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Pharmacognostic Evaluation Of Mussaenda Philippica On Leaf

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ABSTRACT

The use of medicinal plants has gained more importance because of its natural origin and high therapeutic significance. The various species of the genus Mussaenda has many novel phytochemical constituents which has high pharmacological activities such as anti- inflammatory, antioxidant, antimicrobial etc. useful for the treatment of many health disorders. 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 55.8, stomatal index of 23.06%, and epidermal number of 179.8, while the adaxial surface had an epidermal number of 251.9. The plant sample of the leaf also possessed unicellular trichomes. Micromeritic properties of the powdered leaf samples showed bulk volume of 34.33±0.33, tapped volume of 27.00±0.00, bulk density of 0.27±0.00, tapped density of 0.34±0.00 , angle of repose of 34.4°, Carr's Index of 20.89±0.74, Hausner's ratio of 1.25±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: Stomatal, Micromeritic, Pharmacopoeial, Chemomicroscopy, Paracytic Stomata.

INTRODUCTION

Traditional medicine has been used for a long time of history which serves peoples all over the world. The ethno botany provides a rich resource for natural products which provides a step stone for drug research and development. In recent years, the use of traditional medicine of plant source has gained more interest. It has been reported that more than 50% of all modern drugs in clinical usage are of natural products. The medicinal plants have been comprised about 8000 species and among them 50% accounts for higher flowering plant species of India which is yet to be explored 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].

Scientific Classification)[8].

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

Figure 1: Mussaenda Philippica

MATERIALS AND METHOD

Collection, Identification and Preparatioin of the Plant

The Plants were obtained from a local farm at Kanpur. The plant identification and authentication was carried out at the Department of Botany, Christ Church College Kanpur. The fresh plant was air dried, pulverized and packed in a dry container, well labeled and used when needed.

Anatomical Studies

Microscopic Evaluation of Leaf

The plant's adult fresh leaves were cut at the petiole. Placing the leaf on a glass slide allowed for microscopical inspections of the epidermis on both the adaxial and abaxial sides. The sample was irrigated with water and scraped gently with a sharp razor blade until the dermis was reached and loose epidermis cells were rinsed away with water. The epidermal peels were then rinsed gently with water after being cleaned with sodium hypochlorite. The epidermal peels were dyed for (five min with aqueous safranin-O solution and 10% glycerol as mountant. On the microscope, the stained samples were examined. With an Amscope MD500 placed on an Olympus CX21 microscope, photomicrographs of the prepared slides were obtained. The plant's transverse section and powder microscopy were examined, and images were taken. **[9]**.

Quantitative Microscopy of the Leaf

Leaf constant measurements, which included stomatal length and breadth, guard cell length and width, stomatal number, stomatal index, epidermal cell length and width, epidermal cell number, and epithelium thick, were performed using common standards. All measurements were taken with a calibrated ocular micrometre, and ten (10) microscopic fields were chosen at random. Data is shown as mean SEM.

Stomatal Index Determination

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

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 mass volume was calculated by weighing 10 g of the powdered crushed leaf into a 100 ml measuring cylinder and recording the volume occupied (Vb). The cylinder was softly tapped multiple times to get a consistent volume, which was recorded as the tapped volume (Vt). The following formula was used to compute bulk density:

$$B\rho = \frac{M}{Vb}$$

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

$$\textbf{Hausner's ratio} = \frac{\mathbf{T\rho}}{\mathbf{B\rho}}$$

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.

Angle of repose

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

Chemomicroscopic Analysis of Leaf Powder

Using normal protocols, crushed leaflet is analysed for chemomicroscopic properties such as mucilage, lignin, starch, oils, calcium carbonate, and calcium oxalate crystals. **[20]**.

Fluorescence Analysis of Leaf Powders

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

Physico-chemical Evaluation of Leaf Powders

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 measured according to the official method recommended by the WHO guidelines on quality control testing for herbal plant materials [11,15,16].

RESULTS

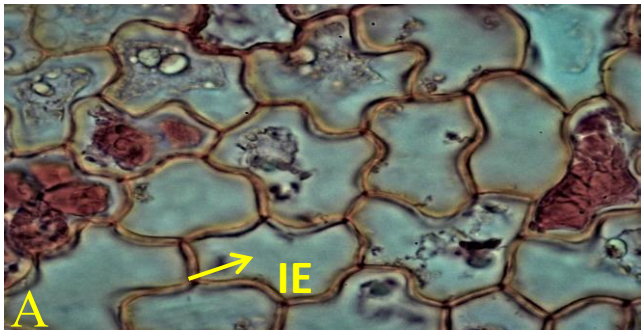
Tables 1–6 provide the findings of anatomical investigations, micromeritic properties, chemomicroscopy, fluorescence properties, soluble extractive values, moisture content, and ash values of the leaf, and Figure 2 (A–E) shows the adaxial, abaxial, transverse section, and powder analysis of the leaf.

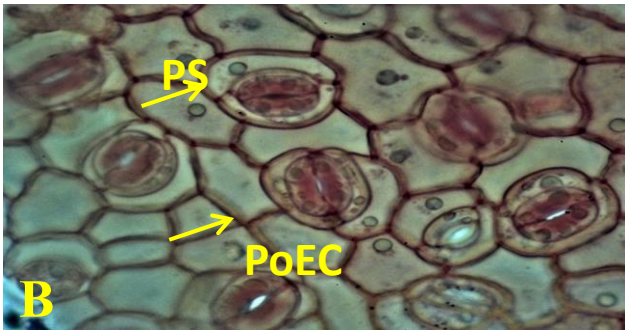
Table 1: Results for the Microscopic Features of M. philippica and Standard Error of Mean (SEM) for the leaf surface;

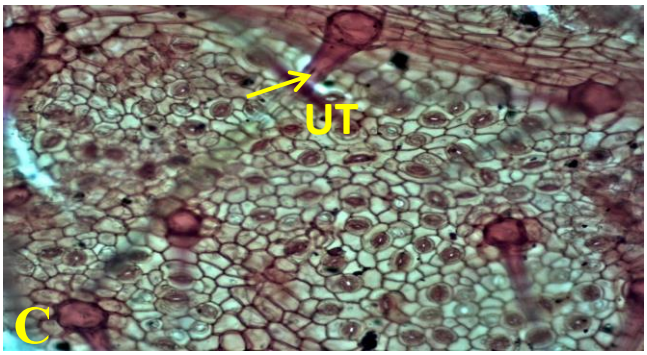
Leaf surface	Abaxial	Adaxial
Stomatal morphology	Paracytic	-
Stomatal length (µm)	13.90 (14.84±0.46) 19.61	-
Stomatal width (µm)	8.39 (8.96±0.28) 11.54	-
Stomatal pore length (µm)	4.07 (6.33±0.42) 7.69	-
Stomatal pore width (µm)	1.62 (2.81±0.19) 3.65	-

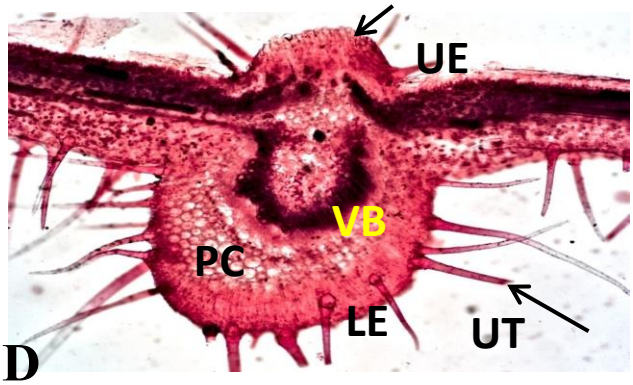
Stomatal number	51 (55.8±1.30) 64	-
Stomatal index	23.06%	-
Epidermal wall pattern	Polygonal	Irregular
Length of epidermal layer (µm)	21.02 (27.24±1.12)31.17	31.01 (40.97±1.62) 49.65
Width of epidermal layer(µm)	8.01 (13.84±0.63) 14.70	14.70 (18.04±1.13) 26.83
Thickness (µm)	3.20 (3.92±0.20) 4.81	3.26 (4.61±0.28) 6.21
Epidermal number	155 (179.8±9.24) 210	215 (251.9±8.83) 271
Trichome type	Unicellular	-
Trichome length (µm)	61.49 (137.60±21.79) 263.25	-
Trichome width (µm)	6.90 (9.84±0.51) 12.41	-

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









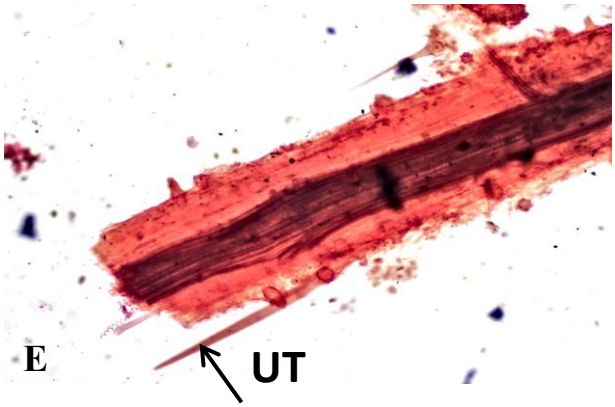


Figure 2: A; Adaxial surface (IE; Irregular epidermal cell shape), B and C: Abaxial surface (PoEc; Polygonal epidermal cell shape), PS; Paracytic stomata, UT; Unicellular trichome. D: Transverse section of the leaf: UE; Upper epidermis, VB; Vascular bundles, UT; Unicellular trichome, PC; Parenchyma cells;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

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

Parameters	Values
Bulk Volume (cm)	34.33±0.33
Tapped Volume (cm)	27.00±0.00
Bulk Density (g/ml)	0.27±0.00
Tapped Density (g/ml)	0.34±0.00
Flow Rate (g/s)	0.67±0.02
Angle of Repose (°)	34.4
Hausner's ratio	1.25±0.01
Carr's Index	20.89±0.74
Diameter of Heap (cm)	6.92±0.08

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

Table 3 : Results for Chemomicroscopy of M. Philippica Leaf

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 + 80% HCL	Calcium Oxalate Crystals seen Crystal dissolves	Calcium Oxalate present 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

Table 5 : Results for Water-Soluble Extractive Value, Ethanol-Soluble Extractive, Methanol-Soluble Extractive Value and Standard Error of Mean for Leaf Powders of M. philippica.

Parameters	Weight(g)	Percentage(%w/w)
Water-soluble extractive value	0.24±0.00	24
Ethanol-soluble extractive value	0.17±0.00	17
Methanol-soluble extractive value	0.18±0.00	18

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	12
Total ash value	0.27±0.00	8
Acid-insoluble ash value	0.03±0.01	2
Water-soluble ash value	0.14±0.01	4

Discussion

On the abaxial surface of the leaf, the results of the microscopy study revealed the presence of paracytic stomata and polygonal epidermal cell shape (Figure 2B), a stomatal number of 55.81.30, stomatal index of 23.06 percent, and epidermal cell number of 179.89.24, while the adaxial surface had no stomata but an irregular epidermal cell shape (Figure 2A) and epi For the abaxial surface of the leaf, the mean stomatal length and width were 14.84 m and 8.96 m, respectively (Table 1). The micromeritic investigation revealed a 34.4° angle of repose, indicating excellent flow. As stated in Table 2, Hausner's ratio and Carr's index were 1.25 and 20.89 percent, indicating acceptable to fair flow characteristics. In recent years,[17] The Compressibility index, as well as the closely related Hausner's ratio, has become a common approach of estimating powder flow characteristics since they are simple, quick, and accurate. The compressibility index has been proposed as an indirect measure of bulk density, size, shape, surface area, moisture content, and cohesiveness of powders, as well as a measure of inter-particulate interactions. Gnetum africanum Welw (Gnetaceae), Buchholzia coriacea Engl Caparidaceae, Umoh et al. and Jatropha tanjorensis J.L. Ellis & Saroja. (Euphorbiaceae) Umoh et al.

As indicated in Table 3, chemomicroscopy analysis of the leaf revealed the presence of lignin, starch, cellulose, calcium oxalate crystals, oil, mucilage, and protein, which were similarly found in Cola pachycarpa leaf and stem powders by Johnny and Bassey [19]. Crystals of calcium oxalate are involved in a number of critical processes, including tissue calcium control, herbivory protection, and metal detoxification [18]. When synthesised into medications, plants with calcium oxalate crystals demonstrate good antioxidant capabilities. When observed in daylight, lower and higher wavelengths of UV light, the fluorescence property of the powdered sample for different solvent extracts displayed distinct colours suggesting the presence of phytochemicals such as anthocyanins, phenols, tannins, and flavonoids.This feature is useful for identifying real samples, identifying adulterants, and defining crude medicines. Fluorescence investigations employ estimations of fluorescence intensity to assist identify a specific drug in a combination of various compounds from two or more species [18].

The extractive values for ethanol-soluble, methanol-soluble, and water-soluble were 17 percent w/w, 18 percent w/w, and 24 percent w/w, respectively. According to the results provided above, water is the optimum solvent for extracting the plant's contents. The presence of water-soluble substances originating from plants, such as sugars, amino acids, and vitamins, is indicated by the water-soluble extractive value.

The moisture percentage was 12 percent w/w, which indicates a moderate moisture level because it falls within the range of vegetable medicines (8 percent to 14 percent) [11]. High moisture content is uneconomical, and when combined with the right temperature, it can lead to enzymatic activation and hydrolytic processes, as well as microbial development and destruction of active ingredients. The plant has moderate moisture content and is suitable for usage, but it should be preserved correctly to maintain its quality as it is susceptible to degeneration.

As shown in Table 6, the total ash content, acid-insoluble ash content, and water-soluble ash content of the sheet were 8% w / w, 2% w / w, and 4% w / w (value limit is 14%). (Do not exceed) w / w) and the acid-insoluble ash value of the leaves were also within the limits (2, should not exceed the European Pharmacopoeia [20]. The ash value should be the reliability and purity of the sample. The ash value indicates that the presence of inorganic ions during ashing oxidizes organic matter and a certain amount of volatile elements are lost.

Conclusion

It may be argued that the information acquired will aid in the appropriate identification of Mussaenda philippica leaves and will serve as a foundation for standardisation.

Author contribution

All author participated Equally**.**

Conflict of Interest

None

Funding

None

Ethical Clearance

In this study there was no need any animals.

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