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Pharmacognostical studies on leaves of *Mussaenda frondosa* Linn.

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ABSTRACT

The *Mussaenda frondosa* Linn. belonging to the family Rubiaceae, commonly known as Sriparnah in Sanskrit, is a scandent shrub traditionally used in the treatment of cough, bronchitis, fever, inflammation, wounds, ulcers, jaundice, leucoderma and pruritus. Though it is an important plant, till date, no pharmacognostical reports have been available on its leaf. Therefore, the present investigation was undertaken to ascertain the requisite pharmacognostical standards for the standardization of the *Mussaenda frondosa* leaves. Various investigations like Pharmacognostical studies, preliminary phytochemical screening and High-Performance Thin Layer Chromatography (HPTLC) analysis were carried out, and the salient qualitative parameters were reported. Microscopical evaluation of the leaf revealed the presence of paracytic stomata, microcrystal's, Idioblast, collenchymas, sand crystals and unicellular unbranched covering Trichomes. The presence of flavonoids, steroids, glycosides, mucilage, saponins and proteins were confirmed through Preliminary phytochemical studies. The HPTLC profile of ethanol extract from *M. frondosa* L. revealed ten phytoconstituents of R_f value ranging from 0.11 to 0.88. The significant peaks are observed at R_f values of 0.11, 0.16, 0.23 and 0.81. These findings provide referential information for correct identification and standardization of the *Musssaenda frondosa* leaves, even in powder form. This information will also be useful to distinguish *Mussaenda frondosa* from the closely related other species of *Mussaenda*. The Pharmacognostic and phytochemical profiles reported in this research work for *Mussaenda frondosa* may play a major role in setting monograph of the plant, which might be helpful in proper identification of the plant.

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INTRODUCTION

According to WHO, the worldwide use of herbal medicines and phytonutrients or nutraceuticals has expanded rapidly, with currently several people resorting to these products for the treatment of various health challenges (WHO, 2004). The standardization of these plant materials is essential in order to meet the global demand of herbal drugs. A big quantum of research works in the area of authentication of the exact plant source has been undertaken to give means of differentiation among many available plant sources (Padashetty and Mishra, 2008). *Mussaenda frondosa* (MF) Linn. belongs to the family Rubiaceae is a scandent shrub found throughout

India (Sastri, 1992). It is commonly known as white flag bush in English, Sriparnah in Sanskrit and Bedina in Hindi (Kirtikar *et al.*, 1984). Traditionally it is used in the treatment of cough, bronchitis, fever, inflammation, wounds, ulcers, leucoderma, pruritus and jaundice. A weak decoction of dried shoots is given to children to relieve coughing. The bitterroot is used in the treatment of white leprosy and eye troubles (Varier, 1996). The Mullu kuruma tribe of Kerala used leaf juice for the treatment of dandruff and eye diseases (Silja *et al.*, 2008). In previous reports, *M. frondosa* L. had significant hepatoprotective, antibacterial and antifungal efficacy (Jayasinghe *et al.*, 2002; Sambrekar *et al.*, 2010; Shanthi and Radha, 2020). The phytoconstituents such as rutin, quercetin, sinapic acid, ferulic acid and stigluside were reported from *M. frondosa* L. (Astalakshmi and Ganapathy, 2017). Despite the abundant therapeutic uses attributed to this plant *M. frondosa* L., there are no pharmacognostical reports on the leaves of this plant. Thus, the present research work focuses on Pharmacognostical studies, preliminary phytochemical screening and HPTLC analysis, which could help us to derive data to layout the standardization protocol for *M.frondosa*.

MATERIALS AND METHODS

Plant material

The plant was collected from Kodaikanal hills, Tamil Nadu, India, in September 2011 and it was authenticated by Dr. P. Jayaraman, taxonomist, Plant Anatomical Research Centre, Chennai, Tamil Nadu, India

Pharmacognostical evaluation

Macroscopic evaluation

The macroscopic features of the fresh leaf of *M. frondosa* L. including the type of leaf, base, presence or absence of petiole and lamina surface characteristics, were studied and reported. (Henry *et al.*, 1987).

Microscopic evaluation

The healthy leaves were collected from the plant and immediately fixed in Formalin: Acetic acid: 70% Ethyl alcohol (5:5:90) for 24h (O'Brien *et al.*, 1964). After fixing, dehydration was performed with graded series of Tertiary-Butyl Alcohol (TBA) (Johansen, 1940). The specimens were cast into paraffin blocks after infiltration with Paraffin wax (melting point 58-60°C). Standard methods of sectioning and staining were followed for microscopical studies (Sass, 1940).

Powder microscopy

Preliminary preparation involves clearing of powder with Chloral hydrate solution (75%) prior to observation using a microscope. Slides of powdered leaves of *M. frondosa* L were prepared according to the prescribed procedures. (Ishtiaq *et al.*, 2016).

Quantitative evaluation

The microscopical examination was employed for the quantitative evaluation using the Camera Lucida. The leaf constants such as palisade ratio, stomatal number, stomatal index, vein-islet and vein – termination number were determined (Kumar *et al.*, 2008).

Physicochemical analysis

Shade dried leaf material was used for the physicochemical analysis in accordance with the World Health Organization. The powdered samples were subjected to analysis (Ministry of Health and Family Welfare, 1996).

Fluorescence analysis

Powdered leaf material was treated with various chemical reagents to study their fluorescence behavior under visible and ultraviolet light (Kokoski *et al.*, 1958).

Preliminary phytochemical analysis

Shade dried leaf powder was extracted with ethanol, filtered and concentrated by means of a Rotary evaporator. The M. frondosa ethanol extract was subjected to phytochemical screening for the identification of various phytoconstituents as per standard procedure (Khandelwal, 2005; Kokate *et al.*, 2002)

HPTLC fingerprint profile

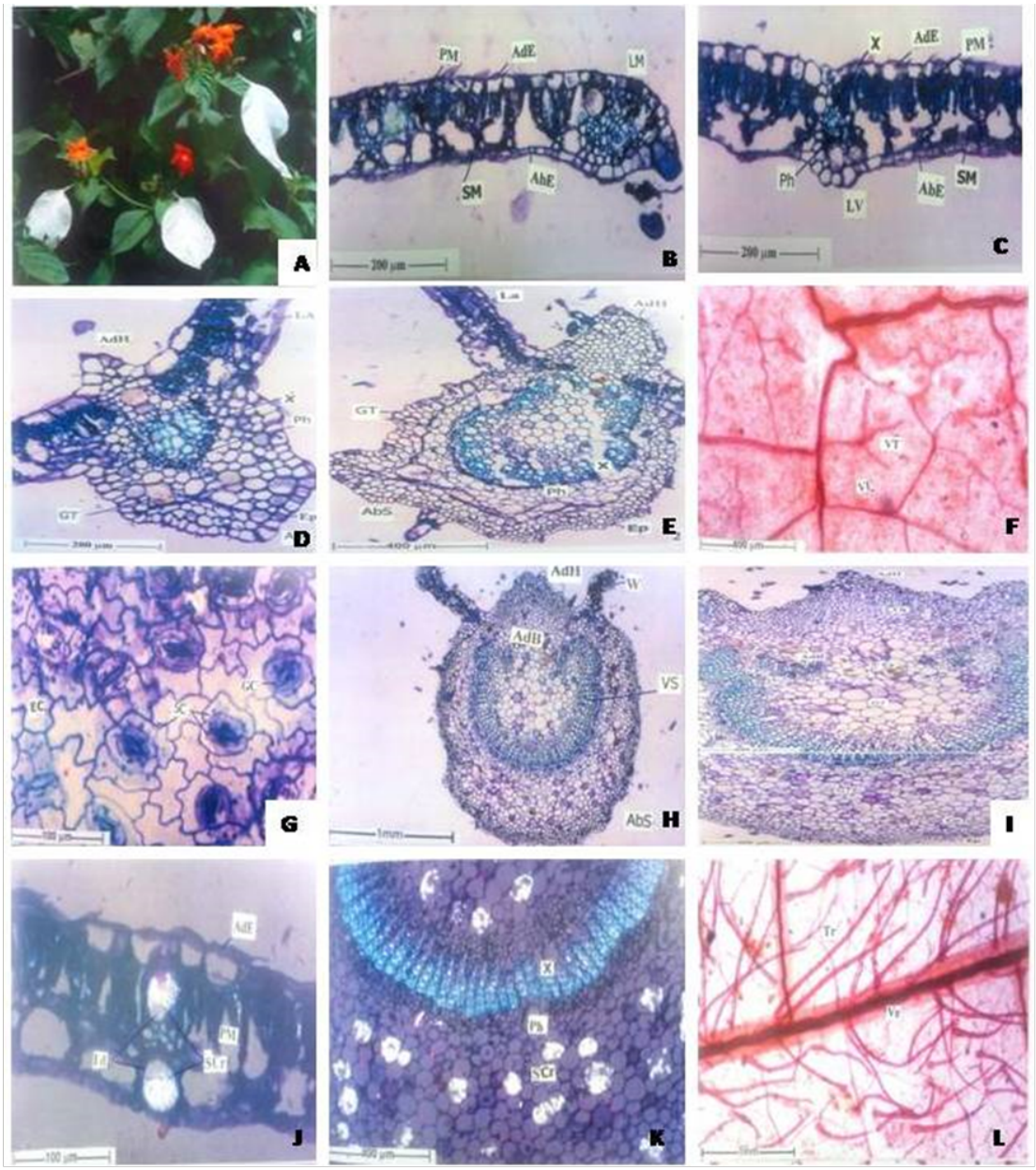
The HPTLC fingerprint profile of ethanol extract of *M. frondosa* L. leaves was developed using the mobile phase, toluene: ethyl acetate: formic acid (7:2.5: 0.5 v/v). After air drying, the plates were scanned at a wavelength of UV 254 nm and using a deuterium lamp (Sethi, 1996).

RESULTS AND DISCUSSION

The Pharmacognosy studies imply a set of methods for the identification and evaluation of crude drugs obtained from natural sources, chiefly from plants.

This study helps to resolve the key problem in the correct identification of genuine drugs of the commercial supply of crude drugs.

So, the application of Pharmacognostical evaluation, including morphology, microscopy, physicochemical analysis, fluorescence analysis, preliminary phytochemical screening and HPTLC studies, will help in identifying genuine drugs. (Alam and us Saqib, 2015; Joshi, 2012)



Abbreviations:
AbE - Abaxial epidermis, AbS -Abaxial side, AdB- Adaxial Bundle, AdE- Adaxial epidermis, AdH- Adaxial Hump, EC-Epidermal cell, Ep- Epidermis, GC-Guard cell, GT - Ground tissue, Id- Idioblast, La- Lamina, LM- Leaf margin, LV-Lateral vein, Ph- Phloem, PM- Palisade mesophyll, SC- Subsidiary cell, SCr- Sandy Crystals, SM - Spongy mesophyll, Tr-Trichome, Ve-Vein, VI - Vein islets, VS-Vascular strand, VT - Vein terminations, W- Wings, X-Xylem.

Figure 1: A) Habit of *Mussaenda frondosa* L. B) TS of the lamina, C) TS of lamina showing lateral vein, D) TS of lamina showing secondary lateral vein, E)TS of the leaf through midrib, F) Venation pattern, G) Paracytic stomata, H) TS of the petiole, I)TS of petiole- a sector enlarged, J) Sandy crystals in the mesophyll of the lamina, K) Microcrystals in the midrib region, L) Covering trichomes of the leaf

Table 1: Quantitative microscopic evaluation reports

S. No.	Leaf constants	Mean ± SEM
1.	Palisade ratio	9.52 ± 0.41
2.	Stomatal Index of the lower epidermis	20.48 ± 0.51
3.	Vein-islet number	7.6 ± 0.47
4.	Vein - termination number	8.3 ± 0.39

Table 2: Physico chemical analysis of *M. frondosa* L.

S. No.	Parameters	Values (% w/w)
1.	Total Ash	4.28 ± 0.015
2.	Acid insoluble ash	1.67 ± 0.001
3.	Water-soluble ash	3.91 ± 0.044
4.	Sulphated ash	0.51 ± 0.02
5.	Loss on drying	10.84 ± 0.32
6.	Ethanol soluble extractive	7.32 ± 0.03
7.	Water-soluble extractive	22.8 ± 0.12

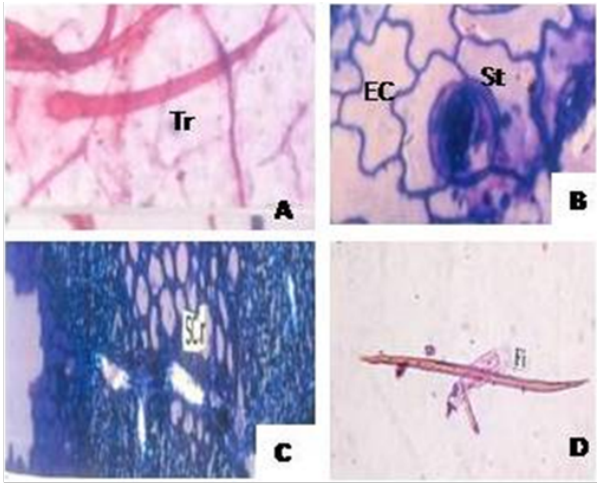
Table 3: Fluorescence analysis of leaves of *M. frondosa* L.

S. No.	Treatments	Observations		
		Ordinary light	UV Light	
			Short wavelength (254 nm)	Long-wavelength (366 nm)
1.	Powder	Light green	Dark green	Dark green
2.	Water	Brown	Dark green	Dark green
3.	Chloroform	Dark Brown	Dark Brown	Greenish Brown
4.	Methanol	Brown	Yellowish green	Dark green
5.	1N NaOH in water	Yellowish-brown	Yellowish green	Yellowish green
6.	1N NaOH in methanol	Pale yellow	Fluorescent green	Dark green
7.	1N HCl	Dark Brown	Dark green	Dark green
8.	50%HNO_3	Yellowish green	Dark green	Yellowish green
9.	50%H_2SO_4	Dark brown	Dark brown	Black-brown
10.	Ammonia solution	Yellowish-brown	Fluorescent green	Greenish-yellow

Table 4: Preliminary phytochemical analysis of *M. frondosa* L.

S. No.	Phytoconstituents	Results
1.	Saponins	+
2.	Tannins	+
3.	Steroids	+
4.	Glycosides	+
5.	Flavonoids	+
6.	Terpenoids	-
7.	Alkaloids	-
8.	Proteins	+
9.	Carbohydrates	+
10.	Lipids	-

+ Present, - absent



Abbreviations: EC- Epidermal cells, Fi – Fibres, SCr- Sandy Crystals, St- Stomata, Tr-Trichome

Figure 2: Powder microscopy of *Mussaenda frondosa* L. leaf: A) Covering trichome, B)Paracytic stomata, C) Sandy crystals, D) Fibres

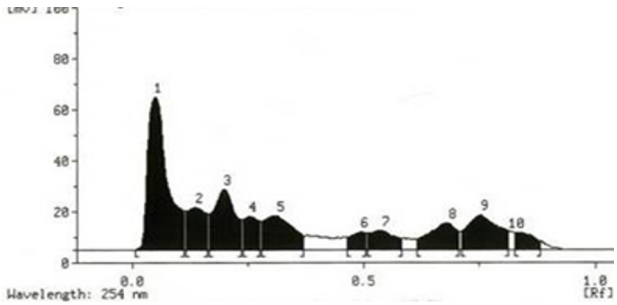


Figure 3: HPTLC fingerprint of ethanol extract of *Mussaenda frondosa* L.

Macroscopic evaluation

Mussaenda frondosa Linn. belongs to the family Rubiaceae is a scandent shrub found throughout India. Leaves are simple, opposite, stipulate, ovate, elliptic, orbicular, lanceolate, oblong (or) obovate with acute apex and cuneate base. Leaves are rather scantily hirsute on the upper surface and densely soft white tomentose on the lower surface. Primary lateral veins are more prominent on the lower surface and more hairy. (Figure 1 A)

Microscopic characters

Lamina

The leaf is thin, dorsiventral and mesomorphic with even and smooth surfaces. The lamina is $100\mu m$ thick. It consists of a thick, prominent adaxial epidermis with dilated squarish epidermal cells, while the abaxial epidermis (AbE) is thin with narrow rectangular cells. The mesophyll composed of an adaxial band of narrow palisade cell zone and three layers of much lobed wide-spaced spongy parenchyma cells. The palisade zone is $50\mu m$ in height with narrow, cylindrical and compact cells. The spongy mesophyll has small lobed cells forming wide air chambers. (Figure 1 B)

The lateral veins are slightly prominent and project beneath the abaxial part. The veinlets are thicker than the lamina. The region of the veinlet is $150\mu m$ thick and consists of a small group of xylem and a few phloem elements with a thin layer of parenchyma bundle sheath and axial extension (Figure 1 C). The secondary lateral vein is more prominent than the veinlets, which have a small adaxial conical hump and abaxial wider, shield-shaped body. The epidermal layer is thick with wide rectangular or circular cells and a prominent cuticle. The ground tissue is seen as dilated compact cells of parenchyma. The single, collateral and semicircular vascular strand ($500\times300\mu m$) were seen (Figure 1 D).

Midrib

The midrib has a thick conical adaxial hump and lobed with a wavy semicircular abaxial body. It is $950\mu m$ vertically. The adaxial hump is $250\mu m$ in height and $200\mu m$ wide, whereas the abaxial midrib is $650\mu m$ thick. The midrib has a thin but distinct layer of epidermal cells. The ground tissue is parenchymatous, in which some of the cells are crushed due to growth pressure. The vascular system has a deep bowl-shaped outline with several radial files of xylem elements and a thin layer of phloem (Figure 1 E).

The secondary veins are thick, while the veinlets are thin. The vein islets are distinct, wide and polygonal in surface view. The vein terminations are also distinct, which are long and slender, unbranched or forked at the tip (Figure 1 F).

Stomata

Stomata occur on the abaxial side of the lamina, which is the predominantly paracytic type with one parallel subsidiary cell on either side of the stomata. The subsidiary cells are either equal in size or unequal, whereas the epidermal cells are large and amoeboid in outline. Their anticlinal walls are fairly thick and undulate (Figure 1 G).

Petiole

The petiole is circular in cross-sectional outline along the proximal part measuring nearly 2 mm thick. Along with the distal parts, towards the lamina, the petiole is elliptical with prominent adaxial –lateral wings (Figure 1 H). The petiole showed a thin epidermal layer composed of small squarish cells followed by four layers of collenchyma and the

remaining portion being parenchymatous. These cells are large, compact and thin-walled and have sand crystals.

The vascular system is bowl-shaped; the two ends of the bowl are bent down, forming adaxial accessory bundles. The vascular bowl consists of radial parallel rows of xylem and a small nest of phloem was seen on the outer boundary of the xylem (Figure 1 I).

Crystals

An abundant Calcium oxalate crystals are seen in the leaf, especially in the mesophyll and midrib. The crystal is microcrystal or sand crystal type. In the lamina, the sand crystals occur in wide dilated idioblasts (Figure 1 J) whereas, in the midrib, they occur in the ordinary unspecialized cells (Figure 1 K). The idioblast is 130 μm wide; the ordinary crystal-bearing cells are 60 μm wide.

Trichomes

Epidermal trichomes are 600 μm long and are abundant, especially along the veins (Figure 1 L). The covering (non-glandular) trichomes are unicellular, unbranched, long and slender with smooth walls and pointed tips.

Powder microscopic characteristics

The powdered plant material is greenish in color, showing unicellular, unbranched covering trichomes and fragments of parenchyma. The palisade cells, fragments of epidermal cells, along with paracytic (or) rubiaceous stomata and fibres, are seen. Idioblast and sandy crystals are also observed. (Figure 2)

The *M. frondosa* L. can easily be identified on the basis of morpho-microscopical characters, but it is not achievable with the powdered material. Therefore, salient diagnostic features have been evolved through Pharmacognostical studies in order to identify the plant material. In this perspective, reliable diagnostic characters are established through microscopical evaluation. They are paracytic stomata, unicellular unbranched covering trichome, sand crystals in idioblast and microcrystals in unspecialized cells.

Quantitative microscopic evaluation

Quantitative microscopic evaluation reports are given in Table 1.

Physicochemical parameter

The results of physicochemical analysis of leaf powder, such as ash values and extractive values, are presented in Table 2. Ash value gives an idea about inorganic content as well as extraneous matter (e.g. soil and sand) clinging to the surface of the plant. As shown in Table 2, the ash values such as Total ash, 4.28 ± 0.015, insoluble acid ash 1.67 ± 0.001, water-soluble ash 3.91 ± 0.044 and sulphated ash 0.51 ± 0.02 % w/w were recorded for the leaves of *M. frondosa.* The extractive values using solvents such as ethanol and water were recorded as 7.32 ± 0.03 % and 22.8 ± 0.12 % w/w, respectively.

Fluorescence analysis

The Fluorescence analysis of leaf powder of MF revealed various colors with different reagents under daylight and UV light (254 nm and 366 nm), signifying the existence of fluorescent compounds in the methanol, as shown in Table 3. The powder treated with an alkali such as NaOH and ammonia showed yellowish-green fluorescence confirmed the presence of flavonoids in the leaf powder. The fluorescence analysis is as well an important tool for the identification of fluorescent compounds such as flavonoids, quinones, etc.

Preliminary phytochemical screening

The preliminary phytochemical screening of leaf powder of MF mainly revealed the presence of flavonoids, steroids, glycosides, saponins, tannins, carbohydrates and proteins (Table 4).

HPTLC fingerprint profile

The HPTLC profile of ethanol extract (10 ml) from *M. frondosa* L. revealed ten phytoconstituents of R_f value ranging from 0.11 to 0.88. As shown in Figure 3, significant peaks are observed at R_f values of0.11, 0.16,0.23 and 0.81.

Microscopical and physicochemical evaluations are carried out in MF samples in order to ascertain proper data that may be utilized for identification besides establishing the purity and standards for crude drugs, particularly for powder drugs. Hence the standard Pharmacognostical parameters established in this study certainly used to distinguish closely related species of Mussaenda or its varieties with similar phytoconstituents or pharmacological activities (Buniyamin *et al.*, 2007).

Qualitative analysis of the MF leaf showed the presence of flavonoids, steroids, glycosides, saponins, tannins, carbohydrates and proteins. The HPTLC profile of ethanol extract of *M. frondosa* L. developed in this study definitely helps in the identification of the plant by the researcher as well industrialists. The Pharmacognostic and phytochemical profiles reported in this research work for *Mussaenda frondosa* may play a major role in setting monograph of the plant, which might be helpful in proper identification of the plant.

CONCLUSION

Mussaenda frondosa L. has abundant uses in traditional medicine to treat several ailments like bronchitis, inflammation, wounds, ulcers, leucoderma, pruritus and jaundice. The Pharmacognostical, Physico-chemical, phytochemical and HPTLC studies of *Mussaenda frondosa* provide data to construct standardization protocol. The Present study offers a standardization profile of the drug *M. frondosa* L. which might be of colossal value in proper identification and authentication of plant drugs in future and can help us to avoid its adulteration.

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Conflict of Interest:

The authors declare that they have no conflict of interest for this study.

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