



PHARMACOGNOSTIC STUDIES AND DEVELOPMENT OF QUALITY CONTROL PARAMETERS FOR LEAVES OF *ALBIZIA CHEVALIERI* HARMS (FABACEAE)

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ABSTRACT

Albizia chevalieri harms is a tree or shrub of 5 - 12 m tall and distributed in the dry savannah from Senegal, Niger and Nigeria. In traditional medicine, the leaf is used in treatment of purgative, dysentery, diarrhoea, taeniasis and also remedy for coughs. The present study was carried out to determine some important pharmacognostic parameters of *A. chevalieri* leaf which will assist in standardization for quality, purity and sample identification. Pharmacognostic standardization of the leaf was assessed based on the macromorphological, micromorphological, chemomicroscopic and some physicochemical parameters. The leaf is greenish in colour and microscopy features showed epidermis to be hypostomatic with numerous paracytic type of stomata, and lignified hair trichomes, prismatic and acicular crystals of calcium oxalates, vascular bundles, fibres, and the epidermal cells of wavy or irregular in shape on both surface with straight anticlinal walls. Study of transverse section of the leaf revealed its dorsiventral nature. Chemomicroscopy showed the presence of tannins, starch, gums and cellulose. Quantitative leaf constants revealed the stomatal number (22.33), stomatal index (21.54), palisade ratio (17.25), vein islets number (5.00) and veinlet termination number (6.65). Physicochemical parameters such as moisture content/loss on drying (9.11), total ash content (6.65), water soluble ash content (1.75), acid insoluble ash content (1.5) and ethanol had high extractive value of (21.00) compared to water which had extractive value of (15.62). The pharmacognostic parameters observed in this study will be of help in correct identification and quality control of *A. chevalieri*.

Keywords: *Albizia chevalieri*, Standardization, Macroscopic, Microscopic, Physicochemical parameters

INTRODUCTION

The use of medicinal plants constitutes an important part of traditional medicine which is a part of African heritage (Odugbemi, 2006). However, standardization of medicinal herbs, which include proper identification, quality control and quality assurance, is the major limitation to their being widely accepted. Evaluation of standards can be done by accessing the macroscopic, microscopic and physicochemical parameters (Elufioye, and Olaifa, 2014; Nuhu *et al.*, 2016). Determination of various components, properties plays a significant role for standardization of the indigenous crude drugs (Hina *et al.*, 2011). Whether they are being taken as dietary supplements by the general public or being evaluated in a clinical study, the authenticity and standardization of botanical products is a matter of paramount concern (Smillie and Khan, 2010). In order to protect consumers and promote development of the herbal medicine, reliable authentication of plant materials is critically important (Hao *et al.*, 2010).

Albizia is a large genus of trees, of the pea family (fabaceae), native to warm regions of the old world. The plant *Albizia chevalieri* is a tree that grows up to 12 m high or a shrub under harsher conditions of dry savannah from Senegal, Niger and Nigeria. It has an open and rounded or umbrella shaped canopy, bark pale-grayish, twigs pubescent with white lenticles, leaves with 8 to 12 pairs of pinnate and 20 to 40 pairs of leaflets each was reported to contain alkaloids and also tannins sufficient for use in tanning in Nigeria and

Senegal (Burkill, 1995). The common name of *A. chevalieri* is *jaree-hi/je*, Hausa name is *kasari*, is a tree of the dry deciduous forest. Found in well watered places, sandy terraces, not gregarious, nor common (Abdel-Kader *et al.*, 2001). *Albizia chevalieri* leaf is used in Borno-North eastern Nigeria as purgative, dysentery, diarrhoea, taenicide and also remedy for coughs (Le Houèrou, 2009). The leaf extract of *A. chevalieri* is used either as cold water decoction or dried, ground and sieved leaf mixed with pap, for the management of diabetes mellitus by traditional medical practitioners in some parts of Niger Republic and Sokoto, Nigeria (Saidu *et al.*, 2007). There are also reports on the local use of the leaves extract for cancer treatment in Zaria city, Kaduna state and also anti-oxidant activity with a significant hypoglycemic effect (Aliyu *et al.*, 2009; Yusuf *et al.*, 2007). The preliminary phytochemical screening of methanol leaf and bark extracts of *A. chevalieri* revealed the presence of saponins, triterpenes, flavonoids, tannins, and alkaloids (Aliyu *et al.*, 2009; Le Houèrou, 2009). Despite the medicinal importance of *A. chevalieri*, there is no information on the pharmacognostic parameter and quality control of the leaves. Therefore, the present work was carried out to determine some important pharmacognostic parameters of *A. chevalieri* leaf which will assist in standardization for quality, purity and sample identification.

MATERIALS AND METHODS

Collection, Identification and Preparation of the Plant Material

The aerial part leaf of *A. chevalieri* was collected from Kufena village, Zaria Local Government Area of Kaduna state, Nigeria. The plant was identified at the Herbarium unit, Biological Sciences Department, Ahmadu Bello University, Zaria, as compared with a voucher specimen with voucher number (900247) available in the herbarium. The leaves was dusted, cleaned and all foreign matter removed, it was then air-died and comminuted to powdered form, stored in an air-tight container for subsequent use.

Macroscopic Examination

The general feature of the fresh leaf of the plant was studied. The size (length and width) of the lamina were measured with a ruler. The shape, composition, venation, type of the margin, apex and base of the lamina was observed and recorded (WHO, 2011).

Organoleptic characters were conducted on the dried leaf sample of the plant and these include; colour, odour, taste, texture, shape and fracture (Evans, 2009).

Colour: The colour of the dried leaf was determined under diffused daylight result was recorded.

Odour: A piece of the dried leaf was placed on the palm of the left hand and air was blown using the right hand and perceived the odour using the organ of smelling, the strength of the odour was determined.

Taste: A piece of the dried leaf was tasted using the organ of taste (tongue) and the taste was determined, noted and result was then recorded.

Microscopic Examination

The microscopic evaluation of the anatomical section and powdered sample of the leaves was carried out using standard

methods (Brain and Turner, 1975; Evans, 2009). The prepared sections were cleared using 70% chloral hydrate solution and boiled on a water-bath for thirty minutes to remove obscuring materials. The cleared sample was mounted on a microscope slide, using dilute glycerol. This was then observed under the microscope and appropriate images were taken and documented. The micrometric evaluation of some of the diagnostic feature was also done.

Quantitative Leaf Microscopy

Quantitative leaf microscopy to determine palisade ratio, stomata number, stomata index, vein – islet number and veinlet termination number was carried out on epidermal peelings and examined under microscope with aid of Camera Lucida (Brain and Turner, 1975, Kokate, 2009).

Chemomicroscopic Examination

The histochemical detection of cell walls and contents of the powdered leaves such as cellulose cell wall, lignin, starch, cutin, tannins and calcium oxalate, calcium carbonate etc. was carried out using standard method (Evans, 2009; Kokate, 2009).

Physicochemical Parameters

Powdered sample was subjected to physicochemical analysis; water and alcohol soluble extractives, total ash, acid insoluble ash, water soluble ash and moisture content was determined (Evans, 2009; WHO, 2011).

RESULTS

Macroscopical Examination

Albizia chevalieri leaves are compound paripinnate, glabrous, and elliptical in shape with entire margin. Apex of leaf is obtuse and petiolate base with equal. Reticulate venation with midrib/vein depressed. Leaf is greenish in colour with characteristics taste and odourless. Mature leaves are 0.7 - 0.9 x 0.3 - 0.5 cm with smooth texture.



Plate 1: Leaf of *Albizia chevalieri* showing arrangement and size in its natural habitat at Kufena village, Zaria Local Government Area of Kaduna state, Nigeria.

Microscopical Examination

Microscopical examination of the leaf of *A. chevalieri* revealed the presence of some important diagnostic characters

such as wavy or irregular epidermal cells with anticlinal walls on both adaxial (upper) and abaxial (lower) epidermal layers. The presence of paracytic stomata or rubiaceous (irregular-

celled) types with two subsidiary cells with their long axes parallel to the pore of stomata present only on the abaxial (lower) epidermal layers. Lignified hair trichomes were present on both the adaxial (upper) and abaxial (lower) epidermal layers. The calcium oxalate crystals present are the

prisms type along the veins as shown in Table 1 and figure 2 – 5. The transverse section of the leaf through the midrib tissue was examined and revealed different anatomical features namely adaxial (upper) and abaxial (lower) epidermis, mesophyll cells and vascular bundle with fibers.

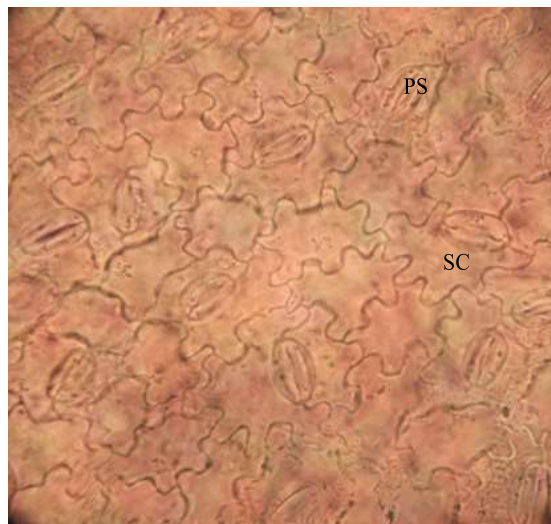


Plate 2: Lower epidermal layer of *A. chevalieri* Leaf (x400); PS- paracytic stomata, SC- subsidiary cell

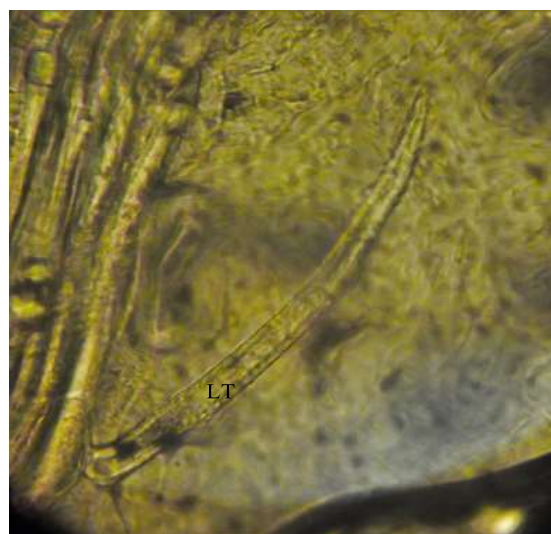


Plate 3: Upper epidermal layer of *A. chevalieri* leaf showing lignified trichome (x400); LT- lignified trichome

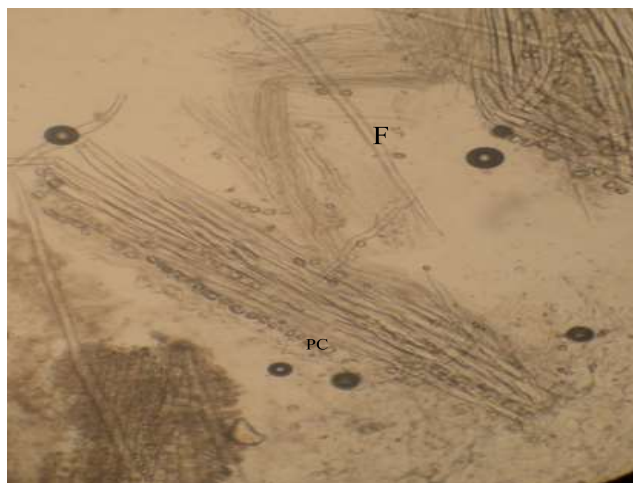


Plate 4: Powdered microscopy of *A. chevalieri* leaf showing some Features (x100); F- fibre, Pc- prism crystals.

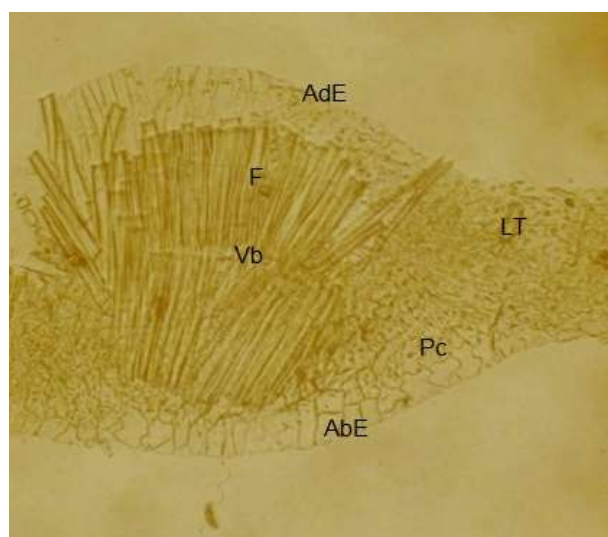


Plate 5: Transverse section of the leaf of *A. chevalieri* showing some Features (x400); AdE- adaxial epidermis, F- fibre, LT- lignified trichome, VB- vascular bundle, Pc- palisade cell, AbE- abaxial epidermis.

Table 1: Microscopical features of *A. chevalieri* leaf

PARAMETERS		OBSERVATIONS
Epidermal cells	Shape	Wavy or irregular
Stomata	Type	Paracytic type
	Position	Abaxial (lower) epidermis
	Frequency	Numerous
	Size*	$2.31 \pm 0.10 \times 1.19 \pm 0.08 \mu\text{m}$
Trichomes	Type	Lignified trichomes
	Position	Adaxial (upper) and abaxial (lower) epidermis
	Frequency	few
	Size*	$26.11 \pm 2.12 \times 1.00 \pm 0.00 \mu\text{m}$
Calcium oxalate crystals	Type	Prism crystals
	Position	Along the veins
	Frequency	Numerous
	Size*	$1.12 \pm 0.13 \times 0.99 \pm 0.10 \mu\text{m}$

*Micrometric values of diagnostic character in length and breadth (μm)

Qualitative Microscopical Values for the Leaf of *A. chevalieri*

On the average, stomatal number (22.33), and index (21.54), palisade ratio (17.25), vein termination numbers (6.65) and vein islets (5.00) were determined and recorded (Table 2).

Table 2: Qualitative Microscopical Values for the Leaf of *A. chevalieri*

Evaluative Parameter	Values*
Stomatal number	18.98 - 22.33 - 25.68
Stomatal index	18.31 - 21.54 - 24.77
Palisade ratio	14.66 - 17.25 - 19.84
Veinlet termination number	5.67 - 6.67 - 7.67
Vein islet number	4.25 - 5.00 - 5.75

*Values are presented as a range from the lower to upper limit [lower limit - (average mean) - upper limit] obtained from five (5) replicate experiments

Chemomicroscopical examination

Chemomicroscopical examination of the powdered leaf of *A. chevalieri* revealed the presence of cellulose cell wall, lignified cell wall, tannins, starch, calcium oxalate prisms like, and suberin or cutin while calcium carbonate was found to be absent.

Physicochemical Parameters

The physicochemical results for the leaves of *A. chevalieri* are presented in Table 3.

Table 3: Physicochemical Parameters of plant leaf of *Albizia chevalieri*

Parameter	*Value obtained \pm SEM (%w/w)
Moisture contents	9.11 \pm 0.29
Total Ash value	4.83 \pm 0.33
Water soluble ash	1.75 \pm 0.25
Acid insoluble ash	1.50 \pm 0.00
Water soluble extractive value	15.67 \pm 0.88
Alcohol soluble extractive value	21.00 \pm 1.00

*Average values of five determinations. SEM: Standard Error of Means

DISCUSSION

In this research, various macroscopical, microscopical, chemomicroscopical and physicochemical standards have been developed, that will help in standardization for quality, purity, and sample identification.

Organoleptic testing of a crude drug is the technique for qualitative evaluation based on the observation of morphological and sensory profile (Kokate *et al.*, 2009).

Microscopically, the leaf was observed to be hypostomatic distribution with numerous paracytic type of stomata, with lignified trichomes on the abaxial (lower) surface only. The epidermal cells are wavy or irregular in shape on both surfaces with straight anticlinal walls. It also reveals the presence of calcium oxalate which is prism in nature along the veins. Study of transverse section of the leaf across the midrib showed hypostomatic leaf arrangement with palisade cells and fibre found at the vascular bundle. The occurrence of the above mentioned characteristic features was observed among members of the Fabaceae (Rahul *et al.*, 2010). Anatomical features of the internal structures of plant drugs provides an important diagnostic features for the identification of fresh and powdered crude drugs and detection of adulterants in plant materials (Ghani, 1990).

Chemomicroscopical features of powdered leaf of *A. chevalieri* revealed the presence of cellulose cell wall, lignified tissues, mucilage, tannins, starch, suberin and calcium oxalate crystals while calcium carbonates was found to be absent, this result is in agreement with the finding of Agboola and coworkers who detected the presence of calcium oxalate crystals on the leaf of *A. altissimum*, there were prismatic and located along the veins (Agboola *et al.*, 2012). The microscopic structures are most valuable in the identification of powdered drug as their identification is largely based on the form, the presence or absence of certain cell types and cell inclusions (Eggeling *et al.*, 2000).

Physicochemical parameters such as moisture content/loss on drying, total ash content, water soluble ash content, acid insoluble ash content and extractable matter content serve an important role in standardization and quality control by means of purity, stability and phytochemical composition of plant drugs (Bharat and Parabia, 2010). The moisture content in the *A. chevalieri* powdered leaf was found to be 9.11%. The general requirement of moisture content in crude drug is that, it should not be greater than 14% (*British Herbal*

Pharmacopeia, 1990) and the value observed in this research work was within the accepted range. Determination of the moisture content helps prevent degradation of drug during storage. The lower the value, the less likelihood of degradation of drug and suggests better stability of product. Moisture is considered an adulterant because of its added weight as well as the fact that excess of it promotes mold and bacterial growth (WHO, 2011).

Ash values are used to determine purity and quality of crude drug. It indicates the presence of various impurities such as carbonate, oxalate and silicate. The water soluble ash (1.75%) contains mainly silica, particularly in sand and it indicates contamination with earthy material. The acid insoluble ash value obtained in this study was 1.5%. The total Ash value (4.83%) represents both the physiological and non-physiological ash from the plant. The non-physiological ash is an indication of inorganic residue after the plant drug is incinerated. Total ash value is a reliable aid for detecting adulteration in drugs (WHO, 1996). Extractive values are useful to estimate the chemical constituents present in the crude drug and are a measure to determine the solubility of phytoconstituents from the crude drug in a given solvent (Thomas *et al.*, 2008). This study showed (Table 3) that ethanol had high extractive value of 21.00 % w/w compared to water which had extractive value of 15.61 % w/w.

CONCLUSION

The present study on pharmacognostic standization of *A. chevalieri* has revealed various pharmacognostical and physicochemical standards which will help in setting a suitable plant profile for the proper identification, quality control and compilation of a suitable monograph on *A. chevalieri*

REFERENCES

- Abdel-Kader, M., Hoch, J., John, M., Evans, R., James, S., Stephen, W., Dalton, James, M., Kingston, G. I. (2001). Two biologically active saponins from *Albizia subdimidiata* from the Suriname rainforest. *Journal of Natural Products*, 64: 536-539.
- Agboola, O. I., Chidiobi, C., Omobuwajo, O. R. (2012). Pharmacognostic Studies and Establishment of Quality

- Parameters for *Albizia altissimum* (hook.f). Hutch Dandy Leaf. *Pharmacognosy Journal*, 4: 27,
- Aliyu, A. B., Musa, A. M., Ibrahim, M.A., Ibrahim, H. and Oyewale, A.O. (2009). Preliminary Phytochemical Screening and Antioxidant activity of Leave extract of *Albizia chevalieri* harms (leguminoseae-mimosoideae). *Bayero Journal of Pure and Applied Sciences*, 2(1):149 – 153.
- Bharat, G and Parabia, M. H. (2010). Pharmacognostic evaluation of bark and seeds of *Mimusopselengi* L. *International Journal of Pharmacy and Pharmaceutical Sciences*, 2: 110-113.
- Brain, K.R., and Turner, T.D (1975). The Practical Evaluation of Phytopharmaceuticals, Wright scientechnica, Bristol, UK. Pp. 166.
- British Herbal Pharmacopoeia (1990). *British Herbal Medicine Association*. Bournemouth: Dorset.1st edition. Pp, 1-2.
- Burkill, H.M. (1995). The Useful Plants of West Tropical Africa Vol. 3. Families J-L, Royal Botanical Gardens, Kew. Pp. 207-208.
- Eggeling, W. J., William, J. and Ivan, R. D. (2000). *The Indigenous Trees of the Uganda Protectorate*. The Government Printer Entebbe, Uganda and the Crown Agents for the Colonies, MB, London, U.K. Pp, 130-132.
- Elufioye, T.O. and Olaifa, O. (2014). Pharmacognostic evaluation of the leaves and stem bark of *Euphorbia lateriflora* Schum&Thonn. *Nigerian Journal of Natural Product and Medicine*, 19(1): 18-23.
- Evans, W. C. (2009). *Trease and Evans pharmacognosy*, 16th edition. Elseviers Ltd., UK, Pp.560-562, 568-570.
- Ghani, A. (1990). *Introduction to Pharmacognosy*, Ahmadu Bello University, Press Ltd. First Edition. Zaria, Nigeria. Pp 187.
- Hao, D., Chen, S., Xiao, P. and Peng Y, (2010). Authentication of Medicinal Plants by DNA-based Markers and Genomics. *Chinese Herbal Medicines*, 2(4): 250-261.
- Hina, F., Nisar, A and Mir, A. K. (2011). Physico-chemical, Phytochemical Evaluation and DPPH- Scavenging Antioxidant potential in Medicinal Plants used for herbal formulation in Pakistan, Pak. *Journal of Botany*. 43: 63-67.
- Kokate, C. K., Purohit, A. P. and Gokhale, S. B. (2009). *Pharmacognosy*. 43th ed. Nirali Prakashan; Pune. Pp. 6.1-6.44.
- Le Houèrou, H. (2009). <http://www.fao.org/ag/AGP/AGPC/doc/GBASE/Data/pf000366.HTM> accessed on 20 -03-2018.
- Nuhu A., Danmalam, U. H., Ilyas, N., Zakariya, A. M., Abdulhamid, Z. and Abubakar, A.Z. (2016). Pharmacognostic Evaluation of the Leaves and Stem-Bark of *Commiphora africana* (A. Rich) Engl. (Bursereaceae). *Nigerian Journal of Natural Products and Medicine*, 20 (1): 56-60. <http://dx.doi.org/10.4314/njnpm.v20i1.10>
- Odugbemi, T. (2006). Outlines and pictures of Medicinal plants from Nigeria, pp.1 – 85.
- Rahul, C., Pradhan, P., Sharma, K. S. and Jhajharia, K. M. (2010). Phytochemical screening and antimicrobial activity of *Albizia lebbek*. *Journal of Chemical and Pharmarceutical Research*. 2(5): 476-484.
- Saidu, Y., Lawal, M., Isezuo, S. A., Shehu, R. A., Sahabi, D. M. and Bilbis, L. S. (2007). Partial purification and elucidation of mechanism of hypoglycaemic agent of aqueous leaf extract of *Albizia chevalieri* Harms (Leguminosae). *Journal Pharmacology Toxicology*; 2(6):513-23.
- Smillie, T. J. and Khan, I. A. (2010). A comprehensive approach to identifying and authenticating botanical products. *Clinical Pharmacology Therapy*. 87: 175-186.
- Thomas, S., Patil, D. A., Patil, A. G. and Chandra, N. (2008). Pharmacognostic evaluation and physicochemical analysis of *Averrhoa carambola* L. fruit. *Journal of Herb Toxicology*; 2(2): 51-54.
- WHO (1996). Quality assurance of pharmaceuticals. A compendium of guidelines and related materials, good manufacturing practices and inspection. WHO, Geneva, Switzerland. Pp 40.
- WHO (2011). *Quality Control Methods for Medicinal Plants*. WHO, Geneva, Switzerland. Pp 31.
- Yusuf, S., Lawal, S. B., Mansur, L., Simeon, A. I. and Rabi, A. U. (2007). Hematotoxicity study of the leaf extract of *Albizia chevalieri* harms (Leguminosae). *Biochemia Medica*; 17(2):20