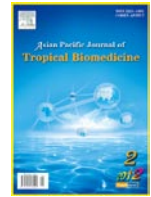




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Morpho anatomical studies of leaves of *Abutilon indicum* (Linn.) Sweet

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ABSTRACT

Objective: To evaluate the pharmacognostic parameters of the leaves of *Abutilon indicum* (Linn.) Sweet which will assist in standardization, quality assurance, purity and sample identification of the species. **Methods:** The pharmacognostic studies were carried out in terms of organoleptic, microscopic, powder microscopic, leaf constants and fluorescence analysis. **Results:** Macroscopic study showed that the leaf shape –cordate, Size –2–4 cm long, Colour – Green, Odour –Characteristic, Taste –Characteristic, Surface – Smooth, Apex –Acute to acuminate, Lamina–Simple, Cordate, Reticulate, Dentate, Margin–Crenate–Dentate. The microscopic features of leaves were observed as covering trichomes, glandular trichome, vascular bundles, crystals, stomata ,mucilage secretory cells adaxial epidermis, palisade mesophyll, spongy mesophyll and lateral vein. Further the study was evaluated leaf constants, powder microscopy and fluorescence study of leaf powder. **Conclusion:** Various pharmacognostic characters observed in this study help in the identification and standardization of *Abutilon indicum* (Linn.) Sweet.

1. Introduction

Over the last decade there has been a growing interest in drugs of plant origin in contrast to the synthetics that are regarded as unsafe to human and environment [1]. *Abutilon indicum* (Indian Mallow) is a small shrub in the Malvaceae family, native to tropic and subtropical regions and sometimes cultivated as an ornamental plant [1]. In traditional medicine, *A. indicum* is used as a demulcent, aphrodisiac, laxative, diuretic, and pulmonary and sedative (leaves). The bark is astringent and diuretic; laxative, expectorant and demulcent (seeds); laxative and tonic, anti-inflammatory and anthelmintic (whole plant); analgesic (fixed oil); diuretic and for leprosy (roots) [2]. The whole plant is uprooted, dried and is powdered. In ancient days, maidens were made to consume a spoonful of this powder with a spoonful of honey, once in

a day, for 6 months until the day of marriage, for safe and quick pregnancy. The leaves can also be used to treat ulcers, headaches, gonorrhoea & bladder infection.[2] The plant is very much used in Siddha medicines. In fact, the root, bark, flowers, leaves and seeds are all used for medicinal purposes by Tamils. The leaves are used as adjunct to medicines used for pile complaints. The flowers are used to increase semen in men [3]. A methanol extract of *A. indicum* had some antimicrobial properties [4]. A chemical compound, β –sitosterol, which has been identified as the active ingredient in many medicinal plants, is present in *A. indicum* and a petroleum ether extract provided larvicidal properties against the mosquito larvae *Culex quinquefasciatus* [5]. Microscopy is an important tool for authentication of crude drugs and study of powdered drugs [5]. It is important to interpret morphological and anatomical descriptions of crude drugs as well as characteristic features of drugs and adulterants of commercial significance [6]. Establishment of the pharmacognostic, morphological and microscopical characters of leaves and bark of the plant will assist in standardization, which can guarantee quality, purity and identification of samples.

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2. Materials and methods

2.1 Chemicals

All the chemicals used were of analytical grade and were obtained from SD Fine Chemicals Mumbai and Qualigen Chemicals Ltd., Mumbai, India.

2.2 Collection of specimens

Abutilon indicum leaves were collected from Dharmapuri and they were identified by Dr.P.Jayraman, Plant Taxonomist (Plant Anatomy Research Centre, Tamil Nadu). The specimen was deposited (Specimen no ANU/BOT2131/2011/AP). The leaves were collected and dried in shade and then coarsely powdered. Powders of leaves were examined for their microscopical characters. Care was taken to select healthy plants and for normal organs. The required samples of different organs were cut and removed from the plant and fixed in FAA (Formalin –5ml+Acetic acid –5ml+70% ethyl alcohol –90ml). After 24 hours of fixing the specimens were dehydrated with graded series of tertiary-butyl alcohol as per the schedule given by Sass, 1940 [6]. Infiltrations of the specimens were carried by gradual addition of paraffin wax (melting point 58–60°C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

2.3 Organoleptic evaluation

Various sensory parameters of the plant material (such as colour, odour, size, shape, and taste) were studied by organoleptic evaluation

2.4 Macroscopic evaluation

Various macroscopic characters of fresh leaves of *A.indicum* were recorded such as duration, type of leaf base, presence or absence of petiole and characters of lamina. Lamina consists of characteristic features such as composition, incision, shape, venation, margin, apex, base, surface and texture.

2.5 Microscopic evaluation

In microscopic evaluation, studies were conducted on both grounds qualitatively and quantitatively.

2.6 Qualitative microscopy

The paraffin embedded specimens were section with the help of rotary microtome. The thickness of the sections was 10–12 micrometers. Dewaxing of the sections was by customary procedure given by Johanson 1940 [7]. The sections were stained with Toluidine blue as per the method published by O'Brien, et.al., 1964 [8]. Since Toluidine blue is a polychromatic stain, the staining results were remarkably good and some

cytochemical reactions were also obtained. The rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc. wherever necessary sections were also stained with saffranin and fast green and iodine (for starch). For studying the stomatal morphology, venation pattern and trichome distribution, paradermal (sections taken parallel to the surface of leaf) as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffery's maceration fluid (Sass, 1940) were prepared [6]. Glycerin mounted temporary preparation were made for macerated/cleared materials.

2.7 Photomicrographs

Microscopic description of tissues or supplemented with micrographs were necessary photographs of different magnifications were taken with Nikon Labphot 2 microscopic unit. For normal observations bright field was used for the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark backgrounds. Magnifications of the figures are indicated by the scale bars [9]

2.8 Powder microscopy

The dried leaves were powdered and studied under microscope. Different staining reagents (such as iodine for detection of starch grains and phloroglucinol for detection of lignified components) were used. A little quantity of root bark powder was taken onto a microscopic slide; 1–2 drops of 0.1% w/v phloroglucinol solution and a drop of concentrated hydrochloric acid were added and covered with a cover slip. The slide preparation was mounted in glycerol and examined under microscope. The presence of starch grain and calcium oxalate crystal was detected by the formation of blue colour on addition of 2–3 drops of 0.01 M iodine solution [10]. The characteristic structures and cell components were observed and their photographs were taken using photomicrography.

2.9 Quantitative microscopy

2.9.1 Leaf Constants

The quantitative values such as palisade ratio, stomatal number, stomatal index and vein islet number were carried out on the pieces of lamina of the *Abutilon indicum* (Linn.) Sweet leaves [10]

2.9.2 Palisade ratio

The average number of palisade cells beneath each upper epidermal cell is termed as palisade ratio. Pieces of leaf about 2mm square were cleared by boiling with chloral hydrate solution for about 1 hour, mounted and examined with a 100mm objective. Camera Lucida was fixed on the microscope.

Epidermal cells were traced on a paper fixed on the board. Firstly, groups of four epidermal cells were traced. Then the palisade cells lying beneath each group were focused and traced. The palisade cells in each group were counted. The palisade cells those were more than half covered by the epidermal cells were also included to be inside the epidermal cells. The figure obtained divided by four group the palisade ratio of that group. The result obtained were tabulated in Table 1.

Table 1

Data showing the palisade ratio, stomatal number & stomatal index of *Abutilon indicum* (Linn.)Sweet

S.No	Leaf Constants	Upper Surface	Lower Surface
1	Palisade ratio	3.21	5.75
2	Stomatal number	3.10	5.10
3	Stomatal index	13.11	21.20

2.9.3 Stomatal Number

The average number of stomata per sq.mm. of epidermis is termed as stomatal number. Fragments of leaf from the middle of the lamina were cleared by boiling with chloral hydrate solution for about 2 hours, camera Lucida was fixed on the microscope, cleared pieces mounted and using 45mm. objective, different fields were selected and the epidermal cells are stomata were traced on the paper in each field. Knowing the area of each selected field, stomatal number was calculated .The result obtained were tabulated in Table 1.

2.9.4 Stomatal Index

The percentage proportion of the ultimate division of the epidermis of a leaf which have been converted into stomata is termed as stomatal index and is represented by the formula

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

Where S=number of stomata per unit area; E=number of ordinary epidermal cells in the same unit area

Pieces of the leaf other than extreme margin or midrib were suitably cleared by boiling with chloral hydrate solution for about 2hours mounted and examined by a microscope using 45mm objective. Using Camera–Lucida, the epidermal cells and stomata were traced on a paper and from the area traced the number of stomata and epidermal cell were counted in a unit area and stomatal index was calculated ,the result obtained were tabulated in Table 1.

2.9.5 Vein–Islet and Vein Termination Number

The term vein islet is used to denote the minute area of photosynthetic tissue encircled by the ultimate divisions of the conductive strands, the number of vein islets per mm in the central post of lamina midway between the midrib and the margin is termed as the vein–islets number. The leaves were cleared by boiling with chloral hydrate solution for about

two hours .Then they are boiled with chlorinated soda and left overnight and again boiled in chloral hydrate solution to clear them. Using the Camera–lucida and 10mm objective the squares were drawn in the paper and then the veins and vein–islets were the traced and by counting the vein islets, the vein islet and vein termination number was determined and the result obtained were tabulated in Table 2.

Table 2

Data showing the vein islets & vein termination number of *Abutilon indicum* (Linn.)Sweet

S.No	Leaf Constants	Range Of Vein Islet Number
1	Vein islet number	2.00–4.50
2	Vein Termination Number	3.40–4.60

2.10 Fluorescence analysis

The shade dried powder was treated with various chemical like acetic acid , FeCl₃, 0.5N H₂SO₄ , 1N H₂SO₄ ,0.5N HCl , 1N HCl, 0.5N NaOH, 1N NaOH,0.5 N KOH 1N KOH, CHCl₃ and their fluorescence behaviors was observed in U.V and in visible day light . The result obtained is given in table no.3

3.Results

3.1 Macroscopical Characters

3.1.1 Morphology of Leaf

Size	:	2–4 cm long
Colour	:	Green
Odour	:	Characteristic
Taste	:	Characteristic
Surface	:	Smooth
Margin	:	Crenate–Dentate
Apex	:	Acute to acuminate
Lamina	:	Simple, Cordate, Reticulate, Dentate

3.2 Microscopical Characters

In this cross section view, the leaf has thick and prominent midrib and thin uneven densely hairy lamina (Figure 1 and 2.).The midrib has broad, pyramidal shaped adaxial part transverse hemispherical wide abaxial body

3.2.1 Lamina

The lamina is 150 μ m thick. The epidermal layers become uneven due to dense trichomes, the epidermal layer are thin with a small squarish or rectangular. Some of the epidermal cells are dilated and contain dense mucilage. When the mucilaginous epidermal cell breaks, mucilage oozes out as cloud. The mesophyll tissue is differentiated into adaxial palisade zone and abaxial spongy mesophyll tissue. The palisade mesophyll consists of narrow, cylindrical cells which

are in two rows .the spongy parenchyma consists of about 5 layers of lobed, loosely arranged cells. The lamina becomes slightly thicker in the region of the lateral vein. The vascular bundle of the lateral vein consists of the vertical file of cells and a small group of phloem elements; parenchymatous bundle sheaths with adaxial and abaxial extension are seen in the lateral vein.

3.2.2 Midrib

The midrib is very prominent projecting both on the adaxial and abaxial sides. It is 1.4mm. in vertical plane and the adaxial dome is 600 μ m.in horizontal plane. The abaxial body is 1.35mm. Horizontally it consists of a thin epidermal layer of less conspicuous, small squarish cells. Dense trichomes are seen on all sides of the midrib the ground tissue consists of an outer zone of 4 or 5 layers of collenchyma cells of abaxial midrib. The adaxial dome has a thick hemispherical mass of collenchymas cell beneath the epidermis. The remaining part of the ground tissue is homogenous, parenchymatous thick walled and compact. The vascular bundle, large and arch shaped extending up to the lateral margin. Xylem consists of several parallel rows of cells. The phloem occurs in broad dense, beneath the xylem(Fig 1.).

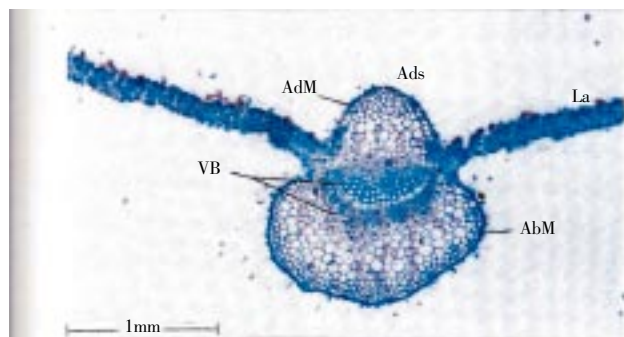


Figure-1 T.S. of Leaf through midrib with lamina

- AbM : Abaxial midrib
- AdM : Adaxial midrib
- Ads : Adaxial side
- La : Lamina
- VB : Vascular bundle

3.2.3 Trichome

All the parts of plant are densely clothed with epidermal trichomes. The trichomes are of two types which occur intermixed with other(Fig 5.).

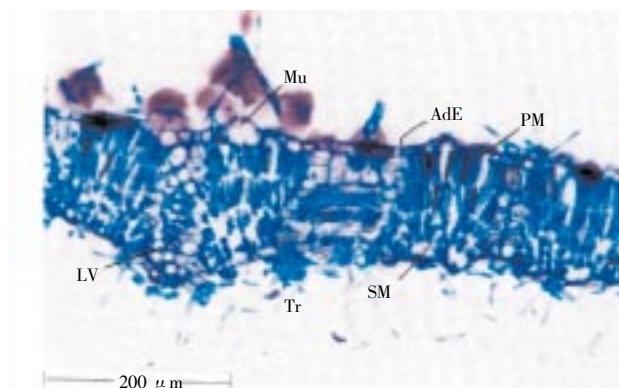


Figure 2 T.S. of Lamina enlarged.

- Mu : Mucilage secretory cells
- AdE : Adaxial epidermis
- PM : Palisade mesophyll
- SM : Spongy mesophyll
- Tr : Trichome
- LV : Lateral vein

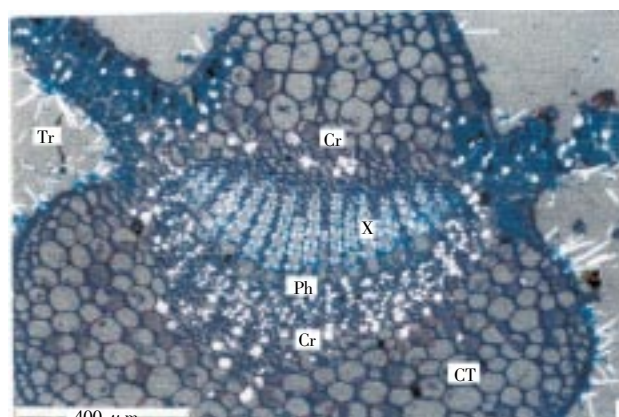


Figure 3 T.S. of midrib showing crystal distribution

- Cr : Crystals
- Tr : Trichome
- Ph : Phloem
- X : Xylem

Table 3

Fluorescence analysis of leaves of Abutilon indicum

TREATMENT	DAYLIGHT	UNDER UV LIGHT	
		Short wavelength (254nm)	long wavelength (366nm)
Powder	Brownish green	Bluish brown	Dark green
Powder+acetic acid	Greenish brown	Dark brown to emerald green	Dark green
Powder+ water	Pale green	Light green	Green
Powder+FeCl3	Pale brownish green	Light green	Jade green
Powder + 1N H2SO4	Light reddish brown	Light green	Green
Powder + 0.5N H2SO4	Light reddish brown	Light green	Green
Powder + 1N HCl	Light brown	Dark brown	Dark green
Powder + 0.5N HCl	Light brown	Greenish brown	Dark green
Powder + 1N NaOH	Green	Light green	Dark green
Powder + 0.5N NaOH	Light green	Brown to light green	Green
Powder + 1N KOH	Dark green	Dark brown	Dark green
Powder + 0.5N KOH	Dark green	Blackish green	Green
Powder + CHCl3	Dark green	Light green	Green

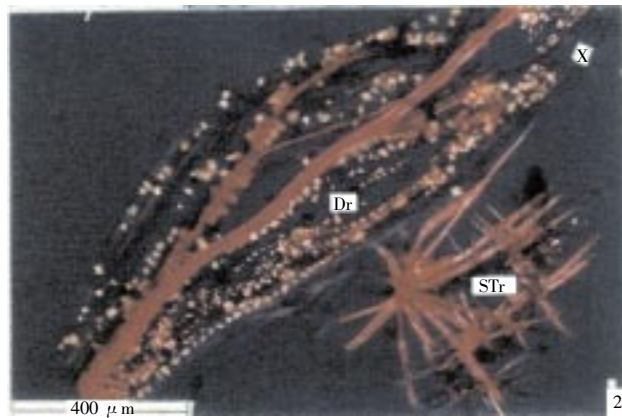


Figure 4: Powder microscopy showing Xylem fibers, Crystal & Stellate trichome

- Dr : Druses
- STr : Stellate trichome
- X : Xylem

3.2.4 Covering trichome

These are more abundant and crowded on the lower and upper part of the leaf. The trichome is known as stellate trichome. It consists of bunch of trichomes deviating in all the direction is obliquely horizontal, plane. The bunch of trichomes originates from a group of epidermal cells. The individual arms of the trichomes are heavily thickened walled and lignified.

3.2.5 Glandular Trichomes

The glandular trichomes are multicellular, uniserrate, unbranched bodies. They attach to the epidermis by means of rectangular basal stalk cell; the lower part of the trichome is wider and becomes gradually tapering towards the tip. The cells contain dark inclusions and are secretory in function.

3.2.6 Crystals

Calcium oxalate crystals are abundant in the lamina and the midrib/lateral veins. The crystals are druses. They occur in close vertical rows, especially along the xylems elements. In the midrib the crystals are denser in the phloem zone, they are smaller than those that occurs in the ground cells(Fig 4.)

3.2.7 Veination Pattern

The lateral veins and veinlets are prominent. The vein islets are distinct and mostly squarish to rectangular. The veins are surrounded by a single layer of hyaline parenchymatous sheath cells. Vein terminations are present in most of the islets. The terminations are distinct, either short or long forked once at the tip occasionally simple(Fig 6.).

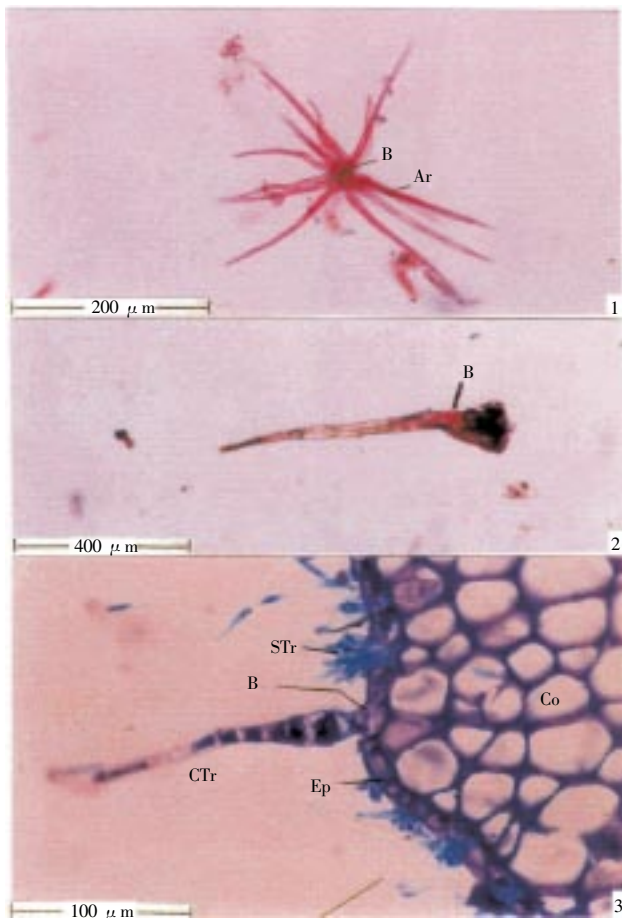


Figure 5 Trichome morphology of *Abutilon indicum*(L) Leaf

- 1) Stellate trichome separate from the leaf.
- 2) A Glandular trichome removed from the leaf.
- 3) Glandular trichome enlarged in the midrib

- Ar : Arms of the trichome
- B : Basal cells of the trichome
- Co : Collenchyma
- Ep : Epidermis
- CTr : Glandular trichome
- STr : Stellate trichome

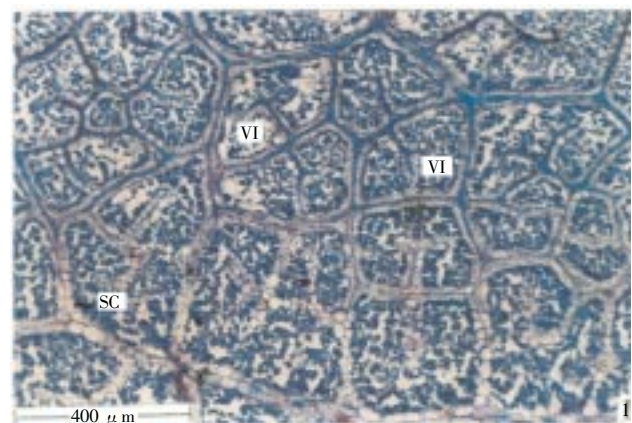


Figure 6: .Venation pattern of *Abutilon indicum*(L) Leaf

- Vein-islet and vein termination
- VI : Vein islets
- VT : Vein termination
- SC : Sheath cells

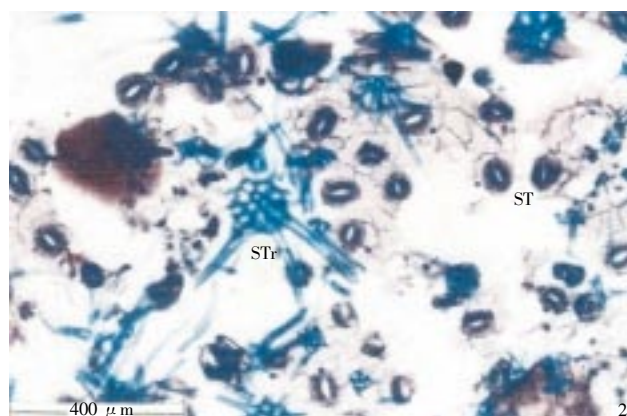


Figure 7 .Abaxial epidermis with stomata of *Abutilon indicum*(L) Leaf
STr : Stellate trichome
ST : Stomata

3.2.8 Stomata

Stomata occur mostly on the lower surface of the leaf. They are of anamocytic type, lacking distinct subsidiary cells. The epidermal cells are polygonal with this, waxy anticlinal walls.

3.3 Powder Microscopy

Fresh leaves of *Abutilon indicum* (Linn.) Sweet were collected, shade dried and finally powdered. It was sieved through sieve no 100 and used for the study. The powder of the leaves showed the following characteristics.

Covering Trichomes	: Uniserate and Unicellular
Epidermal cells	: Epidermis with palisade cells
Calcium oxalate crystals	: Both prismatic and cluster type
Straight Fibers	: Long straight pericyclic fibers
Stomata	: Anamocytic stomata

4. Discussion

Standardization is an important tool for herbal drugs in order to establish their identity, purity, safety and quality. In order to standardize a drug, various macroscopic, microscopic, fluorescence analysis are done. Microscopic method is one of the cheapest and simplest methods to start with establishing the correct identification of the source material [11]. Morphological and microscopical studies of the leaf will enable to identify the crude drug. The quantitative determination of some pharmacognostical parameters is useful for setting standards for crude drugs. Stomatal number, stomatal index value and palisade ratio, vein islet and vein termination value determination are equally important in the evaluation of crude drugs. These values help in the evaluation of purity of drugs . Morphological and microscopic studies of leaves act as a reliable aid for detecting adulteration. These simple but reliable standards will be useful to a lay person in using the drug as a home remedy. These studies can also help the manufacturers for identification and selection of the raw

material for drug production. This study revealed the various morphological and microscopical characteristics. The powder characters of leaves of *Abutilon indicum* are inevitable finding in this study .Hence these characteristics could be used to identify and to know the adulterants if any with this species.

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Conflict of interest statement

We declare that we have no conflict of interest

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