



Phytopharmaceutical Standardization of Leaves of *Jatropha tanjorensis* J. L. Ellis & Saroja. (Euphorbiaceae)

**Romanus A. Umoh^{1*}, Uwemedimo F. Umoh¹, Imoh I. Johnny¹,
Omodot T. Umoh², Victor U. Anah³, Anwanabasi E. Udoh⁴, Akwaowoh A. Elijah⁵
and Blessing E. Andrew¹**

¹Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo,
Akwa Ibom State, Nigeria.

²Department of Botany and Ecological Studies, Faculty of Sciences, University of Uyo,
Akwa Ibom State, Nigeria.

³Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmacy,
University of Uyo, Nigeria.

⁴Department of Pharmacology and Toxicology, Faculty of Pharmacy,
University of Uyo, Nigeria.

⁵Department of Clinical Pharmacy and Biopharmacy, Faculty of Pharmacy,
University of Uyo, Uyo, Akwa Ibom State, Nigeria.

Authors' contributions

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

Article Information

DOI: 10.9734/JOCAMR/2020/v11i230179

Editor(s):

(1) Dr. B. V. Suma, Ramaiah University of Applied Sciences, India.

Reviewers:

(1) Priscila Orlandini, Universidade Estadual de Campinas (Unicamp), Brazil.

(2) S. Salamma, NSPR GDC (W), India.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/61488>

Original Research Article

**Received 24 July 2020
Accepted 29 September 2020
Published 17 October 2020**

ABSTRACT

Jatropha tanjorensis J.L. Ellis & Saroja. (Euphorbiaceae) is a shrub commonly used as an edible vegetable and is also used as a tonic herb. The study was aimed to evaluate pharmacognostic parameters of *Jatropha tanjorensis* leaves. The plant leaves were collected, air-dried, pulverized

*Corresponding author: E-mail: romaumoh2008@yahoo.com;

and stored in a clean glass container. Standard procedures were employed to obtain the microscopic features of the fresh and powdered samples, micromeritic, chemomicroscopy, fluorescence properties, moisture content, ash values and soluble extractive values were also carried out. The results of the microscopic studies using the fresh and powdered leaf samples revealed the presence of anomocytic, anomalous and paracytic stomata on the abaxial surface and anomocytic stomata on the adaxial surface. The plant sample also possessed unicellular trichomes. Results of micromeritic properties of the powdered samples show bulk volume of 38.67 ± 0.7 , tapped volume of 30.00 ± 0.4 , bulk density of 0.26 ± 0.00 , tapped density of 0.33 ± 0.00 , angle of repose of 35° , Carr's Index of 22.96 ± 2.15 , Hausner's ratio of 1.27 ± 0.03 , pH of 7.51 and 7.52 when hot and cold respectively. Chemomicroscopy studies revealed the presence of lignin, mucilage, calcium oxalate crystals, starch and oil in the powdered leaf. Results for moisture contents was $18.33 \pm 0.01\%$ w/w, total ash value was $9.33 \pm 0.00\%$ w/w, acid-insoluble ash value was $0.67 \pm 0.01\%$ w/w, water-soluble ash value was $4.0 \pm 0.00\%$ w/w and sulfated ash value was $14 \pm 0.01\%$ w/w. Results for ethanol-soluble extractive value was $15 \pm 0.00\%$ w/w, methanol-soluble extractive value was $19 \pm 0.00\%$ w/w and water-soluble extractive value was $27 \pm 0.01\%$ w/w. In conclusion, the above evaluation and parameters could be used to establish pharmacopoeial standard of both fresh and powdered drug of *Jatropha tanjorensis*.

Keywords: Chemomicroscopy; *Jatropha tanjorensis*; micromeritic; pharmacognostic; phytopharmaceutical; psychotherapeutic; standardization.

1. INTRODUCTION

The growth of traditional medicine has long been attributed to ever increasing medicinal plants rich in pharmacologically active components with proven efficacy against some well-known diseases and medical conditions. About 80% of the world populations still rely on the use of herbal drugs for treatment of various diseases [1]. The rising popularity of herbal products, both as food and food supplements and as psychotherapeutic drugs, has also given rise to many reports describing adverse health effects and variable quality, efficacy and contents of herbal products [2,3]. Today, with the present surge of interest in psychotherapeutics, the availability of genuine plant material is becoming scarce. Since crude drugs form the basis for the manufacture of numerous medicinal preparations, accurate determination of drug identity forms an essential part of the study of plants [4, 5]. It becomes extremely important to make an effort towards standardization of plant materials as medicine [6]. The process of standardization can be achieved by step-wise pharmacognostic studies.

Jatropha tanjorensis is a bushy, gregarious shrub of about 6 meters in height with spreading branches and stubby twigs and smooth ray bark, which gives off toxic whitish coloured latex when cut. The leaves are deciduous alternate but

crowded towards the apex, ovate, acute to acuminate basally chordate, 3-5 lobed in outline, 6 - 40 cm broad, the petiole is 3-8cm long [7, 8]. The flowers are whitish and bell-shaped while the sepals are broadly deltoid. *J. tanjorensis* plants are drought-resistant succulent trees and therefore can be grown anywhere. *Jatropha tanjorensis* is believed to be a native of Mexico and Central America region [7]. It originated in the Caribbean, widely grown in Southern Nigeria and used as leafy vegetable and medicinal plant. It is cultivated in many parts of the tropics and it also has traditional uses in the subtropics as a hedge crop. It can grow in areas with extreme climates and even in soil conditions that could not be inhabited by most of these agriculturally important plant species. It is also used for fencing [9]. *Jatropha tanjorensis* possess inflammable property which can easily inflict injury to the skin when in contact after some days.

However, due to the pronounced attention of the population on herbal products as a result of its affordability and acceptability, it is necessary to properly identify this crude drug. Hence, the investigation of *J. tanjorensis* was consequently taken up to establish certain botanical and physicochemical standards like pharmacognostic evaluation of the leaf which would help to prepare a monograph for the proper identification of the plant.

Phylogeny of *Jatropha tanjorensis* (Scientific Classification) According to Angiosperm Phylogeny Group (APG) System [10].

Kingdom	-	Plantae	
Clade	-	Tracheophyta	
Clade	-	Angiosperms	
Order	-	Malpighiales	
Family	-	Euphorbiaceae	
Sub-family	-	Crotonoideae	
Tribe	-	Jatrophae	
Genus	-	<i>Jatropha</i>	
Species	-	<i>tanjorensis</i>	
Common Name	-	Hospital too far, Catholic vegetable	
Local Name	-	- Yoruba	Iyana-Ipaja, Igbo Ugu-oyibo



Fig. 1. Leaves and flowers of *Jatropha tanjorensis* J.L. Ellis & Saroja in its natural habitat

2. MATERIALS AND METHODS

2.1 Identification and Collection of Plant

The leaves of the plant were collected from Urua Ikpa, Uyo Local Government Area, Akwa Ibom state, Nigeria in July 2019. The plant was identified and authenticated in the faculty of Pharmacy, University of Uyo herbarium. The fresh plant material was air-dried, pulverized and packed in a dry container, well labeled and used when needed.

2.2 Anatomical Studies

2.2.1 Microscopic evaluation of leaf

Matured fresh leaves of the plant were cut at the petiole. Microscopic examinations of the epidermis of both adaxial and abaxial surfaces were made by placing the leaf on a glass slide. The sample was irrigated with water and scraped

gently with a sharp razor blade till loosed cells from the epidermis were washed away with water and the desired epidermis was reached. The epidermal peels were further cleared with sodium hypochlorite and rinsed gently with water. The epidermal peels were stained with aqueous solution of safranin-O for (five) 5 minutes and 10% glycerol. The stained samples were mounted on a binocular microscope. Photographs of the microscopic features such as stomatal morphology, epidermal cell wall pattern and calcium oxalate crystals of the prepared slides were taken with an Amscope MD500 mounted on Olympus CX21 microscope. Also, the transverse section and powder microscopy of the plant were observed and photographs taken too [11].

2.2.2 Quantitative microscopy of the leaf

Quantitative microscopy parameters such as leaf constant studies namely 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 \pm SEM.

The stomatal index (S.I) was determined according to Metcalfe and Chalk [12] 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.

2.2.3 Micromeritics

The flow property was determined using standard methods [13] which constitutes;

2.3 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 was also 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 was 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.

2.3.1 Angle of repose

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

2.3.2 pH

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

2.3.3 Chemomicroscopic analysis of leaf powder

Powdered leaf was examined for its chemomicroscopic properties namely mucilage, lignin, starch, oils, calcium carbonate and calcium oxalate crystals using standard procedures [14].

2.3.4 Fluorescence analysis of leaf powders

The fluorescent analysis of dried leaf powder was carried out using standard method [15].

2.3.5 Physico-chemical evaluation of leaf powders

The physicochemical parameters such as moisture content, ash values (total ash, acid insoluble ash, water soluble ash, sulfated ash), soluble extractive values such as ethanol, methanol and water-soluble extractive values were performed according to the official method prescribed by the WHO guidelines on quality control methods for medicinal plant materials [16,17,18].

3. RESULTS

The results for the microscopic studies are summarized in Table 1 and Figs. 2 and 3.

The results for the micromeritic, chemomicroscopy, fluorescence, extractive and ash values are summarized in Tables 2, 3, 4, 5 and 6 respectively.

Table 1. Results for the microscopic features of the leaf of *Jatropha tanjorensis* and Standard Error of Mean (SEM)

Leaf surface	Abaxial	Adaxial
Stomatal morphology type	Anomocytic and Paracytic	Anomocytic
Stomatal length (μm)	243.63 (269.94 \pm 7.40) 303.74	269.35 (287.47 \pm 5.84) 326.25
Stomatal width (μm)	163.52 (197.53 \pm 4.78) 213.94	43.48 (156.53 \pm 16.09) 214.77
Stomatal number	2.0 (4.4 \pm 0.39) 6.0	1.0 (1.2 \pm 0.21) 2.0
Epidermal number	12 (16.4 \pm 0.76) 19	14 (17.9 \pm 0.94) 22
Stomatal index	21.15%	6.45%
Length of guard cell (μm)	242.59 (258.86 \pm 3.77) 274.29	206.13 (250.22 \pm 7.33) 280.74
Width of guard cell (μm)	55.00 (73.22 \pm 4.14) 95.56	50.40 (64.04 \pm 3.10) 83.83
Length of epidermal layer (μm)	344.56 (468.06 \pm 27.03) 574.11	336.58 (434.92 \pm 17.09) 491.15
Width of epidermal layer (μm)	80.55 (166.93 \pm 17.07) 248.30	134.21 (229.12 \pm 15.70) 281.46
Thickness (μm)	3.01 (9.62 \pm 1.19) 13.62	3.45 (16.89 \pm 2.19) 23.89

Results presented as Mean \pm SEM of Ten (10) Replicates**Table 2. Results for micromeritic properties of *J. tanjorensis* powdered leaf**

Parameters	Results
Bulk Volume (cm)	38.67 \pm 0.7
Tapped Volume (cm)	30.00 \pm 0.04
Bulk Density (g/mL)	0.26 \pm 0.00
Tapped Density (g/mL)	0.33 \pm 0.00
Flow Rate (g/s)	09.18 \pm 0.46
pH	Cold – 7.51 Hot – 7.52
Angle of Repose ($^{\circ}$)	35
Hausner's Ratio	1.27 \pm 0.03
Carr's Index(%)	21.21 \pm 2.15
Diameter of Heap (cm)	6.71 \pm 0.02

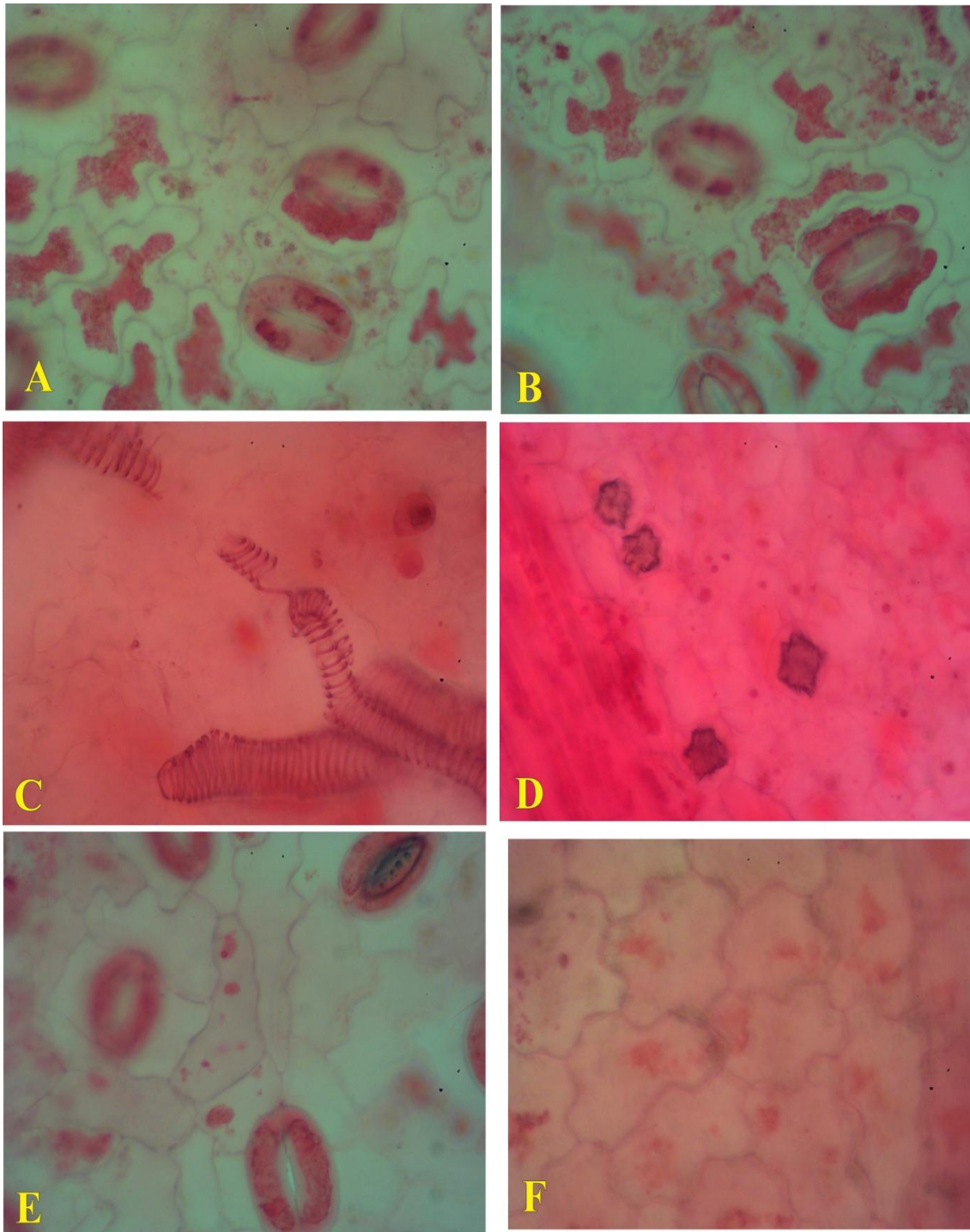
Results presented as Mean \pm SEM (Standard Error of Mean) of Three (3) Replicates**Table 3. Showing chemomicroscopic evaluation of *J. tanjorensis* powdered leaf**

Parameters	Leaf
Lignin	+
Starch	+
Oils	+
Calcium Carbonate	-
Calcium Oxalate crystals	+
Mucilage	+

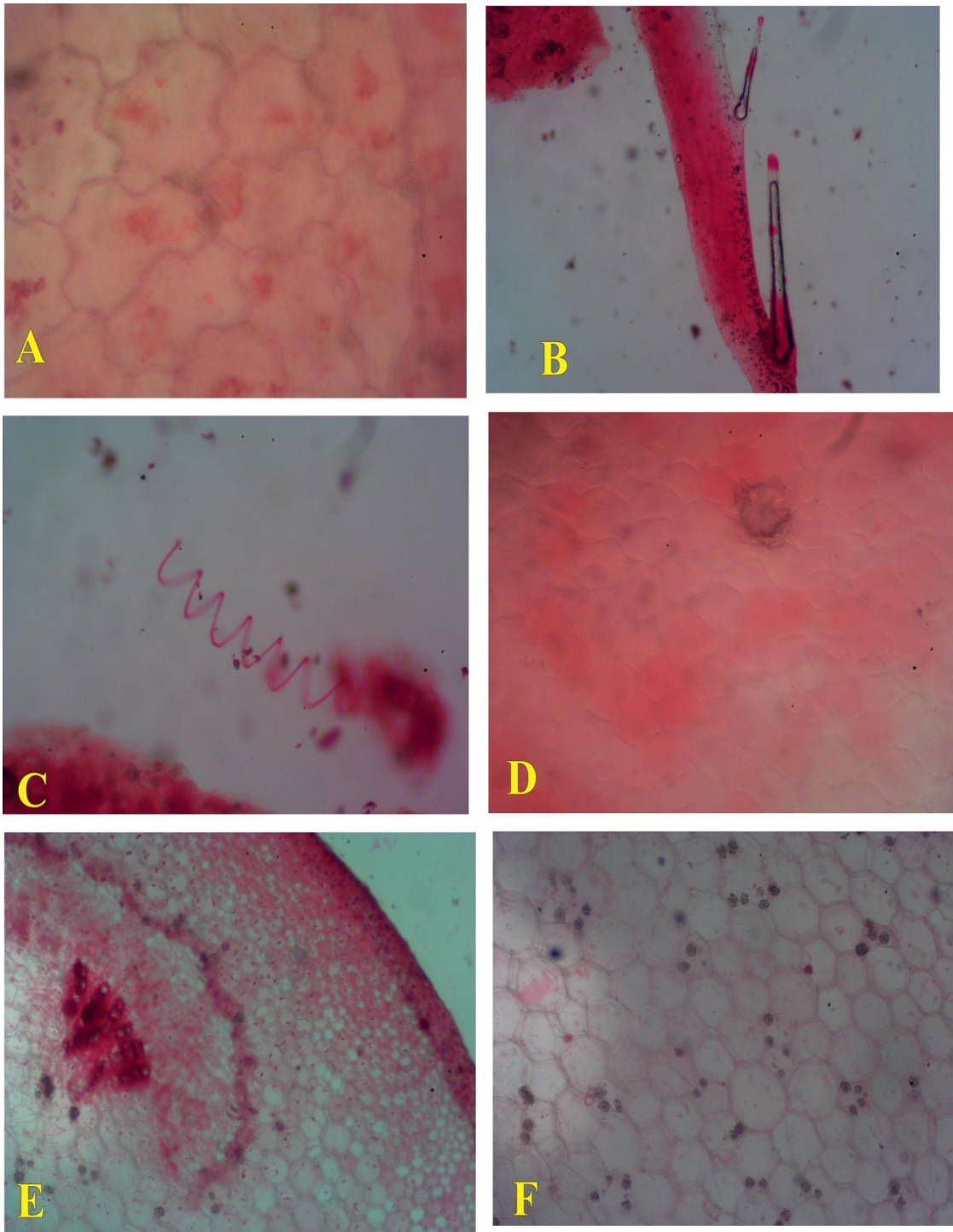
+ = Present, - = Absent

Table 4. Results for fluorescence properties of *J. tanjorensis* powdered leaf

Extract	Sample	Physical Observation Color	UV-365 nm Color	UV- 253.7 nm Color
Water	Leaf	Colourless	Dark Red	Grey
Methanol	Leaf	Green	Red	Dark Grey
Ethanol	Leaf	Green	Orange	Dark Grey
DCM	Leaf	Dark Green	Red	Black
N-hexane	Leaf	Yellow	Dark Red	Black
Ethyl Acetate	Leaf	Yellowish Green	Yellow	Grey



**Fig. 2. A: Abaxial (X40) Anomocytic and Paracytic stomata
B: Abaxial (X40) Anomocytic and Paracytic stomata
C: Adaxial (X40) Annular rings of vascular bundle
D: Adaxial (X40) Druse Calcium oxalate crystals
E: Abaxial (X40) Undulating Epidermal Cell
F: Adaxial (X40) Undulate epidermal cell**



**Fig. 3. A: TS (X40) meta and protoxylem.
B: (X40) Powder Unicellular trichome
C: (X10) Powder: Annular ring of vascular bundle
D: (X400) Powder: Epidermal cells and calcium oxalate crystals
E: (X4) phloem and xylem tissues of the petiole
F:(X4) parenchyma of the petiole**

Table 5. Results for water-soluble extractive value, ethanol-soluble extractive value, methanol-soluble extractive value and standard error of mean of *J. tanjorensis* powdered leaf

Parameters	Weight (g)	Percentage (% w/w)
Water-soluble extractive value	1.08 ± 0.01	27
Ethanol-soluble extractive value	0.60 ± 0.00	15
Methanol-soluble extractive value	0.76 ± 0.00	19

Results presented as Mean±SEM of three (3) replicates

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 of *J. tanjorensis* powdered leaf

Parameters	Weight (g)	Percentage (% w/w)
Moisture content	0.55 ± 0.01	18.33
Total ash	0.28 ± 0.00	9.33
Acid-insoluble ash value	0.02 ± 0.01	0.67
Water-soluble ash value	0.12 ± 0.00	4.00
Sulfated ash value	0.28 ± 0.01	14.00

Results presented as Mean±SEM of three (3) replicates

4. DISCUSSION

In recent times, however there has been an increase in consciousness of the need for standardization of medicinal plant extracts, especially for those with potential therapeutic uses [4, 19, 20]. Microscopic examination of sections and powdered drugs, aided by stains, help in distinction of anatomy in adulterants. From the observation of the understudied plant, the microscopy of the epidermal layers revealed the presence of paracytic and anomocytic stomata only on the abaxial surface (hypostomatous), while anomocytic on the adaxial surface and unicellular trichomes which occur at the margin of the plant leaf. The epidermal cell wall pattern is undulate and sinuous in nature especially on the abaxial surface. The microscopic studies of the epidermal layers reveal stomatal index of 21.45% and 6.45% on abaxial and adaxial surfaces respectively, mean stomatal length of 269.94µm and the mean stomatal width of 197.53µm for the abaxial surface while mean stomatal length and mean stomatal width for adaxial surface is 287.47µm and 156µm respectively. The transverse section of the midrib revealed the presence of vascular bundles. Powdered microscopy also revealed the presence of sclariform tracheid as shown in figures 2C and 3C. In micromeritic evaluation, Umoh *et al* [21] reported a fair flow property for *Culcasia scandens* P. Beauv. with the angle of repose of 38° as in this study the drug showed a good flow property with an angle of repose of 35° which is a preformulation characteristic related to interparticulate friction. Hausner's ratio and Carr's

index were 1.27 and 21.21% respectively as shown in Table 2. According to the USP (United State Pharmacopoeia) standard, the powder has passable flow property. In recent years, the compressibility index and the closely related Hausner's ratio have become simple, fast and popular methods of predicting powder flow characteristics. The compressibility index has been proposed as an indirect measure of bulk density, size and shape surface area, moisture content and cohesiveness of materials because all of these can affect the observed compressibility index.

Chemomicroscopic study revealed the presence of mucilage, lignin, calcium oxalate crystals and oil as shown in Table 3. The ethanol-soluble extractive value was 15.00%^{w/w}, methanol-soluble extractive value was 19%^{w/w} and water-soluble extractive value was 27%^{w/w} as shown in Table 5. The water-soluble extractive value indicated the presence of water-soluble matters such as sugars, amino acids and vitamins derived from plants. The ethanol and methanol-soluble extractive values indicate the presence of polar compounds.

The moisture content obtained was 18.33%^{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 lead to degradation of active constituents [16]. The plant possesses a high moisture content thus if not dried properly before storage could lead to sample degradation. The African Pharmacopoeia limit of moisture content for

vegetable drug ranges from 8% ^w/_w to 14%^w/_w. The total ash value was 9.33%^w/_w, acid-insoluble ash value was 0.67%^w/_w, water-soluble ash value was 4.00%^w/_w and sulfated ash value was 14%^w/_w as shown in Table 6. Ash values are used to determine the quality and purity of crude drug. The ash value indicates the presence of inorganic ions. During the process of ashing, organic matter gets oxidized and certain amount of volatile elements are lost. High ash value indicates the presence of impurities.

5. CONCLUSION

It is concluded that there should be advancements in future researches and pharmacological evaluation of *Jatropha tanjorensis*. The data obtained can assist in the proper identification, collection and authentication of this plant. The parameters could be used to establish pharmacopoeial standard of both fresh and powdered drug of *Jatropha tanjorensis*.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

- World Health Organization. Geneva. Quality Control Methods for Medicinal Plant Materials. 1998;204.
- Taylor DA. Botanical supplements: weeding out the health risks. Environmental Health Perspectives. 2004;112(13):750-753.
- Patil SG, Wagh AS, Pawara RC, Ambore SM. Standard tools for evaluation of herbal drugs. An Overview. The Pharma Innovation Journal. 2013;2(9):56-65.
- Chanda S. Importance of pharmacognostic study of medicinal plants: An Overview. Journal of Pharmacognosy and Phytochemistry. 2014;2(5):69-73.
- Smita L, Arinsha C, Patil V. Need of Herbal Drug Standardization. International Ayurvedic Medical Journal. 2015;3(3):874-877.
- Mukherjee PK. Quality control of herbal drugs. an approach to evaluation of botanicals. Business Horizons; India. 2002;1:113-119.
- Ellis JL, Saroja TL. A new species of *Jatropha* from South India. Journal of Bombay Natural History Society. 1961;8:834-836.
- Egbon EE, Ize-Iyamu OK, Okojie VU. Proximate and mineral composition of *Jatropha tanjorensis*. 2013;17:21-23.
- Oboh FOJ, Masodje HI. Nutritional and antimicrobial properties of *Vernonia amygdalina* leaves. International Journal of Biomedical and Healthcare Sciences. 2009;5:51-56.
- Angiosperm phylogeny group. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. Botanical Journal of the Linnean Society. 2016;181(91):1-20.
- Killedar GS, Harianth N, Sameer J, Nadaf S, Karade R. Phytochemical potential of *Memecyclon umbellatum*. Burm. Leaf extracts. Journal of Drug Delivery and Therapeutics. 2014 4(2):30-35.
- Metcalfe CR, Chalk L. Anatomy of the Dicotyledons. Clarendon Press, Oxford. 1979;1(2):279.
- Mbah CC, Builders PF, Akuodor GC, Kunle OO. Pharmaceutical characterization of *Bridelia ferruginea* Benth (Euphorbiaceae). Tropical Journal of Pharmaceutical Research. 2012;11(4):637-644.
- Trease GE, Evans WC. Pharmacognosy. 16th edition. Elseviers Ltd., United Kingdom. 2009;560-570.
- Kokate CK, Purohit AP, Gokhale SB. Analytical Pharmacognosy, Nirali publication, 30th edition. 2005;199.
- African pharmacopoeia. General Methods of Analysis Pharmacopoeia. 1986;11:121-208.
- Khandelwal KR. Practical pharmacognosy techniques and experiments. New Delhi: Nirali Prakashan. 2002;15-163.
- Kumar D, Gupta J, Kumar S, Arya R, Kumar T, Gupta G. Pharmacognostic evaluation of *Cayratia trifolia* (Linn.) leaf. Asian Pacific Journal of Tropical Biomedicine. 2012;2(1):6-10.
- Thomas S, Patil DA, Patil AG, Naresh C. Pharmacognostic evaluation and

- physiochemical analysis of and Conservation. 2012;3:101–
averrhoa carambola. Journal of 112.
- Herbal Medicine and Toxicology. 21. Umoh RA, Umoh UF, Johnny II,
2009;2:51–59. Effiong DE, Umoh OT. Pharmacognostic
20. Kunle OF, Egharevba OH, studies of *Culcasia scandens* P.
Ahmadu PO. Standardization of BEAUV. (Araceae). World Journal of
herbal medicines. A Review. Pharmaceutical Research. 2020;9(10):32-
International Journal of Biodiversity 43

© 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.

Peer-review history:

The peer review history for this paper can be accessed here:
<http://www.sdiarticle4.com/review-history/61488>