Original Research Article

TAXONOMIC AND PHARMACOGNOSTIC EVALUATION OF LEAF OF *Mussaenda philippica* L. (RUBIACEAE)

ABSTRACT

Mussaenda philippica Linn. belongs to the family Rubiaceae. The aim of this work was to use the quality control parameters in the evaluation of the leaf of this plant. The leaves were collected, identified, air dried and pulverized. Standard procedures were carried out to obtain microscopic features of the fresh and powdered samples, micromeritic, chemomicroscopy, flourescence properties, soluble extractive values, moisture contents and ash values. The results of the microscopy study of the fresh leaf revealed an hypostomatic distribution of stomata with paracytic stomata on the abaxial surface of the leaf only, stomatal number of 56.8, stomatal index of 25.06%, and epidermal number of 169.8, while the adaxial surface had an epidermal number of 241.9. The plant sample of the leaf also possessed unicellular trichomes. Micromeritic properties of the powdered leaf samples showed bulk volume of 35.33±0.33, tapped volume of 28.00±0.00, bulk density of 0.28±0.00, tapped density of 0.35±0.00 , angle of repose of 35.4°, Carr's Index of 20.90±0.74, Hausner's ratio of 1.26±0.01. Chemomicroscopy study on the leaf powder revealed the presence of lignin, starch, cellulose, calcium oxalate crystals, oil, mucilage and protein. The moisture content was 11 %w/w. Results for the total ash, acid-insoluble ash and water-soluble ash values were 9 %w/w, 1%w/w and 5 %w/w respectively. Results for the ethanol-soluble, methanol-soluble and water-soluble extractive values were 18%w/w, 17 %w/w and 25 %w/w respectively. The above results could be used to establish pharmacopoeial standard of fresh and powdered drug of *M. philippica.*

Key Words: Chemomicroscopy, hypostomatic, micromeritic, *Mussaenda philippica*, pharmacognostic, taxonomic

INTRODUCTION

Mussaenda philippica belongs to the family Rubiaceae, is a large shrub or small tree found growing in semi-shaded or open areas in secondary and primary forests, savannahs and forest edges [1]. It is used in high doses to treat appendicitis and hepatitis [2]. It is usually used as ornamental plant. Phytochemical constituents include Iridoids, flavonoids and triterpenes. The most recognized compounds in *M. philippica* are the iridoids and triterpene saponins [3]. The plant is extensively grown as an ornamental in botanical gardens, parks and along roadsides [4]. In Nigeria, this species is used to treat dysentery, antidote for snakebites, affections of the chest and lungs and stomachache [5]. Pharmacologically, Sanshiside methyl ester posssess antiviral].property[6]. Non-glycosidic iridoids like Mussaein are cytotoxic [7

Phylogeny of *Mussaenda Philippica* (white flower) (Scientific Classification)[8].

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Plantae	-	Kingdom:
Tracheophytes	-	*Clade:*
Angiosperms	-	*Clade:*
Eudicots	-	*Clade:*
Asterids	-	*Clade:*
Gentianales	-	Order:
Rubiaceae	-	Family:
Mussaenda	-	Genus:
M. Philippica	-	Species:
Mussaenda philippica ’Aurorae’	-	Botanical Name
White Mussaenda, Bankok Rose	-	Common Name
Afia rose abankuk	-	Local Name

Figure 1: *Mussaenda philippica* in its natural habitat

MATERIALS AND METHOD

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Collection, Identification and Preparatioin of the Plant

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The plants was collected from Brooks street off Nwaniba road, Uyo Local Government Area, Akwa Ibom State, Nigeria in January 2021. It was identified by Prof. Mrs. Magaret Bassey of the department of Botany and Ecological Studies, Faculty of Sciences, University of Uyo with the voucher number: UUPH 67 (m). The fresh plant was air dried, pulverized and packed in a dry container, well labeled and used when needed.

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Anatomical Studies

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Microscopic Evaluation of Leaf

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Matured fresh leaves of the plant were cut at the petiole. Microscopical 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 loose 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 as mountant. The stained samples were viewed on the microscope. Photomicrographs of the prepared slides were taken with an Amscope MD500 mounted on Olympus CX21 microscope. The transverse section and powder microscopy of the plant were observed and photographs taken **[9]**.

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Quantitative Microscopy of the Leaf

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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

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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

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Stomatal Index Determination

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].The stomatal index (S.I) was determined according to Metcalfe and Chalk [10,11

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using the formula:

The sample (quantitative microscopy) was placed under the microscope and the stomatal index was determined using the formula;

$$S.I = \frac{S}{E+S} X100$$

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 **[12,13]** 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;

Where;

$B\rho$ = Bulk density Where

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

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While Carr's Index is measured as

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Where; $T\rho$ = Tapped density

$B\rho$ = Bulk density.

Angle of repose

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Chemomicroscopic Analysis of Leaf Powder

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Powdered leaf was examined for its chemomicroscopic properties namely mucilage, lignin, starch, oils, calcium carbonate and calcium oxalate crystals using standard procedures **[20]**.

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Fluorescence Analysis of Leaf Powders

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The fluorescent analysis of dried leaf powder was carried out using standard method [21].

Physico-chemical Evaluation of Leaf Powders

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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 [11,15,16

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RESULTS

The results for the anatomical studies, micromeritic properties, chemomicroscopy, fluorescence properties, soluble extractive values, moisture content and ash values of the leaf are represented in Tables 1- 6 and the adaxial, abaxial, transverse section and powder analysis and the leaf was as represented in Figure 2 (A-E) respectively.

Table 1: Results for the Microscopic Features of *M. philippica* and Standard Error of Mean (SEM) for the leaf surface;

Leaf surface	Abaxial	Adaxial
Stomatal morphology	Paracytic	-
Stomatal length (µm)	13.96 (15.84±0.46) 19.61	-
Stomatal width (µm)	8.40 (9.96±0.28) 11.54	-
Stomatal pore length (µm)	4.08 (6.33±0.42) 7.69	-
Stomatal pore width (µm)	1.63 (2.81±0.19) 3.65	-
Stomatal number	52 (56.8±1.30) 64	-
Stomatal index	25.06%	-
Epidermal wall pattern	Polygonal	Irregular
Length of epidermal layer (µm)	21.05 (27.24±1.12)31.17	31.01 (40.97±1.62) 49.65
Width of epidermal layer(µm)	9.01 (13.84±0.63) 14.70	14.70 (18.04±1.13) 26.83
Thickness (µm)	3.22 (3.92±0.20) 4.81	3.26 (4.61±0.28) 6.21
Epidermal number	156 (169.8±9.24) 210	215 (241.9±8.83) 271
Trichome type	Unicellular	-
Trichome length (µm)	64.49 (137.60±21.79) 263.25	-
Trichome width (µm)	6.97 (9.84±0.51) 12.41	-

Result presented as Highest range (Mean and standard Error of Mean) Lowest range of 10 determinations

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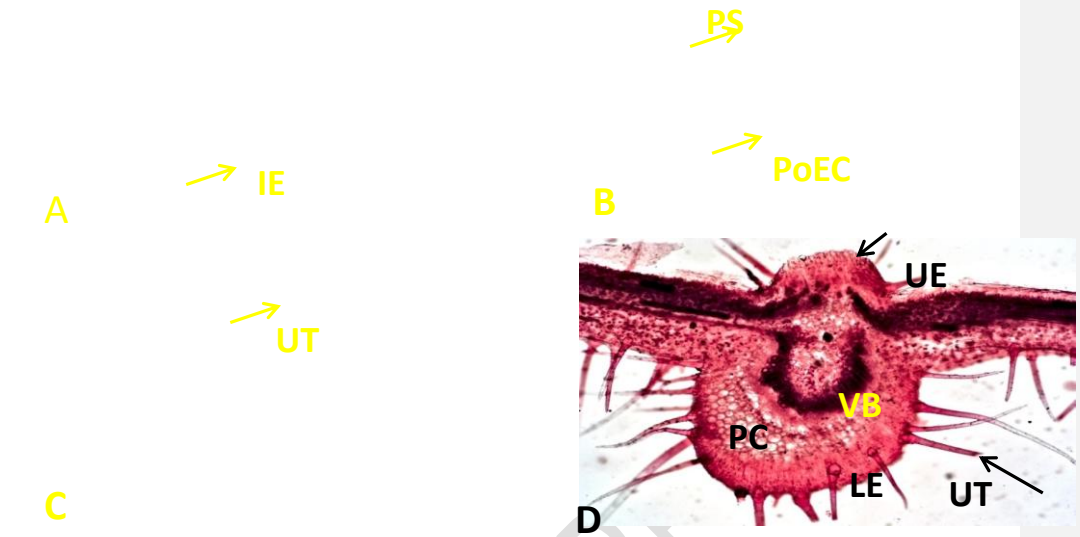

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Figure 2: A; Adaxial surface (IE; Irregular epidermal cell shape), B and C: Abaxial surface (PoEc; Polygonal epidermal cell shape), PS; Paracytic stomata, UT; Unicellular trichome. D: Transverse section of the leaf: UE; Upper epidermis, VB; Vascular bundles, UT; Unicellular trichome, PC; Parenchyma cells; LE; Lower epidermis. E: Powder analysis showing UT; Unicellular trichome

Table 2 : Results for Micromeritic Properties of *M. Philippica* Leaf

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Parameters	Values
Bulk Volume (cm)	35.33±0.33
Tapped Volume (cm)	28.00±0.00
Bulk Density (g/ml)	0.28±0.00
Tapped Density (g/ml)	0.35±0.00

Flow Rate (g/s)	0.68±0.02
Angle of Repose (°)	35.4
Hausner's ratio	1.26±0.01
Carr's Index	20.90±0.74
Diameter of Heap (cm)	6.93±0.08

Result presented as Mean±Standard Error of Mean of 3 determinations

Table 3 : Results for Chemomicroscopy of *M. Philippica* Leaf

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Constituents	Qualitative Test	Observation	Inference
Lignin	Phloroglucinol+ con.HCL	Red stain on sample	Lignin present
Starch	N/50 iodine	Blue-black coloration	Starch present
Cellulose	N/50 iodine+ 66%H_2SO_4	Blue coloration	Cellulose present
Calcium Oxalate Crystals	Sample cleared and viewed under microscope	Calcium Oxalate Crystals seen	Calcium Oxalate present
	+ 80% HCL	Crystal dissolves	Calcium Oxalate crystals present
Oils	Sudan IV, view under microscope	Sample stains pink	Oil present
Mucilage	Ruthenium red, view under microscope	Sample stains pink	Mucilage present
Protein	1%picric acid and millions reagent	Yellow stain strands present	Protein present

Table 4: Results For the Florescence Properties of *M.philippica* Leaf

Extract	Sample Physical Observation	Color	UV-254nm Color	UV-365nm Color
N-hexane	Leaf	White	White	Brown
DCM	Leaf	Green	Green	Orange
Ethyl Acetate	Leaf	White	White	Pink
Ethanol	Leaf	Green	Green	Orange

Methanol	Leaf	Green	Green	Brown
Water	Leaf	Brown	Purple	Grey

Results for Water-Soluble Extractive Value, Ethanol-Soluble Extractive, Methanol-Soluble Extractive Value and Standard Error of Mean for Leaf Powders of *M. philippica.*

Table 5 :

Parameters	Weight(g)	Percentage(%w/w)
Water-soluble extractive value	0.25±0.00	25
Ethanol-soluble extractive value	0.18±0.00	18
Methanol-soluble extractive value	0.17±0.00	17

Table 6: Results for Moisture Content, Total Ash Value, Acid-Insoluble Ash Value and Standard Error of Mean for the Leaf of *M. philippica*

Parameters	Weight(g)	Percentage (%w/w)
Moisture content	0.33±0.00	11
Total ash value	0.27±0.00	9
Acid-insoluble ash value	0.03±0.01	1
Water-soluble ash value	0.15±0.01	5

Discussion

The results of the microscopy study using the fresh leaf revealed the presence of paracytic stomata and polygonal epidermal cell shape (Figure 2B), a stomatal number of 56.8±1.30, stomatal index of 25.06%, and epidermal cell number of 169.8±9.24 on the abaxial surface of the leaf, while the adaxial surface had no stomata but had an irregular epidermal cell shape (Figure 2A) and epidermal cell number of 241.9±8.83. The mean stomatal length and width for the abaxial surface of the leaf were 15.84 µm and 9.96 µm (Table 1). The micromeritic study showed angle of repose of 35.4° indicating a good flow. Hausner's ratio and Carr's index were 1.26 and 20.90% as shown in Table 2 indicating a passable to fair flow characteristics. In recent times, the Compressibility index and the closely related Hausner's ratio have become simple, fast, and a popular method of predicting powder flow characteristics. The Compressibility index has been proposed as a measure of inter-particulate interactions and as an indirect measure of

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bulk density, size, shape, surface area, moisture content and cohesiveness of powders. Umoh *et al*. [17] reported using same parameters for establishing standards in *Gnetum africanum* Welw (Gnetaceae), *Buchholzia coriacea* Engl Caparidaceae, Umoh *et al*. [18] and *Jatropha tanjorensis* J.L. Ellis & Saroja. (Euphorbiaceae) Umoh *et al*. [19].

Chemomicroscopy study on the leaf revealed the presence of lignin, starch, cellulose, calcium oxalate crystals, oil, mucilage and protein as shown in Table 3 as also reported by Johnny and Bassey [20] for *Cola pachycarpa* leaf and stem powders. Calcium oxalate crystals play a central role in a variety of important functions, including tissue calcium regulation, protection from herbivory and metal detoxification **[**21**]**. Thus, plants with calcium oxalate crystals shows good antioxidant properties when formulated into pharmaceuticals. The fluorescence property of the powdered sample for different solvent extracts revealed different colors indicating the presence of phytochemicals like: anthocyanins, phenols, tannins and flavonoids when viewed in daylight, lower and higher wavelengths of UV light. This property is useful in characterizing crude drugs, identifying authentic samples and recognizing adulterants. In a mixture of different drugs of two or more species, fluorescence studies help to identify a particular drug by the use of estimates of intensity of fluorescence [22**]**.

The ethanol-soluble, methanol-soluble and water-soluble extractive values were 18 %w/w, 17 %w/w and 25 %w/w respectively. From the result obtained above, water is the best solvent for the extraction of the constituents of the plant. The water-soluble extractive value indicates the presence of water-soluble matters such as sugars, amino acids and vitamins derived from the plants.

The moisture contents was 11 %w/w which suggests a moderate moisture content as it is within the limit (8 % to 14 %) for vegetable drugs [11]. 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. The plant possesses moderate moisture content and is stable for use but for better quality should be stored properly as it is subject to degradation. The total ash, acid-insoluble ash and water-soluble ash values for the leaf were 9 %w/w, 1 %w/w and 5%w/w as shown in table 6. The total ash values for leaf was within the limit (Total ash value limit should not exceed 14 %w/w) and the acid-insoluble ash values of leaf was also within the limit (should not be greater than 2, European Pharmacopoeia [23]. Ash value is useful in determining authenticity and purity of sample. The ash value indicates the presence of Inorganic ions. During ashing, organic matter gets oxidized and certain amount of volatile elements are lost.

Conclusion

It can be said that the data obtained can assist in the proper identification and provides basis for standardization of the leaf of *Mussaenda philippica*.

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