International Journal of Research and Development in Pharmacy and Life Sciences
Available online at http//www.ijrdpl.com
August - September, 2013, Vol. 2, No. 5, pp 567-573
ISSN: 2278-0238

Research Article

PHARMACOGNOSTIC INVESTIGATION ON ROOTS & LEAVES EXTRACT OF *ABUTILON INDICUM LINN.*

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(Received: March 18, 2013; **Accepted:** May 02, 2013**)**

ABSTRACT

Herbal medicines are natural products and their phytoconstitutents vary depending on time and region, processing and storage. Variations in the collection, processing or storage of an herb could impact its efficacy profile. Since prior knowledge regarding appropriate collection and usage of most medicinal plants exists in tradition, it can be used as a guide to quality standardization. The present study is aimed to carry out research work to evaluate Pharmacognostic investigation on Root & Leaf extract of *Abutilon indicum*. From the literature study it is found that a very little work is done on root & leaf part of plant for setting standardization limits. So, our aim is to find out again various standardization limits according WHO guideline like macroscopic & microscopic study, to find out LOD, ash values, extractive values etc. for root & leaf part of drug with a view to control the quality of crude drug.

Keywords: *Abutilon indicum* Linn., Taxonomic Classification, Microscopic Study, Physicochemical Investigation.

INTRODUCTION

Since ancient time, plant- based product has been used for health care, search is continuing for new plant material and their interaction with biological system. Whenever such plant material is found to be useful, it is taken up for further investigation, as regards to the constituents present for its biological action. On confirmation of its biological activity, the suitable extracts or isolated phytoconstituents are prepared from the plant material and put into usage. Currently this is an area, which is gaining considerable importance and leading to evaluation of green chemicals'. Many consumers preferred to treat themselves with phytopharmaceuticals or herbal preparation and the sale of these are increasing in most first world countries. All these have led to the development of new field called herbal drugs extraction. Many manufacturers are making efforts to improve yields, as well as composition of total extracts and also of phytoconstituents of intrest, whenever desired**.** [1]

The microscopic examination of different parts of the drug provides several diagnostic characters. In case of leaves, surface preparation and transverse section, preferably through midrib, are made and nature of epidermis, trichomes, and stomata, arrangement of tissues like palisade cells, vascular bundles and nature of cell content are studied. Similarly in case of bark, root, rhizome and wood, transverse and longitudinal sections are made and from characteristic arrangements of tissues of each drug and from diagnostic elements like stone cells, fibers, vessels etc. as also from the study of the cell deposits like crystals, starch etc., and the drugs are identified.[2]

Abutilon indicum Linn.

Synonyms[3]: *Abutilon indicum* G. Don.

About $1^{1/2}$

Taxonomic Classification[4]

Kingdom	:	Plantae
Class	:	Magnoliopsida
Order	:	malvales
Family	:	malvaceae
Genus	:	*Abutilon*
Species	:	*indicum*

Vernacular name [5]

English	:	Indian mallow
Hindi	:	Kanghi
Bengali	:	Badela
Sanskrit	:	Kankatika,Rsyaprokta
Telgu	:	Tutturubenda
Oriya	:	Pedipidika
Tamil	:	Tutti,Thuthi

Figure 1: *Abutilon indicum Linn.*

Ayurvedic properties: [5]

Rasa :	Madhura
Guna :	Snigdha
Veerya :	Sita
Vipaka :	Madhura
Karma:	Balya,Vatahara,Vrsya,Grahia

Traditional Uses

Almost all the parts of Atibala are of medicinal importance and used traditionally for the treatment of various ailments. The roots of the plant are considered as demulcent, diuretic, in chest infection and urethritis. The infusion of the root is prescribed in fevers as a cooling medicine and is considered useful in strangury, haematuria and in leprosy. The leaves are found to be good for ulcer and as a fomentation to painful parts of the body. The decoction of the leaves is used in toothache, tender gums and internally for inflammation of bladder. The bark is used as febrifuge, anthelmintic, alexeteric, astringent and diuretic. The seeds are used in piles, laxative, expectorant, in chronic cystitis, gleets and gonorrhea.[6]

Traditionally the plant is used in inflammation, piles, gonorrhea treatment and as an immune stimulant. Root and bark are used as aphrodisiac, anti diabetic, nervine tonic, and diuretic. Seeds are used as aphrodisiac and in urinary disorders.[16] Along with other therapeutic applications,*The Ayurvedic Pharmacopoeia of India* indicates the use of the root in gout, polyuria and haemorrhagic diseases.[3]

MATERIAL & METHODS

1. Collection and Authentication of Plant material

For Root and Leaf of *Abutilon indicum* Linn. **(**Family-Malvaceae**)**, plants were collected in the month of September from village Ismailpur, Tha. Najibabad, Distt. Bijnor (U.P.). It was authenticated by Dr. Arvind Kumar, Research Scientist, Patanjali Ayurved Limited, D-38, Haridwar (U.K.). The authentication no. of the plant is RUBL-20649.

2. Drying of Plant material

Roots & Leaf parts were separated from the ***Abutilon indicum*** plant then washed with water. Kept in sun light for thirty minutes and then dried under shade at room temperature for 15 days. Grounded to a coarse powder and passed through a 60 # sieve for Uniform particle size.

3. Macroscopic study

Macroscopic characteristics of leaf and root were carried out by naked eyes & with the aid of magnifying lens. Shape, Size, Colour, Odour, Taste, Surface characteristics, fractures etc. were identified of root part.[7,8]

4. Microscopical study

T.S. of the Leaf

The pieces of leaves were boiled in a test tube with chloral hydrate for several minutes until complete removal of chlorophyll. The leaf can be examined from both the dorsal and ventral surfaces. Transverse section was obtained by cutting the leaf portion including mid rib with the help of sharp blade and staining was done by safranin to impart

a red color to the lignified tissue.

T.S. of the Root

The root part having a diameter 3 to 5 mm and a length of approx 2.5 cm was selected & boiled in water for a few minutes to soften the hard drug sample. Now the soften samples were used for taking fine sections. Transverse sections were obtained by cutting along the radial plane of a cylindrical portion of the root and perpendicular to the long axis. [9, 10]

Staining & mounting of sections

For staining of cut fine sections, below mentioned steps followed.[11]

i). **Preparation of 20% HCl (100 ml)**

- 37.5 ml distilled water
- 62.5 ml concentrated HCl (10.18 M, dens = 1.16 kg/l)

ii). **Preparation of Phloroglucinol stain**

Prepared a saturated solution of Phloroglucinol in 20 % HCl. filtered the solution to remove crystals. It was protectd with aluminum-foil to avoid exposure to the light.

iii). **Staining & mounting**

Sections were dehydrated with ethanol series (30%, 50%, 90%, and 100%). The dehydrated fine sections were placed in the center of the glass slide, applied few drops of phloroglucinol in 20% HCl on glass slide. Covered the section with cover slip and observed it under compound microscope in visible light.

Microscopic study of Root & Leaf powder [7,8,9,10]

Microscopic study of root & leaf powder is used to find out the characteristics (like presence of calcium oxalate crystals, starch grains, lignified cell walls, tannins, fatty substance etc.) of root powder.

Procedure

The following reagents were used for Microscopic study of root & leaf powder.

1. Iodine solution
2. $FeCl_3$ Solution
3. Lectochloral
4. Sudan red

1. Iodine solution: It was prepared by dissolving 2.6g Iodine & 3g KI in 100 ml of water (q.s). With iodine solution starch turns blue. So presence of starch in root & leaf powder was detected with iodine solution.

2. $FeCl_3$ Solution – $FeCl_3$ Solution was prepared by dissolving 5g of $FeCl_3$ in 100 ml of 90% alcohol. This solution is also known as 5% $FeCl_3$ Solution. With $FeCl_3$ Solution tannins give blue black color. So presence of tannins in the root & leaf powder was detected with $FeCl_3$ Solution.

3. Lectochloral – Lectochloral reagent was prepared by mixing of 50g chloral hydrate & 50g lactic acid using of gentle heat. Chloral hydrate in the lectochloral reagent is clearing agent; after treating with lectochloral reagent of root& leaf powder calcium oxalate crystals are observed clearly.

4. Sudan red reagent prepared by dissolving 0.5g 0f Sudan red GR in 100 ml of glacial acetic acid. With fatty substances Sudan red reagent gives orange red to red color. So presence of fatty substances in the root & leaf powder was detected by this reagent.

5. Physicochemical Investigation [8, 9]

Loss on drying

Loss on drying is the loss of mass expressed as per cent w/w. Loss on drying determines both water and volatile matter in the crude drug. Moisture is an inevitable component of crude drug, which must be eliminated as far as possible.

An accurately weighed quantity of about 2 g of powdered drug was taken in a tared glass petridish. The powder was distributed evenly. The petridish kept open in vacuum oven and the sample was dried at a temperature between 100 to 105^{0}C for 2 h until a constant weight was recorded. Then it was cooled in a desiccator to room temperature, weighed and recorded. % Loss on drying was calculated using the following formula.

$$\% \text{ Loss on drying} = \frac{\text{Loss in weight of the sample}}{\text{Weight of the sample}} \text{ X } 100$$

Determination of Ash values

Ash values are helpful in determining the quality and purity of a crude drug, especially in the powdered form. The objective of ashing vegetable drugs is to remove all traces of organic matter, which may otherwise interfere in an analytical determination.

a) Total Ash value:

It is the total amount of material remaining after ignition. This includes both "Physiological ash", which derived from the

plant tissue itself, and "Non physiological ash", which is residue of the extraneous matter. Total ash value was found out after putting the drug (about 2 gm) in crucible by using furnace at temp. of 600°C for 2hr. Now calculated the percentage yield of ash value with reference to air dried drug.

b) Acid-Insoluble Ash:

It is residue obtained after boiling the total ash with dilute hydrochloric acid, and igniting the remaining insoluble matter. Boiled the ash for 5 to 10 minutes with 25ml of dilute hydrochloric acid, collected the insoluble matter in a crucible on an ash less filter –paper, ignited, and weighed. Now calculated the percentage yield of acid-insoluble ash with reference to the air-dried drug.

$$\%\,\text{Acid insoluble ash value} = \frac{\text{Wt. of acid insoluble ash}}{\text{Wt. of crude drug taken}} \times 100$$

c) Water soluble Ash value:

Boiled the total ash for five minutes with 25 ml of water; collected the soluble matter in a crucible, ignited, and weighed. Calculated the percentage or water soluble ash with reference to air dried drug.

$$\text{\% Water soluble ash value} = \frac{\text{Wt. of total ash} - \text{Wt. of water insoluble ash}}{\text{Wt. of crude drug taken}} \times 100$$

Determination of extractive values

Determination of extractive values is useful for evaluation of crude drug. It give idea about the nature of the chemical constituents present in a crude drug.

a) Alcohol soluble extractive value

Macerated 5 gm accurately weighed coarse powdered drug with 100 ml of alcohol (90%v/v) in a stoppered flask for 24 h, shaking frequently during first 6 h. Filtered rapidly through filter paper taking precaution against excessive loss of alcohol. Evaporated 25 ml of alcoholic extract to dryness in a tared dish and weighed it. Calculated the percentage w/w of alcohol soluble extractive with reference to the air-dried drug using following formula.

% Alcohol soluble extractive value = 80 X (Wt. of residue)

b) Water soluble extractive value

The procedure as above was followed using chloroform water I.P. instead of alcohol.

RESULT AND DISCUSSION

Macroscopic characteristics of Root of *Abutilon indicum*

Shape - Cylindrical or slightly straight

Surface characteristic - Surface was fissured & longitudinal corrugations was present on its surface.

Colour – Yellowish Brown

Odour – odorless

Taste – characteristic

Macroscopic characteristics of Leaf of *Abutilon indicum*

Shape - oval

Surface characteristic - Surface was Rough

Colour – Light Green

Odour – Characteristic & persistent

Taste – Slightly Sweet

Histological study of Root: Performed by using of compound microscope.

Transverse section of root shows a thin cork of 4-7 or more tangentially elongated rectangular cells, cork cambium, single layered, and at the lenticel regions followed by 2-3 layers of secondary cortex of thin-walled, almost cubical or rectangular cells, containing small clusters of calcium oxalate in most of cells, phellogen followed by 3-4 layers of thin-walled cells of cortex.

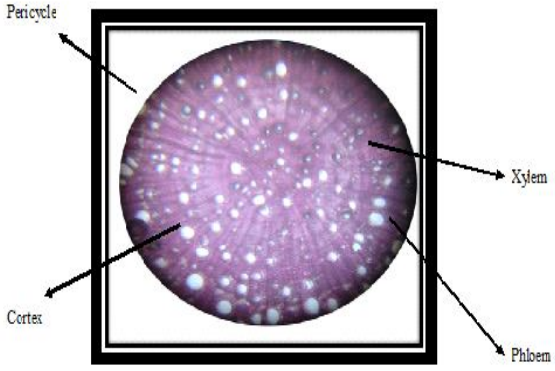


Figure 2: T.S. of *Abutilon indicum* Root

Study of Root powder

The root powder was bright yellow to brownish yellow in colour, had a slight bitter taste and a characteristic odour. Starch grains, tannins & calcium oxalate crystals were observed in the root powder using following reagents.

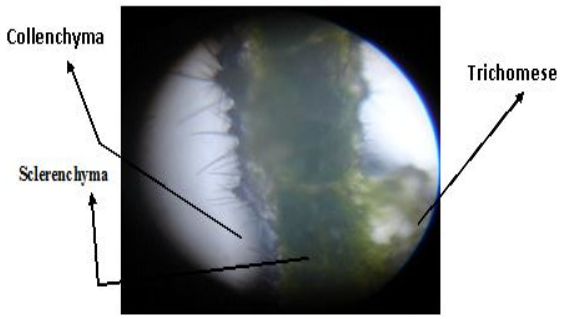
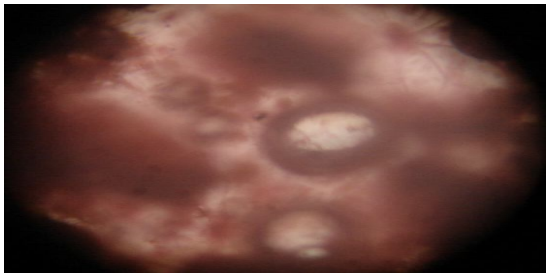


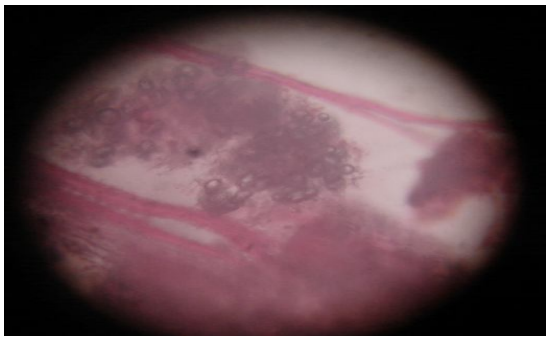
Fig. 3: T.S. of *Abutilon indicum* Leaf Study of Powder

Table 1: Characteristics of Powder microscopy of *Abutilon indicum* Root

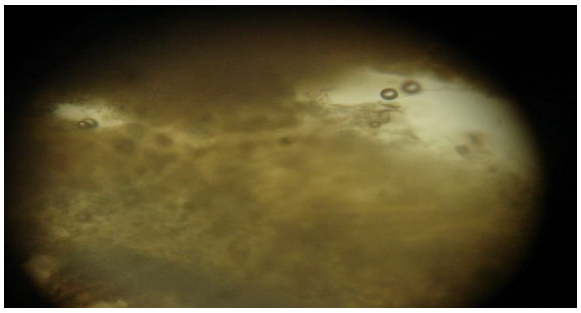
S. N.	Reagents	Observation	Inference
1.	Iodine solution	Blue color	Starch grains present
2.	$FeCl_3$ Solution	Blue black color	Tannins were present
3.	Lectochloral	Observed calcium oxalate crystals	Calcium oxalate crystals Present



[A]- starch present



[B]- Tannin is present



[C]- Calcium oxalate crystals Present

Figure 4: Powder Microscopy of Root (Fig. A-C)

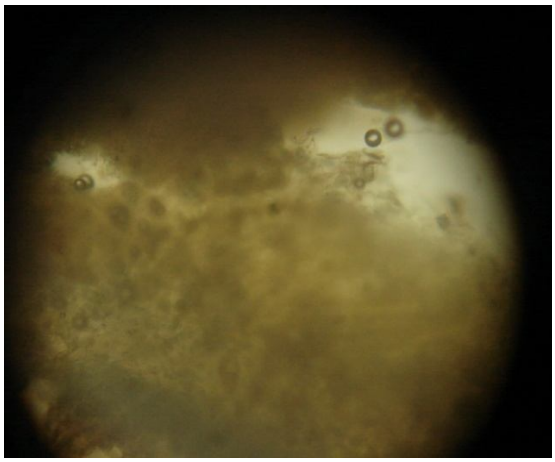
Study of leaves powder

The leaves powder was bright yellow to Greenish in colour, had a slightly sweet taste and a characteristic odour. Starch grains, tannins & calcium oxalate crystals were observed in the leaf powder using following reagents.

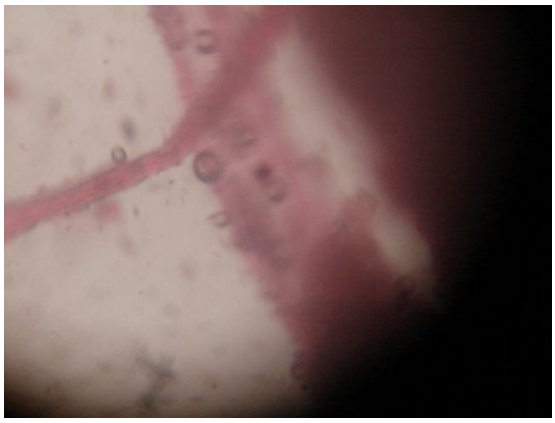
Table 2: Characteristics of Powder microscopy of *Abutilon indicum* Leaf

S. N.	Reagents	Observation	Inference
1.	Iodine solution	Blue colour	Starch grains present
2.	$FeCl_3$ Solution	Blue black colour	Tannins were present
3.	Lectochloral	Observed calcium oxalate crystals	Calcium oxalate crystals Present

[A]- Starch present



[B]- Tannin is present



[C]- Calcium oxalate crystals Present

Figure 5: Powder Microscopy of Leaves (A to C)

Physicochemical Evaluation for Root

In the present study, Root powder was investigated for the physicochemical characterization according WHO guideline and following results were found of loss on drying, ash values & extractive values.

Table 3: Physicochemical Parameters of *Abutilon indicum* Root

S.N	Physical Constants	Results
1	Loss on drying	5.23%
2	Total ash value	7.8%
3	Acid-insoluble ash value	1.%
4	Water soluble ash value	4.30%
5	Alcohol soluble extractive value	4.07%
6	Water soluble extractive value	9.6%

Physicochemical Evaluation for Leaves

In the present study, Leaf powder was investigated for the physicochemical characterization according WHO guideline and following results were found of loss on drying, ash values & extractive values.

Table 4: Physicochemical Parameters of *Abutilon indicum* Leaves

S.N	Physical Constants	Results
1	Loss on drying	6.67%
2	Total ash value	7.4%
3	Acid-insoluble ash value	1.%
4	Water soluble ash value	4.30%
5	Alcohol soluble extractive value	3.97%
6	Water soluble extractive value	9.36%

SUMMARY AND CONCLUSION

In the present study, Root & Leaf of ***Abutilon indicum*** was subjected to Pharmacognostic study.

In pharmacognostic study, various standardization parameters like macroscopic & microscopic study, loss on drying, ash values, extractive values etc. were determined on the Root and Leaf of *Abutilon indicum*. The water soluble extractive value of the crude drug was found to be higher than alcohol soluble extractive value.

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