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PHARMACOGNOSTIC STANDARDIZATION OF *MADHUCA INDICA* LEAF AND STEM, AN IMPORTANT MEDICINAL PLANT

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ABSTRACT: Objective: To evaluate the pharmacognostic characters of *Madhuca indica* J.F. Gmel leaf and stem an important traditional medicinal plant. **Method:** In the present investigation, pharmacognostic parameters like macroscopic and microscopic and powder characters of leaf and stem were studied. Preliminary phytochemical and physiochemical analyses were done by using reported methods. Fluorescent behavior of the leaf and stem powder were also tested. **Results:** The macroscopic study showed that the leaf was simple and ovate with sinuate margin, acuminate at apex, decurrent at base and surface glabrous and venation was pinnate. The microscopic study of leaf revealed the presence of dorsiventral type of cellular arrangement, unicellular trichomes, prismatic crystals and anomocytic stomata; while the microscopic study of stem revealed the presence of vascular bundles in zig zag form, large central collenchymatous pith. Pith cells were polygonal in shape with minor angular thickenings. Physiochemical analysis of leaf showed total ash, water soluble ash and acid insoluble ash as 4.6, 0.83 and 0.5 %w/w respectively while in stem it was 5.37, 1.5 and 1.0 %w/w respectively. In both parts maximum extractive value was in methanol. Phytochemical analysis revealed maximum amount of flavonoids in leaf and tannins in stem. **Conclusions:** Various pharmacognostical characters observed in this study will help in identification and standardization of *M. indica*; will also help in quality control and formulation development.

INTRODUCTION: Herbal remedies for any type of disease and disorder is gaining more and more importance as time passes by. This renewed interest is simply because it is a popular belief that green medicine is safe. Moreover synthetic drugs are regarded as harmful to human beings and environment. The use of plants to treat various diseases and disorders is an age old tradition. They are frequently used in most ancient system of medicine like Ayurveda, Unani, Siddha, etc. There is renewed interest in their usage mainly because of many adverse effects associated with synthetic drugs.

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Natural remedies are popular because they are safe, easily available and has less toxic effects. But this is hampered by adulteration and substitution of natural drugs. Therefore it is important to lay down standard parameters for identification of plants. This will ensure reproducible quality of the drug and will also maintain its safety and efficacy. Pharmacognostic study helps in laying down such standards. In such studies the various parameters analyzed are macroscopic, microscopic and powder study, phytochemical, physicochemical and fluorescent analysis [1].

Madhuca indica J.F. Gmel belongs to the family Sapotaceae and its vernacular name is mahudo and in English it is known as Indian butter tree. The name is derived from Sanskrit madhu meaning honey. Various parts of the plant are traditionally used to treat a number of illness for eg. Anti diabetic, antiulcer, hepato-protective, anti pyretic,

anti fertility, analgesic, anti oxidant, swelling, inflammation, piles, emetic, skin problems, laxative, tonic, anti burn, anti earth worm, wound healing headache and many more problems [2]. There are many medicinal uses of this plant for eg. methanolic extract of bark shows antihyperglycemic activity [3]. Ethanolic extract of leaf shows anticancer activity [4], etc. Pharmacognostic studies of flower is reported [5] but to the best of our knowledge the pharmacognostic standardization of leaf and stem are not reported. Hence, the objectives of the present investigation was macroscopic, microscopic, phytochemical, physicochemical and fluorescence characterization of leaf and stem of *M. indica*.

MATERIALS AND METHODS:

Procurement of Plant Material:

Madhuca indica J.F. Gmel leaf and stem were collected from Rajkot, Gujarat, India in August, 2013. The plant was compared with voucher specimen (Voucher specimen number PSN426) deposited by Dr. PS Nagar at the Department of Biosciences, Saurashtra University, Rajkot, Gujarat, India. The plant parts were washed thoroughly with tap water, shade dried, homogenized to fine powder and stored in air tight bottles.

Pharmacognostic studies:

Macroscopic study:

For morphological observations, fresh leaf and stem was collected from Rajkot, Gujarat, India in August, 2012. The macro morphological feature of the leaf and stem was observed under magnifying lens [6].

Microscopic characteristics:

The microscopic evaluation was done by taking free hand sections of fresh leaves and stained by safranine to confirm lignifications. Various identifying characters such as trichomes and cell composition were recorded and then pictomicrography was done. Powder microscopy of dried leaf powder was studied under microscope. The characteristic structures and cell components were observed and their photographs were taken.

Phytochemical analysis:

Qualitative phytochemical analysis:

The crude powder of leaf and stem was subjected to qualitative phytochemical analysis [7]. The phytochemicals analysed were alkaloids, flavonoids, tannins, phlobatanins, triterpenes, steroids, saponinis and cardiac glycosides.

Physicochemical analysis:

The physicochemical analysis of the crude powder *T. bellerica* leaf and stem stem was carried out as per WHO guidelines [8]. The parameters analysed were Loss on drying, Total Ash ,Water soluble ash, Acid insoluble ash, Petroleum ether soluble extractive, Ethyl acetate soluble extractive, Acetone soluble extractive, Water soluble extractive values

Fluorescence analysis:

Fluorescence study of leaf and stem powder was performed as per reported standard procedures [9]. A small quantity of the stem/leaf powder was placed on grease free clean microscopic slide and 1-2 drops of freshly prepared reagent solution was added, mixed by gentle tilting of the slide and waited for few minutes. Then the slide was placed inside the UV chamber and observed in visible light, short (254 nm) and long (365 nm) ultra violet radiations. The colour observed by application of different reagents in different radiations was recorded.

Statistical analysis:

All experiments were repeated at least three times. Results are reported as Mean ± S.E.M. (Standard Error of Mean).

RESULTS:

Organoleptic and macroscopic characteristics:

The organoleptic features of *M. indica* leaf are given in **Table 1**.

TABLE: 1 ORGANOLEPTIC FEATURES OF *M. INDICA* LEAF

Colour	Dark green
Lamina	Ovate
Dimensions	5.5 –25 x 2.0 – 13.5 cm
Leaf	Simple
Margin	Sinuate
Apex	Acuminate
Base	Decurrent
Surface	Glabrous
Venation	Pinnate
Inflorescence	In dense axillary fascicles

The macroscopic study showed that the leaf was simple and alternate, with sinuate margin, apex acuminate and base decurrent with surface appearance and texture glaborous. The inflorescence was in dense axillary fascicles **(Fig. 1).**

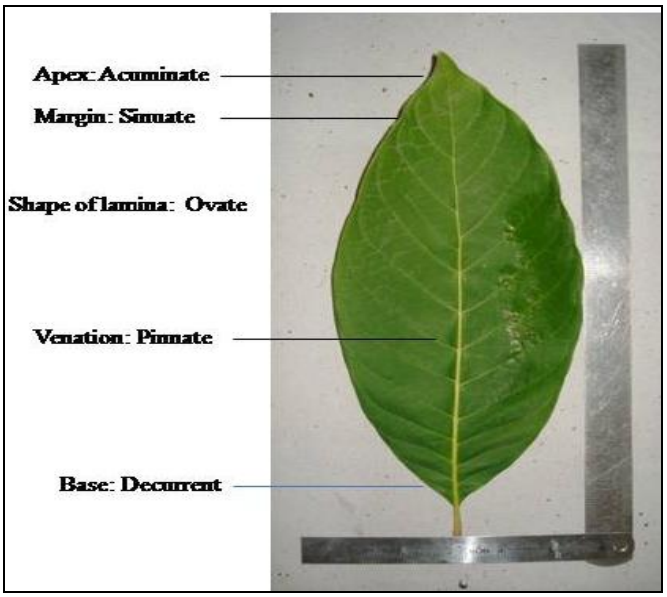

FIG. 1: PHOTOMICROGRAPHS OF MACROSCOPIC CHARACTERISTICS OF *M. INDICA*

Microscopic characteristics of leaf of *M. indica:*

Microscopic studies are useful to establish the botanical identity for the valuable herbal drugs, which forms the basis for the identification and determination of adulterants. The transverse section of the leaf of *M. indica* **(Fig. 2)** through the lamina and the midrib region which showed the presence of dorsiventral type of cellular arrangement with a thick prominent midrib and thin lamina, the upper epidermis was made up of single layer compactly arrange thin cell walled parenchyma cells. Mesophyll was differentiated in to palisade and spongy parenchymatous cells. Palisade cells are single layered, tightly packed, regularly arranged, elongated and does not form a continuous band throughout as it is absent above the vascular bundles of midrib. Multilayered, distinct, irregular cells with large intercellular spaces parenchymatous cells are present below palisade layer and continue till the lower epidermis. Epidermal layers of lamina were in continuity with that of midrib. But, size of the epidermal cell was smaller as compared to the size of cells in lamina portion.

Vascular bundle was surrounded by many layers of cortex. Vascular bundles were prominent occupying the central portion of the midrib. Xylem was towards the center of the leaf and was covered by phloem. Vascular bundle was surrounded by distinct sclerenchymatous cells of midrib and lamina portion. Prismatic calcium oxalate crystals were found in cortex region, unicellular trichome and anomocytic stomata were also present.

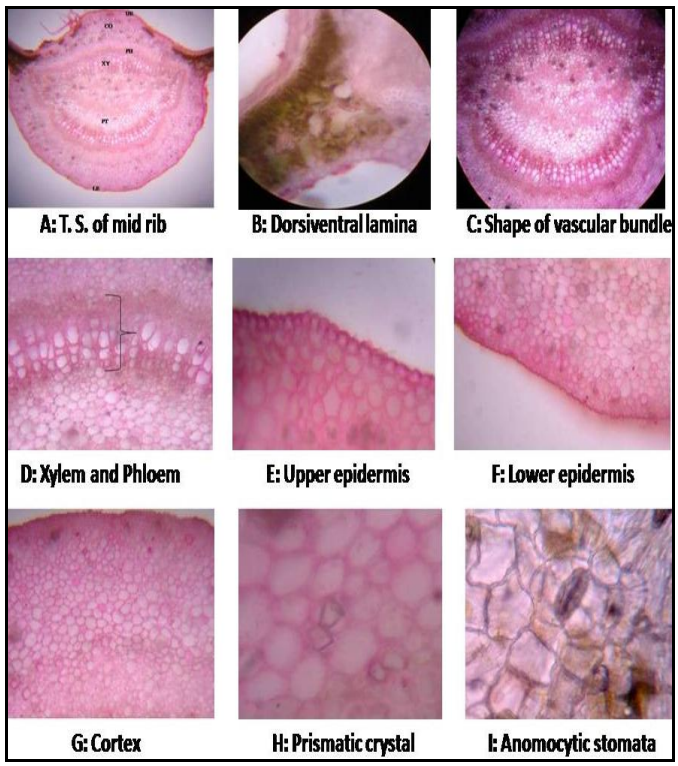

FIG. 2: PHOTOMICROGRAPHS OF MICROSCOPIC CHARACTERISTICS OF *M. INDICA* LEAF

Powder study of leaf of *M. indica:*

The crude powder of leaf was dark green in colour with characteristic odour and astringent taste. The

powdered leaf of *M. indica* under microscopic investigation showed prism like crystals, unicellular trichomes, epidermal cells, annular spiral vessel, bunch of xylem vessels and anomocytic stomata **(Fig. 3).**

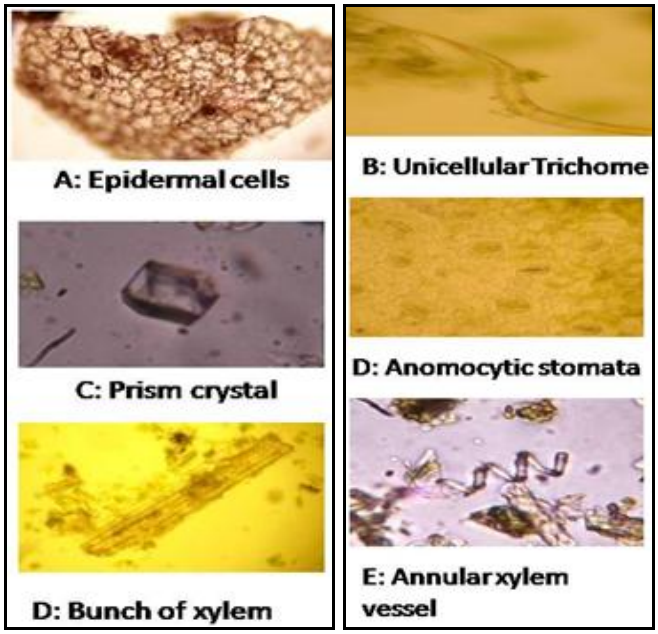

FIG. 3: PHOTOMICROGRAPHS OF MICROSCOPIC CHARACTERISTICS OF POWDER OF *M. INDICA* LEAF

Microscopic studies of stem of *M. indica:*

The microscopic study of *M. indica* stem showed that the stem was round in shape **(Fig. 4).** The outer most single layered epidermis was covered with cuticle; after epidermis, many layers of cortex cells were present. Vascular bundles were arranged in a zig-zag ring. Vascular bundle was surrounded with sclerenchymatous cells.

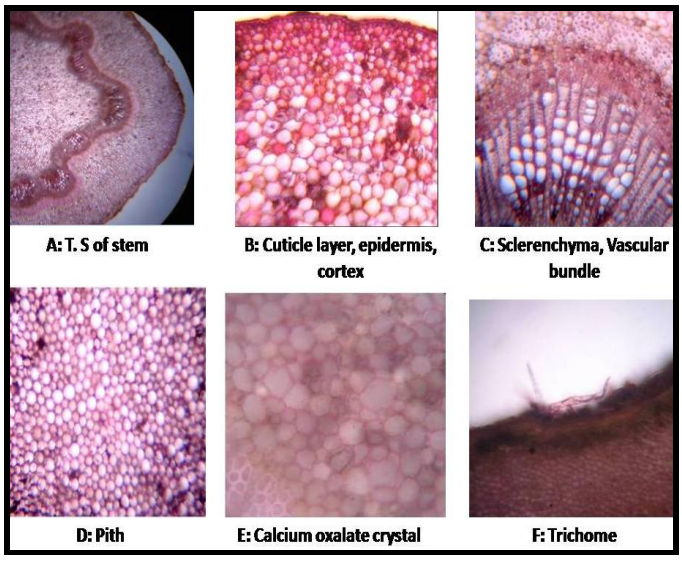

FIG. 4: PHOTOMICROGRAPHS OF MICROSCOPIC CHARACTERISTICS OF *M. INDICA* STEM

Phloem was well developed and made up of sieve tube, companion cells and phloem parenchyma. Xylem was also well developed and consisted of vessels, tracheids, fibers and xylem parenchyma. Major portion of the section was occupied by central collenchymatous pith. Pith cells were polygonal in shape with minor angular thickenings. Calcium oxalate crystals and unicellular trichomes were also present.

Powder study of stem of *M. indica:*

The crude powder of stem was light brown in colour. The stem powder of *M. indica* under microscopic investigation showed prism like crystals, unicellular trichomes, annular spiral vessel, bunch of xylem vessel and epidermal cells **(Fig. 5)**.

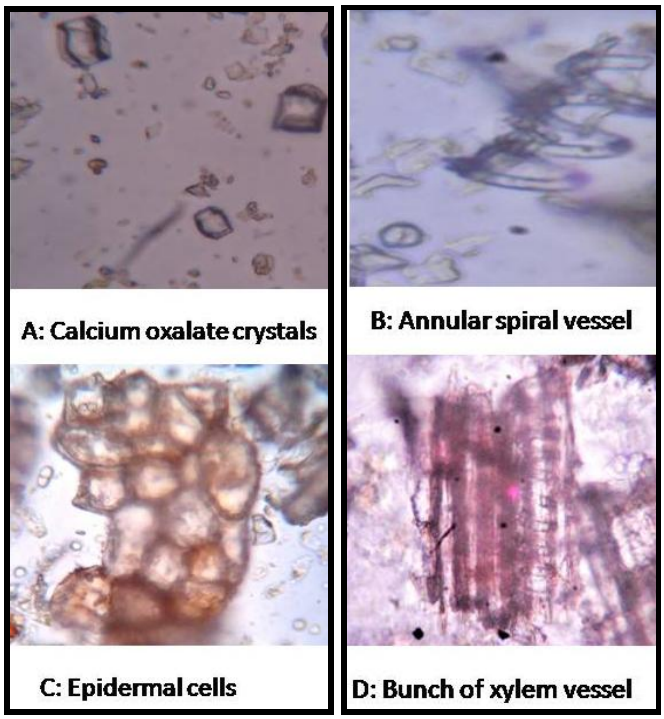

FIG. 5: PHOTOMICROGRAPHS OF MICROSCOPIC CHARACTERISTICS OF POWDER OF *M. INDICA* STEM

Phytochemical analysis:

The results of qualitative phytochemical analysis of the crude powder of *M. indica* leaf and stem are shown in **Table 2**. The leaf had maximum amount of flavonoids followed by tannins while stem had maximum amount of tannins.

The other phytoconstituents like alkaloids, cardiac glycosides, triterpenes and steroids were present in

trace amounts in both leaf and stem while saponins were absent in both the parts **(Table 2).**

TABLE 2: QUALITATIVE PHYTOCHEMICAL ANALYSIS OF *M. INDICA* LEAF AND STEM.

Phytochemicals		Leaf	stem
Alkaloids	Dragondroff's	+	+
	Mayer's	+	+
	Wagner's	+	+
Flavonoids		+++	+
Tannins		++	
		+++	
Cardiac glycosides		+	-
Triterpenes		+	+
Steroids		+	+
Saponins		-	-

Phytochemicals present in less amount (+), high amount (+++) and absent (-)

Physicochemical analysis:

The physiochemical characterization of *M. indica* leaf and stem are shown in **Table 3**. The moisture content of leaf and stem was 8.25 and 6.75 % respectively. The ash value was determined by three different forms viz., total ash, water soluble ash and acid insoluble ash. The total ash in leaf was 4.6%, while water soluble ash and acid insoluble ash was 0.83 and 0.5 respectively. The total ash in stem was 5.37% while both water soluble ash and acid insoluble ash was 1.5% and 1.0% respectively. The extractive values of leaf and stem are shown in **Table 10**. The maximum extractive value was found in methanol and minimum was in petroleum ether in both leaf and stem of *M. indica*.

TABLE 3: PHYSICOCHEMICAL PARAMETERS OF *M. INDICA* LEAF AND STEM

S. No.	Parameters	% Value (w/w*) leaf	% Value (w/w*) stem
1	Loss on drying	8.25 ±0.25	6.75 ±0.29
2	Total ash	4.6 ±0.31	5.37 ±0.30
3	Water soluble ash	0.83 ±0.22	1.5 ±0.24
4	Acid insoluble ash	0.5 ±0.0	1.0 ±0.0
5	Petroleum ether soluble extractive value	0.99 ±0.01	1.03±0.1
6	Ethyl acetate soluble extractive value	2.05 ±0.03	2.25 ±0.01
7	Acetone soluble extractive value	3.67 ±0.02	5.33 ±0.01
8	Methanol soluble extractive value	24.93 ±0.11	15.92±0.17
9	Aqueous soluble extractive value	22.88 ±0.19	11.40 ±0.09

*Average of three readings ± SEM

Fluorescence analysis:

The fluorescence characteristics of leaf and stem powder of *M. indica* are summarized in **Table 4** and **5**. Fluorescence study helps in the qualitative evaluation which can be used as a reference data for the identification of adulterations. The fluorescent analysis under visible light and UV light by treatment of different chemical reagents showed different colour. This is attributed to the ultra violet light which produces fluorescence in many natural products that do not visibly fluoresce in daylight. If substance themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by applying different reagents. Thus fluorescence is used for qualitative assessment of crude drug.

TABLE 4: FLUORESCENCE ANALYSIS OF *M. INDICA* LEAF POWDER

Treatment	Visible light	Under UV Short Wavelength (254 nm)	Light Long wavelength (365) nm
Powder + 1 N NaOH (aq)	Brown	Black	Black
Powder + 1 N NaOH (alc)	Light green	Black	Dark green
Powder + Ammonia	Dark green	Black	Black
Powder +Picric acid	Light yellowish green	Black	Black
Powder +Petroleum ether	Green	Black	Reddish brown
Powder + 50% HCl	Light brown	Black	Black
Powder + 50% H_2SO_4	Light green	Black	Black
Powder + Ethyl acetate	Dark green	Black	Orange
Powder + Ethylalcohol	Green	Black	Yellow
Powder + Methanol	Dark green	Black	Yellow

TABLE 5: FLUORESCENCE ANALYSIS OF *M. INDICA* STEM POWDER

Treatment	Visible light	Under UV light Short Wavelength (254 nm)	Long wavelength (365 nm)
Powder + 1 N NaOH (aq)	Dark brown	Black	Black
Powder + 1 N NaOH (alc)	Brown	Black	Green
Powder + Ammonia	Light brown	Black	Light green
Powder + Picric acid	Yellow	Black	Black
Powder + Petroleum ether	Light brown	Black	Green
Powder + 50% HCl	Brown	Black	Black
Powder + 50% H_2SO_4	Brown	Black	Black
Powder + Ethyl acetate	Light brown	Black	Light green
Powder + Ethyl alcohol	Light brown	Black	Green
Powder + Methanol	Light brown	Black	Light green

DISCUSSION: Standardization is very important and essential to maintain the identity, quality, purity and safety of crude drugs especially in the powder form. Morphological evaluation is a technique of qualitative evaluation based on morphological characters of plant parts or sensory profiles of drugs [10]. The morphological characters can serve as diagnostic characters of a particular plant species and will be very useful in identifying the plant at species level and prevent adulteration or substitution.

However, another difficulty arises when the drug is in dried powder form. Even if the plant is identified correctly, it can be misused or rather substitution can occur in powder form. Hence it is essential to have some characteristics of the particular plant parts in powder form also. Hence, the leaf and stem powder of *M. indica* were analyzed for some diagnostic characters like type of stomata, crystals, trichomes, xylem vessels, etc.

The powder was also analyzed for various phytochemical constituents which revealed maximum amount of flavonoids and tannins in leaf and tannins in stem. The physicochemical parameters like ash values, loss on drying and extractive values evaluated will help in preventing adulteration. In leaf, the extractive value was highest in water followed by methanol while in stem it was just opposite. However, it indicates that in both polar compounds are more.

The extractive values are useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in a particular solvent [11]. Thus, preliminary analysis of phytochemical and physicochemical analysis ensures purity, identity and quality of the drug and also gives an idea about the phytoconstituents present for further analysis [12].

The fluorescent analysis of dried powder with different chemical reagents show different colour in visible and UV light which is characteristic of particular part and particular plant. Thus fluorescence analysis is useful for qualitative assessment of crude drug [13].

Pharmaognotic studies on different plants like *Cissus quadrangularis* stem [14], *Cordia macleodii* stem bark [15], *Tephrosia maxima* Pers root [16], *Woodfordia fruticosa* flower [17], *Barringtonia acutangula* leaf [18], *Brunfelsia Americana* leaf [19], *Mangifera indica* leaf [20], *Holoptelea integrifolia* leaf [21], *Ficus racemosa* fruit [22], *Achyranthes aspera* leaf [23], *Terminalia bellerica* leaf and stem [24], *Punica granatum* fruit rind [25] are also reported.

Establishing standards is an integral part of establishing the correct identity and quality of a crude drug. The microscopic characters, the physiochemical studies and fluorescence analysis can be used for the quality control of the crude drug and these are prime stem for evaluation [1]. *M. indica* is traditionally used to treat many ailments and illness hence it is imperative to standardize it for use as a drug. According to World Health Organization (WHO) the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken. Morphological and microscopic studies are reliable, simple and cheapest in establishing the identity of source materials [26].

CONCLUSIONS: In conclusion, it can be stated that the pharmacognostic results of the present work lays down the standard parameters which can be useful for checking the authenticity *M. indica*, an important useful medicinal plant. The parameters laid down ensure in maintaining the quality of crude drug and can be also useful for the preparation of a monograph.

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