

Original Research Article

MICROMORPHOLOGICAL AND PHARMACOGNOSTIC STUDIES OF THE FLOWER OF *Mussaendaphilippica* L. (RUBIACEAE)

ABSTRACT

Mussaendaphilippica Linn. belongs to the sub-family Ixoroideae which belongs to the family Rubiaceae. The aim of this study was to employ the quality control parameters in the evaluation of flowers of *M. philippica* L. The flowers were collected, identified, air dried and pulverized. Standard procedures were carried out to obtain microscopic features of the fresh and powdered samples, micromeritic, chemomicroscopy, fluorescence properties, soluble-extractive values, moisture content and ash values. The results of the microscopic studies using the fresh and powdered flower revealed the presence of paracytic and anomocytic stomata on the abaxial surface of the flower (hypostomatic) and none on the adaxial. The abaxial surface also had a stomatal number of 13.6, stomatal index of 4.97% and epidermal number of 260.8 while the adaxial surface had an epidermal number of 304.4. The plant samples of the flower also possessed unicellular trichomes. Results of the micromeritic properties of the samples were bulk volume of 67.16 ± 0.16 , tapped volume of 48.00 ± 0.57 , bulk density of 0.14 ± 0.00 , tapped density of 0.20 ± 0.00 , angle of repose of 38.5° , Carr's Index of 28.50 ± 0.81 and Hausner's ratio of 1.39 ± 0.01 . Chemomicroscopy study showed cellulose, mucilage and protein. The moisture content values obtained was $13\%^{w/w}$. Results for the total ash, acid-insoluble ash and water-soluble ash values were $7.7\%^{w/w}$, $1.3\%^{w/w}$ and $4\%^{w/w}$ and for the ethanol-soluble, methanol-soluble and water-soluble extractive values were $33\%^{w/w}$, $33\%^{w/w}$ and $36\%^{w/w}$. The above results could be used to establish pharmacopoeial standards for both fresh and powdered flower of *M. philippica*.

Comment [WW1]: Double subject

Key Words: Hypostomatic, *Mussaendaphilippica*, micromorphological, pharmacognostic

INTRODUCTION

Mussaendaphilippica is a large shrub or small tree, is glabrous to minutely puberulent and the leaves are ovate-elliptic to elliptic, to 17×6 cm, strongly acuminate, base contracted to a slender petiole 1-1.5 cm long, veins 9 to 12 on a side, also the stipules are sericeous, triangular, with a deeply bifid acumen with panicle about 4 times trichotomous, densely sericeous to soft-pubescent, bracts linear, bracteoles irregularly and flowers apparently dioecious with a caducous calyx, lobed almost to base, principal lobes 5 and the outer one greatly enlarged and showy on a few flowers with the calyx usually somewhat less sericeous [1]. It is found growing in semi-shaded or open areas in secondary and primary forests, savannahs, forest edges, coastal scrubs and thickets, disturbed areas, ravines and riparian sites at elevations from sea level to 1400 m. The plant extensively grown as an ornamental in botanical gardens, parks, gardens and along roadsides, byways and highways. It is used in the decoration of compounds of various homes [2].

Comment [WW2]: Double to be in one sentence. This sentence can become several sentences.

Phytochemical constituents of the plant include Iridoids, flavonoids and triterpenes. The most recognized compounds in *M. philippica* are the iridoids and triterpenes [3]. In Nigeria, this species is used to treat dysentery, antidote for snakebites, affections of the

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chest and lungs and stomach ache [4].Pharmacologically,Sanshiside methyl ester posseses antiviral property [5].

Phylogeny of *Mussaendaphilippica*(The white flower species)(Scientific Classification)[6].

Comment [WW4]: Not bold

Kingdom:	-	Plantae
Clade:	-	Tracheophytes
Clade:	-	Angiosperms
Clade:	-	Eudicots
Clade:	-	Asterids
Order:	-	Gentianales
Family:	-	Rubiaceae
Genus:	-	<i>Mussaenda</i>
Species:	-	<i>M.philippica</i>
Botanical Name	-	<i>Mussaendaphilippica</i> 'Aurorae'
Common Name	-	White Mussaenda, Bangkok Rose
Local Name	-	Afia rose abankuk

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Figure 1: Showing leaves and flowers of *Mussaenda philippica* in its natural

Habitat. Source: Field data, (2021)(Umohet al., 2022)[7].

Comment [WW5]: Images should be numbered so that they can be distinguished from one another

MATERIALS AND METHODS

Collection, Identification and Preparation of the Plant Material

Plant sample was collected from Brooks street, Uyo Local Government Area, Akwa Ibom State, Nigeria in January, 2021. The plant was identified by Dr. Imoh I. Johnny, Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo with herbarium identification number: UUPH No. 38(b). The fresh flower material was collected, air-dried, pulverized and packed in a dry container, well labelled and used when needed.

Comment [WW6]: Sub section should be numbered, see guideline of this journal !

Anatomical Studies

Microscopic Evaluation of the Flower

The standard median portion of the flower was obtained. Microscopical examinations of the Epidermis of both adaxial and abaxial surfaces were made by placing the flower on a glass slide. The sample was irrigated with water and scraped gently with a sharp razor blade, loose cells from the epidermis were washed away with water till the desired epidermis was reached. The epidermal peels were further cleared with sodium hypochlorite, rinsed gently with water and stained with aqueous solution of safranin-O for (five) 5 minutes and 10% glycerol. The stained samples were mounted on a binocular microscope. Photomicrographs were taken from good preparations using the Olympus CX21 binocular microscope fitted with an MD500 amscope microscope eyepiece camera. Measurements were done at $\times 10$ and $\times 40$ for photomicrographs [8].

Quantitative Microscopy of the Flower

Quantitative microscopy parameters such as flower 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 [9].

All measurements were made using a calibrated ocular micrometer and 10 microscopic fields chosen at random were used and data presented as mean \pm Standard Error of Mean (SEM).

Stomatal Index Determination

The stomatal index (S.I) was determined according to African Pharmacopoeia [10].

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

$$S.I = \frac{S}{E+S} \times 100$$

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 [11]. Which constitutes;

Bulk Density and Tapped Density

The weight of 10 g of dried powdered flower 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;

$$B_p = M / V_b$$

$$T_p = M / V_t$$

Where B _p	=	Bulk density
M	=	Mass of powder
V _b	=	Bulk volume of powder
T _p	=	Tapped density
V _t	=	Tapped volume

Hausner's Ratio and Carr's index

Hausner's ratio a function of interparticle friction was calculated using the formula

$$\text{Hausner's ratio} = T_p / B_p$$

$$\text{While Carr's index} = T_p - B_p / T_p \times 100$$

Where; T_p = Tapped density

B_p = Bulk density.

Angle of repose(θ) = \tan^{-1} (Heap height of powder / Radius of heap base)

Chemomicroscopic Analysis of Flower Powder

Powdered flower was examined for its chemomicroscopic properties namely mucilage, lignin, starch, oils, calcium carbonate and calcium oxalate crystals using standard procedures [12].

Fluorescence Analysis of Flower Powder

The fluorescent analysis of dried flower powder was carried out using standard method [13].

Physico-chemical Evaluation of Flower Powders

The physicochemical parameters such as moisture content, ash values (total ash, acid-insoluble ash, water-soluble 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 [9].

Table 1: Results for the Microscopic Features of *Mussaendaphilippica* and Standard Error of Mean (SEM) For the Flower Surface

Flower surface	Abaxial	Adaxial
Stomatal morphology	Paracytic, Anomocytic	-
Stomatal length (µm)	19.91 (22.76±0.64)	25.61
Stomatal width (µm)	9.79 (12.85±0.75)	16.52
Stomatal pore length (µm)	7.17 (7.97±0.18)	8.78
Stomatal pore width (µm)	2.45 (3.01±0.17)	3.97
Stomatal number	9 (13.6±0.99)	18
Stomatal index	4.97%	-
Epidermal wall pattern	Sinuuous	Polygonal-Irregular
Length of epidermal layer (µm)	27.03 (32.31±1.38)	41.05 19.78 (26.07±1.56)
Width of epidermal layer (µm)	12.45 (16.78±1.17)	23.03 10.45 (13.88±0.99)
Thickness (µm)	3.21 (4.30±0.23)	5.53 3.99 (4.62±0.51)
Epidermal number	164 (260±17.62)	333 250 (304.4±13.91)
Trichome type	Unicellular	Unicellular
Trichome length (µm)	142.82 (218.33±19.73)	109.70 (198.40±27.13)
	329.29	396.28
Trichome width (µm)	9.30 (11.72±0.43)	13.50 9.93 (14.95±1.31)
		25.08

Table 2: Results for Micromeritic Properties of *Mussaendaphilippica* Powdered Flower

Parameters	Values
Bulk Volume (cm)	67.16±0.16
Tapped Volume (cm)	48.00±0.57
Bulk Density (g/ml)	0.14±0.00
Tapped Density (g/ml)	0.20±0.00
Flow Rate (g/s)	0.18±0.02
Angle of Repose (°)	38.5
Hausner's ratio	1.39±0.01
Carr's Index	28.50±0.81
Diameter of Heap (cm)	8.63±0.06

Table 3: Results for Chemomicroscopy of *Mussaendaphilippica* Powdered Flower

Constituents	Qualitative Test	Observation	Inference
Lignin	Phloroglucinol+ Conc.HCL	No red stain on sample	Lignin absent
Starch	N/50 iodine	No blue-black coloration	Starch absent
Cellulose	N/50 iodine+ 66% H ₂ SO ₄	Blue coloration	Cellulose present
Calcium Oxalate Crystals	Sample cleared and viewed under microscope + 80% HCL	No Calcium Oxalate Crystals seen	Calcium Oxalate absent

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		No dissolution takes place	Calcium Oxalate crystals absent
Oils	Sudan IV, view under microscope	No pink stain on sample	Oil absent
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 *Mussaendaphilippica* Powdered Flower

Extract	Sample	Physical Observation Colour	UV-254nm Colour	UV-365nm Colour
N-hexane	Flower	White	White	Grey
DCM	Flower	Brown	Pink	White
Ethyl Acetate	Flower	Brown	Brown	Black
Ethanol	Flower	Brown	Brown	Black
Methanol	Flower	Brown	Brown	Black
Water	Flower	Brown	Purple	Black

Table 5: Results for Water-Soluble Extractive Value, Ethanol-Soluble Extractive, Methanol-Soluble Extractive Value and Standard Error of Mean for Flower Powders of *Mussaendaphilippica*.

Parameters	Weight(g)	Percentage (%w/w)
Water-soluble extractive value	0.36±0.00	36
Ethanol-soluble extractive value	0.33±0.00	33
Methanol-soluble extractive value	0.33±0.00	33

Comment [WW8]: The title of this table too long, maybe it can be shortened to "Result of solubility tests of *Mussaenda philippica* flower powders"

Table 6: Results for Moisture Content, Total Ash Value, Acid-Insoluble Ash, water-soluble Ash Values and Standard Error of Mean for the Flower of *Mussaendaphilippica*

	Weight(g)	Percentage (%w/w)
Moisture content	0.39±0.00	13
Total ash value	0.23±0.00	7.7
Acid-insoluble ash value	0.04±0.01	1.3
Water-soluble ash value	0.12±0.00	4

Comment [WW9]: The title of this table too long, maybe it can be shortened, example "Result for non specific tests of *Mussaenda philippica* flower"

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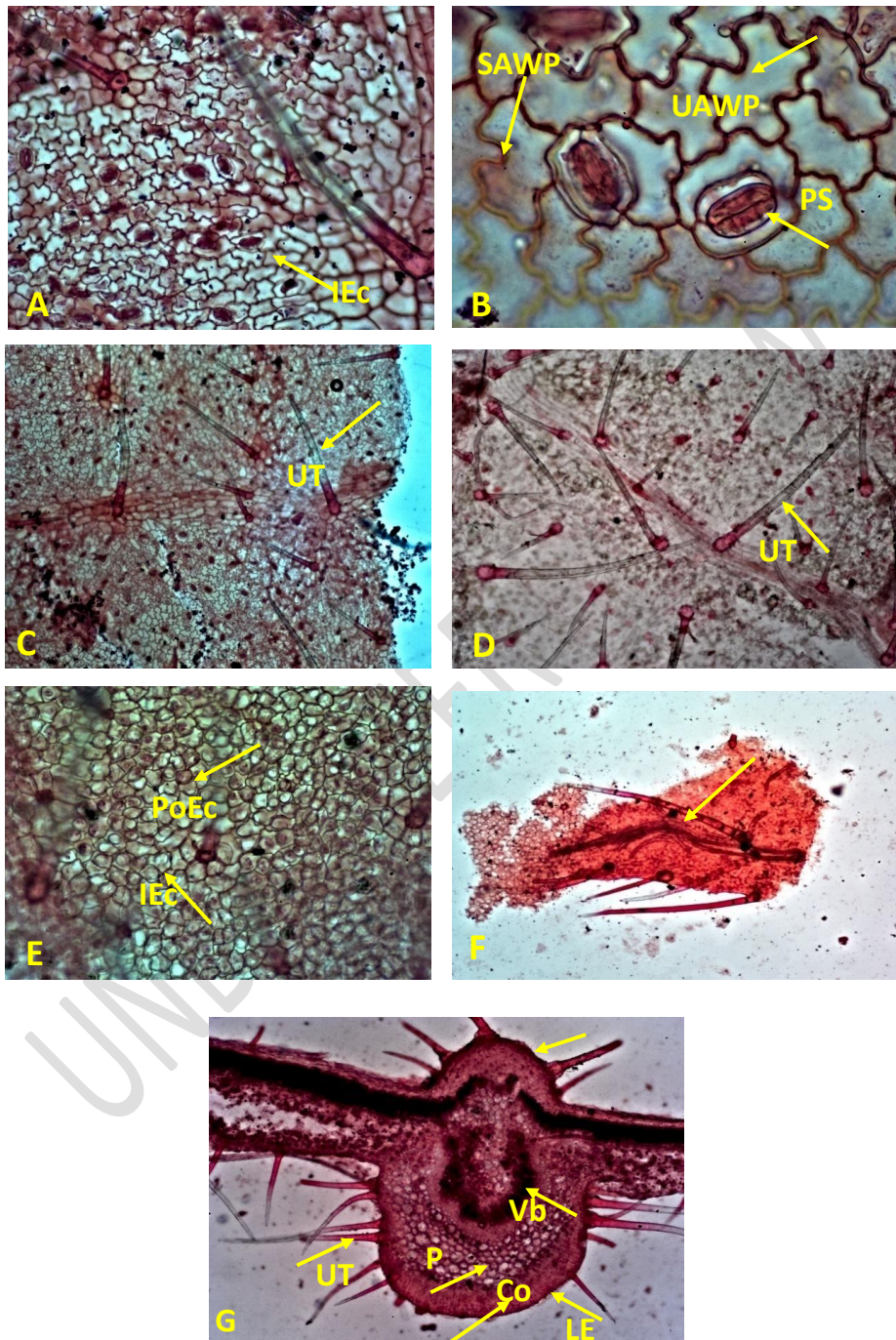


Figure 2: *M. philippica* Flower A: IEC (Irregular Epidermal Cell) $\times 10$ Abaxial surface, B: SAWP (Sinuous Anticlinal Wall Patter), UAWP (Undulate Anticlinal Wall Pattern), PS (Paracytic Stomata) $\times 40$ Abaxial Surface, C: UT (Unicellular Trichome) $\times 4$ Abaxial surface, D: UT (Unicellular

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Trichome) $\times 4$ Adaxial surface, E: PoEc (Polygonal Epidermal Cell) $\times 10$ Adaxial surface, F: UT (Unicellular Trichome) Powder $\times 4$, G: UE (Upper Epidermal Cell), Vb (Vascular bundle), P (Parenchyma), Co (Collenchyma), LE (Lower Epidermis), UT (Unicellular Trichome) $\times 4$

DISCUSSION

The qualitative microscopic studies of the epidermal layers of the flower of *M. philippica* revealed the presence of paracytic stomata (Fig. 2B), sinuous anticlinal wall pattern (Fig. 2B) and unicellular trichomes (Fig. 2C) on the abaxial surface. While the adaxial surface has no stomata but unicellular trichomes and polygonal-irregular wall pattern as shown in Fig. 2 E. Stomatal number of 13.6 ± 0.99 , stomatal index of 4.97% and epidermal number of 260.8 ± 17.62 on the abaxial surface of the flower and the adaxial surface had no stomatal number and stomatal index but had an epidermal number of 304.4 ± 13.91 as shown in Table 1. The mean stomatal length and width for the abaxial surface of the flower were $22.76 \mu\text{m}$ and $12.85 \mu\text{m}$. The powder microscopy also revealed the presence of calcium oxalate crystals. The micromeritic study showed angle of repose of 38.5° , indicating a fair flow. Hausner's ratio and Carr's index were 1.39 and 28.50% as shown in Table 2. From the result obtained, the powder of the flower has a poor flow characteristic. Micromeritics is an important consideration in the development of solid dosage formulation which is mostly used for physical, mechanical and chemical processes [14]. The micromeritic properties indicate flow properties as well as interparticulate resistance between these powders. This information predicts the stability and solubility of crude drugs. These studies influence a number of processing parameters in manufacturing pharmaceutical formulations. The knowledge and effect of particle size distribution of active pharmaceutical ingredient as well as excipients will be useful in solving 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 a direct impact on the flowability, tableting, content uniformity, weight variation and dissolution rate which will inadvertently affect the bioavailability of the 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 powders include: moisture content, temperature, particle 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. It is a characteristic related to interparticulate friction or resistance to movement between particles [15]. An alternative test is to determine Carr's index which relates the bulk density to the tapped density.

Chemomicroscopy study on the flower showed cellulose, mucilage and protein as shown in Table 3. Protein is an important constituent which is useful in body building and repair of body tissues. The fluorescence properties of the powdered sample for different solvent extracts revealed different colours in the daylight, higher and lower wavelengths of UV light indicating the presence of phytochemicals like: anthocyanins, phenols, tannins and flavonoids as shown in Table 4. 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 [16].

The ethanol-soluble, methanol-soluble and water-soluble extractive values were $33\% \text{ w/w}$, $33\% \text{ w/w}$ and $36\% \text{ w/w}$ as shown in Table 5. From the result obtained above, water is the best solvent for the extraction of the constituents of the powdered flower. The water-soluble extractive value indicates the presence of water-soluble matters such as sugars, amino acids and vitamins derived from plants. Extractive values help to measure the amount of constituents which are extractable by the solvents under specified conditions.

Comment [WW11]: All figures and tables should be described and mentioned in this subsection, compressively, (from Figure 1 to Figure 2) and Table 1 until Table 6. Suggestion for this subsection is "Result and Discussion"

They are equally useful in estimating the specific constituent based on its solubility in a particular solvent used for its extraction.

The moisture contents obtained for the flower was 13%^{w/w}. These values suggest a moderate moisture content as it is within the limit (8% to 14%) for vegetable drugs [9]. The obtained moisture content is indicative of the plant's shelf life as high moisture content is uneconomical and at the right 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. The plant possesses moderate moisture content and should be stored properly as it is subject to degradation. The total ash, acid-insoluble ash and water-soluble ash values were 7.67%^{w/w}, 1.33%^{w/w} and 4%^{w/w} as shown in Table 6. The total ash value is within the limit (Total ash limit should not exceed 14%^{w/w}) and the acid-insoluble ash values is also within the limit (should not be greater than 2%^{w/w}, European Pharmacopoeia, 2007) [17]. Ash value is useful in determining authenticity and purity of sample. During ashing organic matter gets oxidized and certain amount of volatile elements are lost. Acid-insoluble ash value gives an idea of the measured amount of silica especially sand and other siliceous material present in the drugs while total ash contains inorganic radicals like phosphates, carbonates and silicates of sodium, magnesium and calcium giving an estimation about the purity and quality of the drug and the water-soluble ash value gives an estimation of the inorganic contents inherent in the sample.

Conclusion

It can be said that the data obtained can assist in the proper identification and in the development of the plant monograph.

Comment [WW12]: The conclusions should represent the overall results of this study

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