf</td>
<td>Colourless</td>
<td>Green</td>
<td>Lemon</td>
</tr>
<tr>
<td>Ethylacetate</td>
<td>Leaf</td>
<td>Light yellow</td>
<td>Maroon</td>
<td>Lemon</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Weight (g)</th>
<th>Percentage (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture Content</td>
<td>0.31±0.00</td>
<td>10.3</td>
</tr>
<tr>
<td>Total Ash value</td>
<td>0.19±0.00</td>
<td>6.3</td>
</tr>
<tr>
<td>Acid-insoluble ash value</td>
<td>0.03±0.00</td>
<td>1</td>
</tr>
<tr>
<td>Water-soluble ash value</td>
<td>0.09±0.00</td>
<td>3</td>
</tr>
<tr>
<td>Sulfated ash value</td>
<td>1.15±0.00</td>
<td>7.5</td>
</tr>
<tr>
<td>Water-soluble extractive value</td>
<td>0.56±0.00</td>
<td>14</td>
</tr>
<tr>
<td>Methanol-soluble extractive value</td>
<td>0.12±0.00</td>
<td>3</td>
</tr>
<tr>
<td>Ethanol-soluble extractive value</td>
<td>0.28±0.00</td>
<td>7</td>
</tr>
</tbody>
</table>

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

Results presented as Mean±SEM of three (3) replicates
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 [32]. 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 carbonate, and oil, but absence of starch. The water-soluble extractive value was 14±0.00% w/w, methanol-soluble extractive value was 3±0.00% w/w, ethanol-soluble extractive value was 7±0.00% 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±0.00% 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±0.00% w/w, acid-insoluble ash value was 1±0.00% w/w (this shows low content of earthly materials), water-soluble ash value was 3±0.00% w/w and sulfated ash value was 7.5±0.00% 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.

5. CONCLUSION

The data generated from this study would be of help in the authentication 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.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.
COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


© 2020 Umoh et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.