Available online at www.ijpsdr.com
International Journal of Pharmaceutical Sciences and Drug Research 2009; 1(1): 28-31

Research Article

Pharmacognostical and Phytochemical Evaluation of Leaf of *Abutilon indicum* (Linn.)

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ABSTRACT
Abutilon indicum (Linn) family (Malvaceae) commonly known as Atibala is used in our Traditional System of Medicine for healing various diseases. It is used in the treatment of piles, uterine discharge, febrifuge and in cases of pulmonary tuberculosis. In the present investigation an attempt was made to study its Pharmacognostical features, including macroscopic, microscopic features, physico-chemical parameters, leaf constants and to investigated the phytochemical present in the extracts in the preliminary level. Thus it was thought worthwhile to explore the plant on the basis of its standardization parameters. The study will provide a referential information for the correct identification of the crude drug.

Keywords: *Abutilon indicum,* Standardization, microscopy, phytochemical analysis.

INTRODUCTION

Plants are the essential and integral part in Complementary and Alternative medicine and due to this they develop the ability for the formation of secondary metabolites like proteins, flavonoids, alkaloids, steroids and phenolic substances which are in turn used to restore health and heal many diseases. .Natural products of plant and animal origin offer vast resource of newer medicinal agents with potential in clinical use.

Abutilon indicum (Linn) family Malvaceae, commonly known as Atibala is an important medicinal plant used in our Traditional System of Medicine to treat various health aliments. The plant is used as demulcent, aphrodisiac, laxative, diuretic, pulmonary and sedative. The leaves are used as astringent [1], bark is used as diuretic and seeds are used as laxative, expectorant and demulcent. [2] The plant contains mucilage, tannins, asparagines, gallic acid and sesquiterpenes. [3] Thus the present investigation was aimed at evaluating the pharmacognostical features and phytochemical analysis for authentication and identification of the plant and also to evaluate the exact extract responsible for the biological activity.

MATERIALS AND METHODS

Plant Material

The fresh leavers of the plant *Abutilon indicum* were collected from the wild sources of the Satupara district of Maharashtra, in the month of February and were identified from the authentic sources. The collected leaves were washed; shade dried and was pulverized with a mechanical pulverizer for the size reduction. It was then passed through # 60 and the fine powder was collected and was used for the experiment for powder microscopy and preparation of extract. The fresh leave sample was used for the microscopy identification.

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Pharmacognostical Studies

Morphological studies were done by using simple microscope to determine the shape, apex, base, margins, taste and odour of the leaves. Microscopic studies were done by preparing a thin hand section of the midrib and the lamina region of *Abutilon indicum*. The section was cleared with chloral hydrate solution and was stained as per the protocol. Histochemical reactions were applied with Concentrated Hydrochloric acid and phloroglucinol and were mounted in glycerin for the identification of lignified elements, iodine solution for identification of starch grains, ruthenium red for mucilage, 60 % sulphuric acid for calcium oxalate crystals and ferric chloride for the phenolic compounds in the powdered bark by reported methods. [4-5]

As a part of quantitative microscopy, stomatal number, stomatal index, vein islet number, vein termination was determined by using fresh leaves of the plant.

Table 1: Flurosence Analysis of the powdered leaf of Abutlion indicum

S. No.	Sample	Colour in day Light	Colour in Short UV 365 nm	Colour in Long UV 480 nm
1.	Powder	Green	Dark Green	Light Green
2.	Powder + 0.1 N Sodium Hydroxide	Light Green	Emerald Green	Pale Green
3.	Powder + 0.1 N Hydrochloric acid	Dark Green	Light Green	Emerald Green
4.	Powder + Water	Green	Light Green	Dark Green
5.	Powder + Acetic Acid	Green	Dark Green	Dark Green
6.	Powder + Acetic Anhydride	Green	Dark Green	Emerald Green

Table 2: Physicochemical Evaluation of the crude drug *Abutlion indicum*

Standardization Parameters	% W/W (Mean ± SEM)
Total Ash	4.52 ± 0.12
Acid Insoluble Ash	2.54 ± 1. 21
Water Soluble Ash	2.01 ± 0.09
Sulphated Ash	3.42 ± 0.02
Alcohol Soluble Extractive Value	6.32 ± 0.23
Water Soluble Extractive Value	4.32 ± 1.24
Loss on Drying	6.34 ± 0.54

Table 3: Quantitative Analysis of leaf constants of *Abutlion indicum*

S. No.	Particulars		Values
1.	Stomatal Number	Upper Epidermis	3.12
		Lower Epidermis	5.23
2.	Stomatal Index	Upper Epidermis	13.12
		Lower Epidermis	20.21
3.	Vein islet Number		2.0 - 4.56
4.	Vein Termination Number		3.23 – 5.67

Table 4: Flurosence Analysis of the leaf extarct of *Abutlion indicum*

S. No.	Sample	Colour in day Light	Colour in Short UV 365 nm	Colour in Long UV 480 nm
1.	Petroleum Ether Extract	Light Green	Dark Green	Emerald Green
2.	Ethyl Acetate Extract	Dark Green	Scarlet Red	Light Green
3.	Chloroform Extract	Dark Green	Dark Greenish Brown	Emerald Green
4.	Methanolic Extract	Green	Scarlet Red	Dark Green
5.	Aqueous Extract	Brown	Pale Yellow	Light Yellow

Table 5: Extarctive Values of Leaf extracts of *Abutlion indicum* with Different solvents

S. No.	Extracts	% Extractability (Mean ± SEM)
1.	Petroleum Ether Extract	4.62 ± 0.89
2.	Ethyl Acetate Extract	6.45 ± 1.23
3.	Chloroform Extract	9.65 ± 2.20
4.	Methanolic Extract	20.34 ± 1.09
5.	Aqueous Extract	12.32 ± 2.89

Physico-chemical parameters

The parameter was done to evaluate the percentage of Total ash; water soluble ash, acid insoluble ash and sulphated ash were calculated as per Indian Pharmacopoeia. [6] Extract of the powdered leaf was prepared with different solvents for the study of extractive value. Flurosecne analysis was also carried out for the powder and for extract as per standard procedures.

Powder Analysis

Preliminary analysis of the powder of leaf of *Abutilon indicum* with different chemical reagents was carried out microscopically. [7- 8]

Preliminary Phytochemical Analysis

For Preliminary phytochemical analysis, extract was prepared by weighing 1kg of the dried powdered leaves and were subjected to hot successive continuous extraction with different solvents as per the polarity, petroleum ether, ethyl acetate, chloroform methanol and finally with aqueous. The extracts were filtered in each step, concentrated and the solvent was removed by rotary evaporator. The extracts were dried over dessicator and the residues were weighed. The presence or absence of the primary and secondary phytoconstituents was detected by usual prescribed methods. [9]

RESULTS

The macroscopical studies of leaf revealed certain characters:
Shape: Ovate to orbicular-cordate
Margin: Acuminate and toothed.
Apex: Pointed
Base: Symmetrical
Venations: Reticulate
Taste: Sweet to characteristic
Odour: Odourless.
Surface: Smooth on both the surfaces.

Microscopy

The Transverse section should the following characteristic features: The Lamina region consisted of upper and lower epidermis with covering and glandular trichomes. Covering trichomes were muticellular and uniseriate in nature while the glandular trichomes were multicelluar with single stalk and mutli head fused together. Stomata were of anomocytic type. Below the epidermis layer the next region was mesophyll which consists of long elongated palisade cells and calcium oxalate crystals.

The midrib region resembles dorsi ventral leaf. It consists of closely packed collenchyma cells with 2-3 layered in upper part and 3-4 layered in lower part. Just below and above the collenchyma the parenchyma cells are arranged in a loosely packed with much of intracellular space. The vascular bunbles are composed of xylem and phloem cells. Figures are shoen in fig. 1

Powder Microscopy

The powder was characterized on its morphological features as colour:emerald green, odour: odourless and taste was sweet to characteristic in nature

The dried fine powder was stained with chloral hydrate to detect the perecnce of calcium oxalate crystals. They were prismatic in nature. When stained with phloroglucinol and concentrated hydrochloric acid vascular bundles, lignified fibers were observed.

With glycerin mounting trichomes were observed both of covering and glandular types. Stomata were anamocytic in nature. All the results are figured in fig. 2a, 2b, 2c and 2d respectively

Fluorescence Analysis

The powder was subject to fluorescence analysis as per the standard procedure and shown in Table 1.

Physico-chemical Parameters

The powdered drug was evaluated for its physico-chemical parameters like Ash values, Sulphated ash, Acid Insoluble ash, Water soluble ash, Extractive values (Alcohol and water soluble values) and loss on drying. All the results are tabulated in Table 2.

Quantitative Analysis

The fresh leaf samples were subjected to quantitative analysis for various leaf constants like stomatal number, stomatal index, vein islet number, vein termination number. The results are shown in Table 3.

Fluorescence Analysis of the extracts

The extracts were prepared as per their polarity in hot successive extraction technique. Further they were treated with reagents and the colour changes were observed under Ultr Violet light. All the results are tabulated in Table 4.

Extractive values

The extracts were prepared according to the polarity and they were concentrated and their values were calculated with reference to air dried drug. The results are tabulated in Table 5.

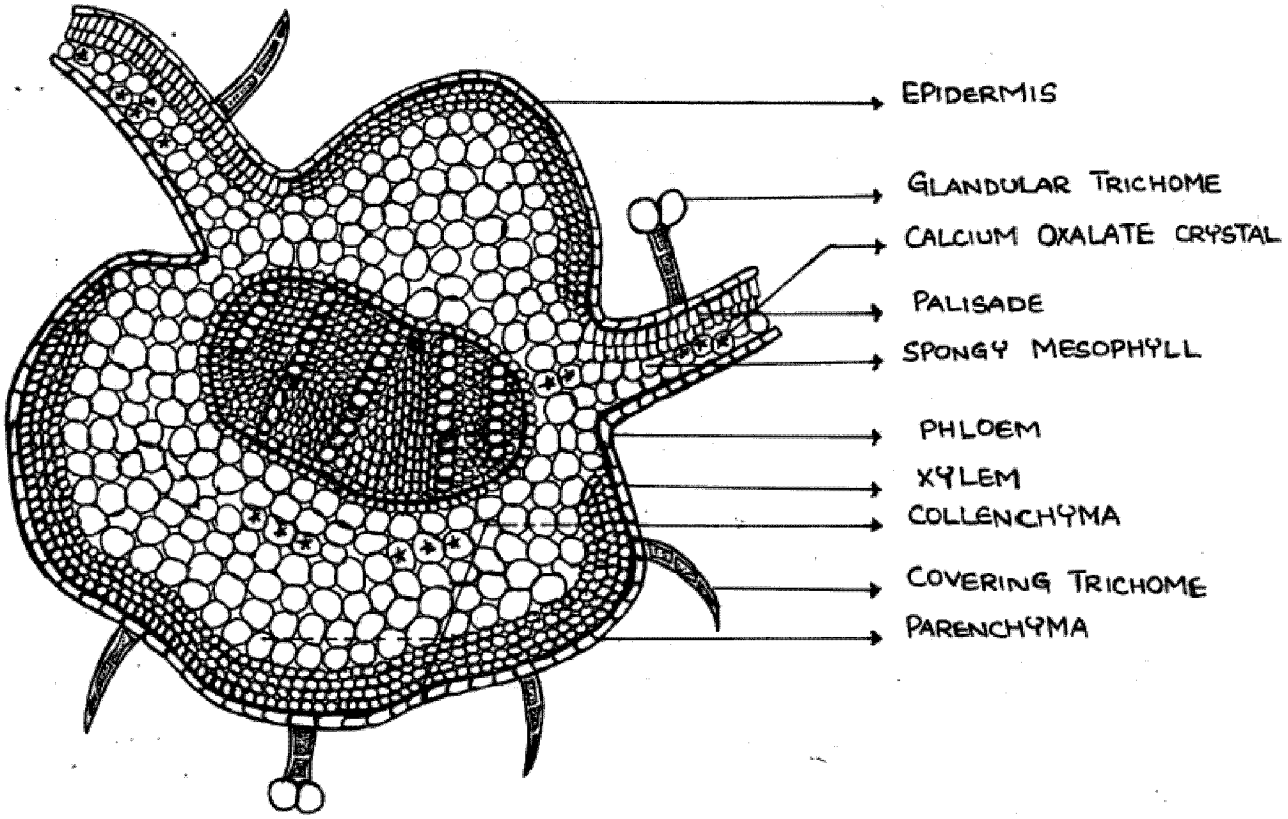

Fig. 1. Transverse Section of Leaf of *Abutilon indicum*

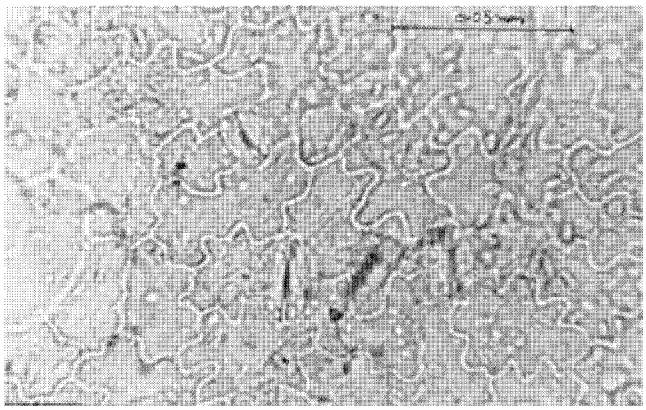

Fig. 2a: Anomocytic Stomata

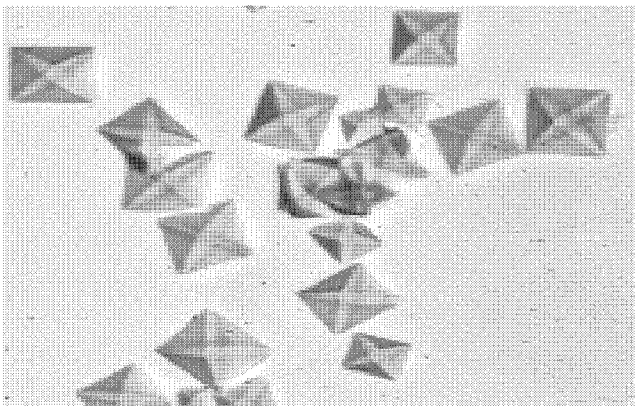

Fig. 2c: Calcium Oxalate Prisms

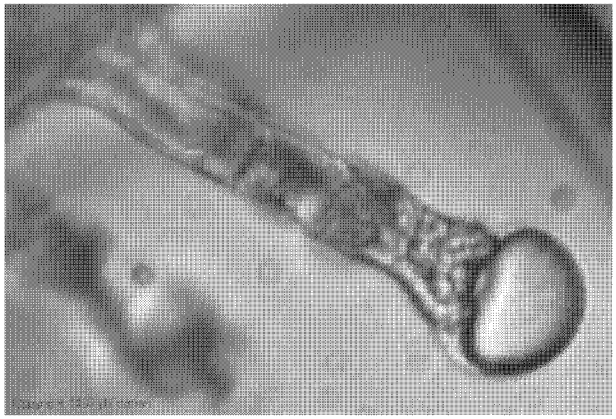

Fig. 2b: Glandular Trichomes

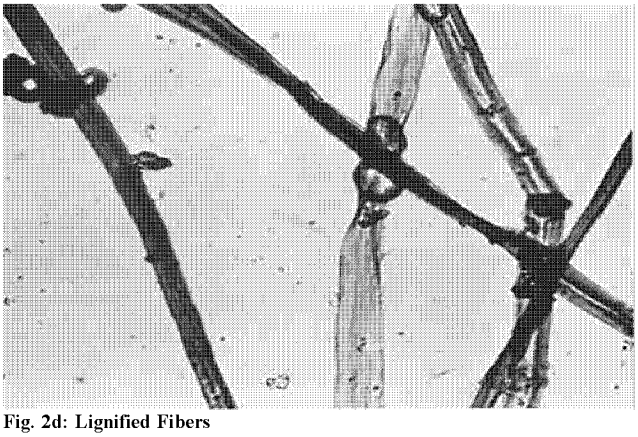

Fig. 2d: Lignified Fibers

Table 6: Preliminary Phytochemical Screening of Leaf Extract of *Abutlion indicum*.

Test	Petroleum Ether Extract	Ethyl Acetate Extract	Chloroform Extract	Methanolic Extract	Aqueous Extract
Alkaloids	-	+	+	+	-
Carbohydrate	-	+	+	+	+
Flavonoids	+	-	+	+	-
Glycosides	+	-	+	+	+
Phenolic Compounds	-	+	+	+	-
Saponins	-	-	-	+	+
Steroids	-	-	-	+	+
Sterols	+	-	+	+	+
Tannins	-	-	-	+	+
Gums	-	-	-	-	+

Preliminary Phytochemical Analysis

The various extracts were subjected to preliminary phytoconstituents analysis for their presence or absence of the constituents. The results are shown in Table 6.

DISCUSSION

Lack of standardization techniques fails to identify the drug from its originalitywhich thereby exploits the usage of drug from its Traditional System of Medicine. The plant *Abutlion indicum* is used from the ancient time for its great medicinal values as a remedy in day to day life but in this aspect adulterations are also done which leads to its extinct. Thus a perfect protocol was designed for its authentication and identification on the basis of microscopy and chemical analysis. Thus the present investigation was aimed and the results were found to be significant and encouraging towards the goal for standardization.

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