WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 10, 32-43. Research Article ISSN 2277– 7105

PHARMACOGNOSTIC STUDIES OF CULCASIA SCANDENS P. BEAUV. (ARACEAE)

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Article Received on
06 July 2020,
Revised on 26 July 2020,
Accepted on 16 August 2020,
DOI: 10.20959/wjpr202010-18500

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ABSTRACT

Culcasia scandens P. Beauv. (Araceae) is a medicinal plant commonly known as Climbing Arum and by the Ibibio speaking people of Akwa Ibom State of Nigeria as Ata Utippe is known for its analgesic, anti-abortifacient and anti-emetic properties. The study was aimed to investigate the pharmacognostic parameters of *Culcasia scandens* leaf. The leaves were identified, collected, air dried, weighed and subjected to evaluation parameters of microscopy, micromeritics, chemomicroscopy, fluorescence, extractive values, moisture content and ash values using standard procedures. The result of microscopy revealed amphistomatic type of stomata with unicellular trichomes on both abaxial and adaxial surfaces, stomatal index of 8.68% on abaxial surface and 3.54% on the adaxial surface. The stomatal number on the abaxial surface was 6(11.3±0.70)13 and adaxial surface was 4(6.1±0.43)8. The micromeritics indicated that the powder had fair flow while chemomicroscopy indicated that the powder contained various constituents including lignin, mucilage, starch and calcium oxalate crystals. The fluorescence properties revealed different colours under Ultraviolet lights. Soluble extractive values were found to be 5.50%$^{w}/_{w}$ for water, 4.25%$^{w}/_{w}$ for methanol and 3.50%$^{w}/_{w}$ for ethanol. The moisture content of the leaf was found to be 10.67%$^{w}/_{w}$, total ash value was 11.33%$^{w}/_{w}$, water- soluble ash value 3.00%$^{w}/_{w}$, acid- insoluble ash was 1.33%$^{w}/_{w}$ and sulfated

ash value was 16.00%$^{w}/_{w}$. In conclusion the results obtained from this study provide information about the identity, purity and quality of *Culcasia scandens* for medicinal and formulation purposes.

KEYWORDS: chemomicroscopy, *Culcasia scandens*, micromeritic, Pharmacognostic.

INTRODUCTION

Despite the remarkable progress in synthetic organic medicinal products of the twentieth century, over 25% of prescribed medicines in industrialized countries are desired directly or indirectly from plants.[1] However, one of the main obstacles to the acceptance of traditional medicine in developing countries is lack of documentation and stringent quality control.[2]

Since proper identification as well as quality assurance of the starting material is important in ensuring reproducible quality of herbal medicines which will in turn contribute to its efficacy and safety[3], it becomes necessary to make effort towards standardization of plant materials to be used as medicine and which can be achieved through pharmacognostic studies.

The plant *Culcasia scandens* commonly known as climbing arum belongs to the family Araceae. *Culcasia scandens* is an epiphytic plant with lean and wiry stems that are up to 5m high. It clings to tree trunks using its clasping roots and growing on forest and stream margins and savanna from Liberia, Ivory Coast, Sierra Leone, Nigeria and Cameroun.[4]

Ethnopharmacologically, *C. scandens* P. Beauv. (ARACEAE) is used to take care of diversity of infirmities and conditions namely; analgesic for earache, toothache, tonsillitis and stomach complaints.[5] In addition, the plant is used as an anti-emetic, for various skin conditions, imbibed during pregnancy as an anti-abortifacient and for venereal diseases.[6] The extracts from *C. scandens* have been reported to show antimicrobial activity against *E. coli*, *S. aureus*, *B. subtilis*, *P. aeruginosa* and *S. typhi*.[6]

Following overwhelming dependence of indigenes on herbal products due to its affordability and acceptability, there is need for proper identification of these crude drugs. Hence, the present investigation *C. scandens* therefore taken up to establish certain botanical and chemical standards like pharmacognostic characterization, physicochemical analysis and preliminary phytochemical testing of the leaf which would help to prepare a monograph for the proper identification of the plant.

Phylogeny of *Culcasia scandens* (Scientific Classification) According to Angiosperm Phylogeny Group (APG) System.[7]

Kingdom:	Plantae
Clade:	Tracheophytes
Clade:	Angiosperms
Clade:	Monocots
Order:	Alismatales
Family:	Araceae
Genus:	*Culcasia*
Species:	*C. scandens*
Local name Ibibio:	Ata Utippe
Common Name:	Climbing Arum

Synonyms

Caladium scandens

Culcasia gracilis N. E. Br.

Calcasia lancifolia N. E. Br.

Culcasia saxatilis A. Chev.

Denhamia scandens (P. Beauv) Schott.

Figure 1: *Culcasia scandens* in its natural habitat.

MATERIALS AND METHODS

Collection and Identification of Plant Material

The leaves of *Culcasia scandens* was collected from University of Uyo farm town campus, Ikpa Road, Uyo. Akwa Ibom State, Nigeria in September 2019. The collected leaves were washed under running tap water, rinsed with distilled water, chopped into pieces, dried under shade at room temperature. The dried leaves were powdered using electric blender, sieved through 350 microns sieve size and stored in airtight bottles to avoid moisture and humidity prior to use.

Macro-morphological Evaluation of Leaf

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

Anatomical Studies

Microscopic Evaluation of Leaf

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 with water and 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 and then viewed using 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.[8]

Quantitative Leaf Microscopy

Quantitative microscopy parameters such as leaf constant studies viz. stomatal length and width, guard cell length and width, stomatal number, stomatal index, epidermal cell length and width, epidermal cell number, epidermal cell thickness was 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[9] 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[10] 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 hot and cold extract of the leaf.

Chemomicroscopic Analysis of Leaf and Stem Powders

Powdered leaf was examined for its chemomicroscopic properties viz. mucilage, lignin, starch, oils, calcium carbonate and calcium oxalate crystals.[11]

Fluorescence Analysis of Leaf and Stem Powders

The fluorescent analysis of dried leaf powder was carried out using standard method.[12]

Physico-chemical Evaluation of Leaf and Stem Powders

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 prescribed and the WHO guidelines on quality control methods for medicinal plant materials.[13,14]

RESULTS

Table 1: Results for the Microscopic Features of *Culcasia scandens* and Standard Error of Mean (SEM).

Leaf surface	Abaxial	Adaxial
Stomatal morphology type	Anomocytic	-
Stomatal distribution	Amphistomatic	Amphistomatic
Stomatal length (µm)	64.60(74.10 ± 2.34)86.96	60.97(73.22±2.21)80.35
Stomatal width (µm)	35.83(45.29 ± 2.14)54.48	36.33(47.80 ±2.23)56.66
Stomatal number	6(11-3 ± 0.70)13	4(6.1±0.43)8
Epidermal number	86(118.9 ± 22.72)139	146(166.9 ± 7.06)185
Stomatal index	8.69%	3.54%
Length of guard cell (µm)	45.48(60.73±3-36)75.21	34.72(46.84± 2.31)56.34
Width of guard cell (µm)	13.3(18.08±1.22)23.89	11.94(19.28± 1.54)24.04
Length of epidermal layer (µm)	96.82(125.67±6.08)157.42	72.12(126-62±9.72)168.61
Width of epidermal layer (µm)	52.66(69.6±4.37)94.09	29.71(62.94±6.19)94.43
Thickness (µm)	2.67(3.99±0.52)7.55	2.87(5.19 ± 0.85)10.68

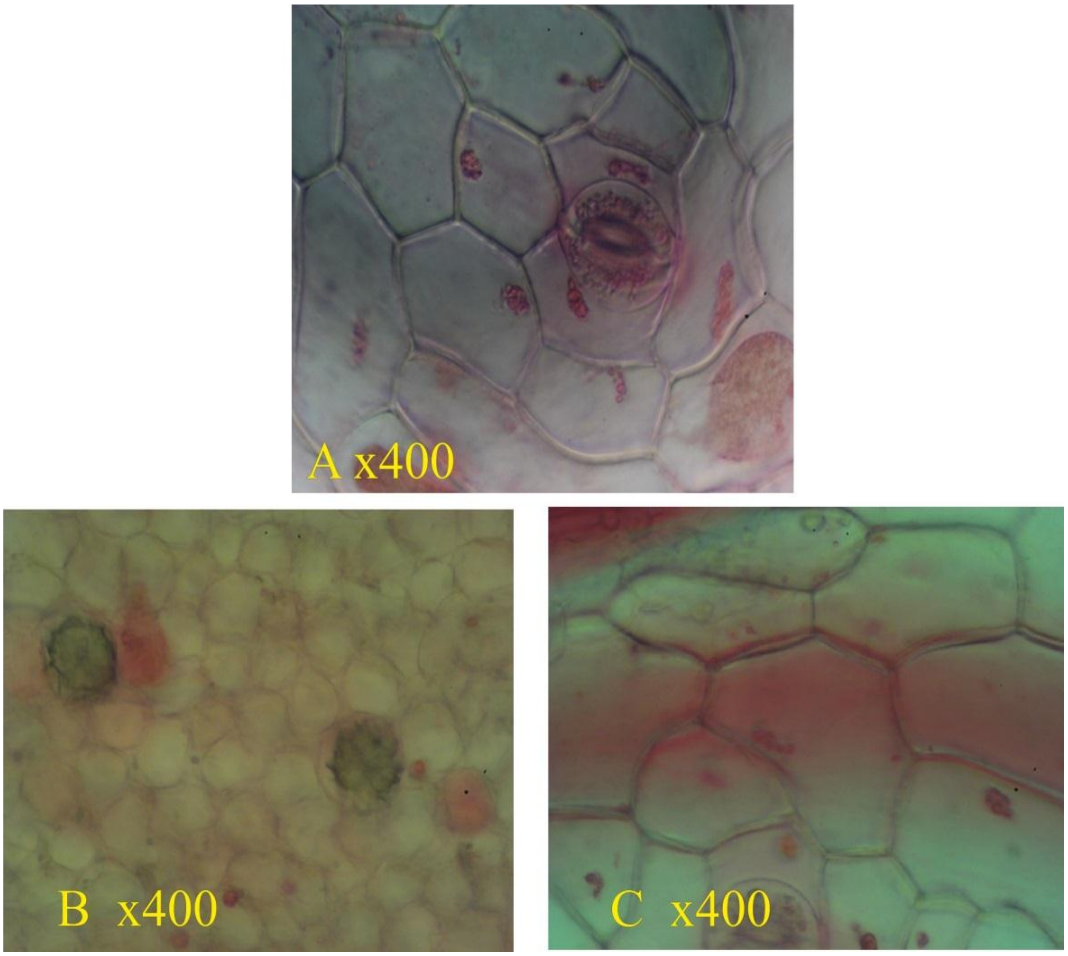

Figure 2: (A) Abaxial Surface showing Anomocytic Stomata (B) Adaxial Surface Showing Druse Crystals (C) Polygonal to Irregular Epidermal cell wall pattern × 400.

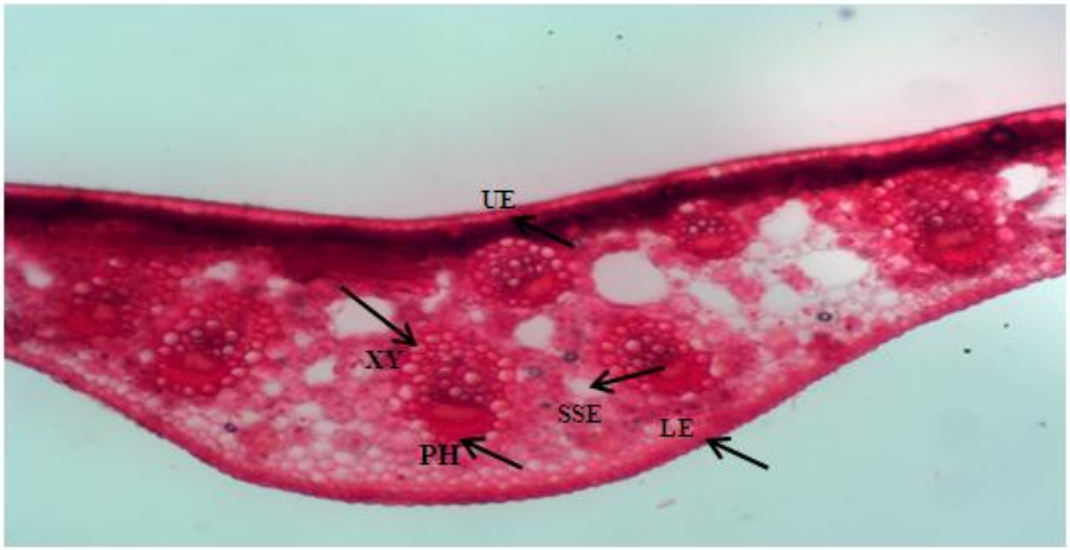

Figure 3: Transverse Section of Leaf through Midrib, XY(Xylem), PH (Phloem), LE (Lower epidermis), UE (Upper epidermis) SSE (Sub-stomatal cavity) × 400.

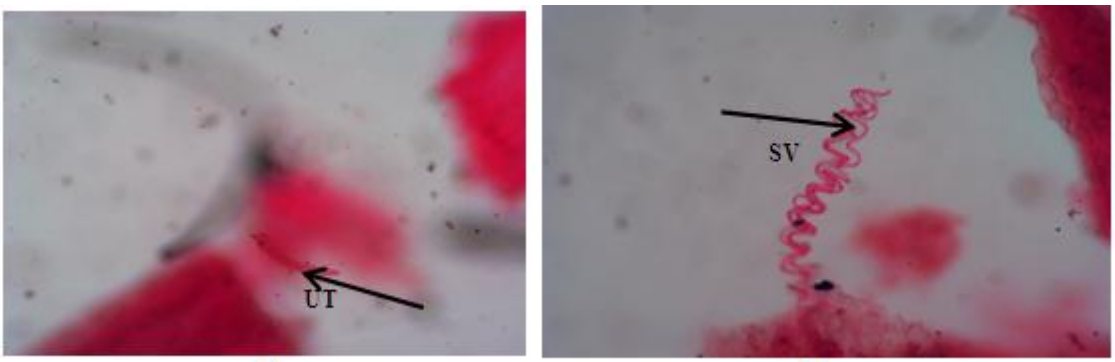

Figure 4: Microscopy of Powdered leaf; (A) Unicellular trichome; (B) Spiral vessel× 100

Table 2: Results for Micromeritic Properties of *Culcasia scandens* leaf.

Micromeritic Parameters	Leaf Powder
Bulk volume (cm)	39.00± 0.61
Tapped volume (cm)	25.50±0.35
Bulk density (g/ml)	0.25±0.00
Tapped Density (g/ml)	0.39±0.00
Hausner's Ratio	1.53 ± 0.04
Carr's Index (%)	35.01 ± 1.55
Diameter of Heap(cm)	6.42 ± 0.12
Height of Heap(cm)	2.53 ± 0.16
Flow time (secs)	22.64 ± 0.42
Angle of Repose(^{0})	38
Flow rate (g/s)	22.64±0.42
pH – COLD HOT	7.78 8.06

Table 3: Results for Chemomicroscopy of *C. scandens* Leaf.

Constituents	Qualitative test	Observation	Inference
Mucilage	Ruthenium red, view under microscope	Sample stains pink	Mucilage Present
Lignin	Phloroglucinal Conc. HCL	Sample Stains red	Lignin Present
Starch	N/50 iodine	Sample Stains blue Black	Starch Present
Calcium Oxalate Crystals	Sample cleared and viewed under microscope + 80% HCl	Calcium Oxalate Crystals Seen Crystal Dissolves	Calcium Oxalate Crystals Present Calcium Oxalate Crystals Present
Calcium Carbonate	Glycerol + acetic acid solution	No gas Evolution	Calcium Carbonate Absent
Oil	Sudan IV, view under Microscope	Sample Stains Pink	Oil present

Table 4: Results for Fluorescence Properties of *C. scandens* Leaf.

Extract	Sample	Physical Observation Color	UV-365nm Color	UV-253.7 Color
Water	Leaf	White	Yellow	Grey
Methanol	Leaf	Green	Red	Grey
Ethanol	Leaf	Green	Orange	Dark grey
Ethyl Acetate	Leaf	Light green	Dark red	Light grey
Dichloromethane	Leaf	Dark green	Dark red	Black
n-Hexane	Leaf	Yellow	Bright red	White

Table 5: Results for water soluble extractive value, ethanol-soluble extractive value methanol-soluble extractive value and standard error of mean (SEM) for leaf powder of *C. scandens*.

	Weight(g)	Percentage (%$^{w}/_{w}$)
Water-soluble extractive value	0.22±0.02	5.50
Ethanol soluble extractive value	0.17± 0.00	3.50
Methanol soluble extractive value	0.14± 0.01	4.25

Table 6: Results for moisture content, total ash value, acid insoluble ash value, water soluble ash value, sulfated ash value and Standard Error of Mean for the Leaf Powders of *C. scandens.*

	Weight(g)	Percentage (%$^{w}/_{w}$)
Moisture Content	0.32 ± 0 .01	10.67
Total Ash value	0.34± 0.01	11.33
Acid- Insoluble Ash Value	0.04 ± 0.00	1.33
Water- Soluble Ash Value	0.25 ± 0.01	3
Sulphated Ash Value	0.32± 0.00	16

DISCUSSION

For plant based formulations, quality and identity of starting material is utmost essential which can be best established by Pharmacognostical Standardization. Pharmacognostical characters are helpful in confirming the identity and in the determination of purity, quality of crude drug.[15] As per WHO microscopic characters help in establishing identity and purity and hence the present Pharmacognostical research was aimed at establishing the Pharmacognostical standards for *Culcasia scandens*.

The result obtained from microscopy of *C. scandens* as shown in Table 1 was found to be amphistomatic, unicellular trichome on both adaxial and abaxial surfaces. The epidermal cell wall pattern is undulated on both of these surfaces. The stomatal index on the abaxial surface was 8.68% and on the adaxial surface is 3.54%. This stomatal index is not affected by factors such as age of plant, size of leaf, environmental factors, etc.

The micromeritics properties like bulk density, tapped density, angle of repose, Hausner's ratio and Carr's index indicate flow properties as well as packing geometry and interparticulate resistance between these powders. This information predicts the ease of storage and conveying of powdered crude drugs as well as uniformity of weight and doses of dosage forms produced with them.[16] Angle of repose of the leaf was 38^0 and this indicated a fair flow. The micromeritics can be used to characterize and standardize the formulation properties of herbal drug powder, in order to determine it suitability for formulation into solid dosage forms.[10]

Chemomicroscopy analysis of the leaf of the plant recorded the presence of mucilage, calcium oxalate, starch, oil and protein as shown in table3. In fluorescence analysis, the powdered leaf extracts was tested with water, methanol, ethanol, dichloromethane and n-hexane and was observed under ordinary light, 253.7nm of UV light and 365nm of UV light. The colour changes were distinctive and reproducible as shown in Table 4. The various colours signifies different phyto-constituents that may be present.

For the extractive values, the water- soluble extractive value was found to be $5.50\%^{w}/_{w}$, methanol- soluble extractive value was $4.25\%^{w}/_{w}$ and Ethanol-soluble extractive value was $3.50\%^{w}/_{w}$. This indicated that water is the most suitable solvent for extraction of constituents of this plant.

The moisture content of *C. scandens* (10.67% $^{w}/_{w}$) as shown in Table 6, falls within the recommended range of 8-14%$^{w}/_{w}$ for vegetable drug according to the African pharmacopoeia, 1986. This is an indication that the plant can be stored for a long period of time, with less probability of microbial attack. Higher moisture content could even provide environment for hydrolytic degradation thus affecting chemical stability of the herbal drug.[17] Ash values are a useful indicator of the purity of any drug and give information relative to adulteration/contamination with inorganic matter. Total ash value of *C. scandens* leaf of 11.33%$^{w}/_{w}$ was within the limit indicated in the European Pharmacopoeia 2007 not exceeding 14%$^{w}/_{w}$. The Acid-insoluble ash value was found to be 1.33%$^{w}/_{w}$ and this is also within the accepted limit of the European pharmacopoeia (not exceed 2%$^{w}/_{w}$). The water-soluble ash value was 3%$^{w}/_{w}$ and the sulfated ash value was 16%$^{w}/_{w}$. The sulfated ash determination is useful in assessing the amount of inorganic substances contained as impurities in an organic substance.

CONCLUSION

The result obtained from the pharmacognostic studies provide information about the identity, quality and purity of *Culcasia scandens*. The result collectively might also be useful to supplement information for further studies on *C. scandens* leaf.

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