Pharmacognostic and phytochemical analysis of *Acacia nilotica* (Lam.) Willd. ex Del. (Mimosaceae)

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ABSTRACT

The present study deals with pharmacognostic and phytochemical analysis of *Acacia nilotica* (Lam.) Willd. ex Del. of the family Mimosaceae. The various parts of this plant like leaves, stem bark, root and gum are used by the tribal people in traditional medicine for curing the different diseases such as diarrhoea, dysentery, diabetes, acidity, etc. The evaluation of quality and purity of crude drugs by means of various parameters is the most important aspect of pharmacognosy. The parameters like micromorphological, anatomical, physical constant and fluorescence analysis have been employed for the pharmacognostical evaluation of different parts of this plant. Microchemical and histochemical parameters are used for phytochemical screening. Leaves are amphistomatic and stomata are diacytic with few anaisocytic types. Stomatal index is 29.79 on the upper surface and 30.08 on the lower surface. Palisade ratio is 2.16. Non-glandular, unicellular with pointed apex trichomes are present on both surfaces. The T.S. of petiole is horse-shoe shaped in outline and 3 vascular bundles are present of which one larger, centrally located and two smaller vascular bundles are present in the periphery. Methanolic extracts of leaf indicate presence of tannins, proteins, flavonoids, steroids, etc. Ash value and moisture content of the leaves were found to be 09.17% and 67.09 % respectively. This study will provide some diagnostic features by which the crude drug of this plant can easily be identified.

Keywords: *Acacia nilotica* (Lam.) Willd. ex Del., Pharmacognostic and phytochemical studies.

I. Introduction

Plants have been playing a vital role in curing the diseases and ailments of human being from the time immemorial. Herbal medicines are gradually being popular for primary health care of the human's world-wide because of their efficacy, easy availability, no or very negligible side effect and low price. Approximately 2,400–3,000 medicinal plants species are in use in different Indian systems of medicine including Ayurveda and many of those plants are constantly being screened for their biological activity (Bhukani, 1985). Many of the important medicinal plants in India have pharmacognostically characterized and they have been enumerated in standard literature on pharmacognosy (Mitra, 1985). In this context, the present study has been undertaken to analysis this ethnomedicinal plant pharmacognostically and phytochemically. The leaves of the investigated plant have been considered here in this investigation because leaves are commonly used by the tribal and common people for curing the diseases. Use of micromorphology and anatomy is now a recognized tool in the field of plant systematic (Rahaman *et al.*, 2008, 2009). Importance of epidermal characters in general and those of trichomes in particular and comparative wood anatomy are widely employed in taxonomic consideration of angiosperms (Cutler, 1984; Ogundipe and Olatunji, 1991; Parveen *et al.*, 2000; Banerjee *et al.*, 2002). Ontogeny and structure of stomata are now also considered as an important taxonomic character for many of the angiospermic taxa (Inamdar, 1970; Kothari and Shah, 1975; Rajagopal, 1979). Different members of the family Mimosaceae have been studied anatomically by the previous workers with special emphasis on stem and leaf epidermal micromorphology (Metcalfe and Chalk, 1950; 1979). Chemical analysis and biological assays are very important aspects in pharmacognostic evaluation of medicinal plants (Trease and Evans, 1978; 1985; Evans, 2008). Therefore, in this investigation the foliar micromorphology, stem xylem elements, phytochemical screening and physical evaluation of this medicinally important taxon have been carried out. This investigation will provide some useful markers for identification of the crude drug obtained from the investigated plant.

II. Materials and methods

Plant Material: *Acacia nilotica* (Lam.) Willd. ex Del. (Mimosaceae)
Common English name: Indian gum Arabic tree, Black babon.
Local/Tribal name: Babla, Kikar, Gubul-daru, Babul (Bengali).
Botanical characteristics: Small branched tree; branches with straight axillary stipular spines; leaves bipinnate, paripinnate, leaflets 1-25 pairs, entire, glabrous; flowers yellow, numerous in globose heads, corolla tubular with subtriangular teeth, stamens numerous; pod stalked, compressed, constricted at suture, moniliform; seeds 8-12.
Parts used: Gum and leaf
Medicinal uses: Gum used to treat diarrhoea, dysentery, diabetes; leaves treated with warm water cures dysentery and acidity.
Chemical constituents: Bark and pod yield tannins (20% and 12.19%), galloylated and tetrahydroxyflavan-3, 4-diols; seeds also contain tannin as the major component apart from amino acids and ascorbic acid; flowers give kempferol-3-glucoside, isoquercetin, leucocyanidin; gum yields aldobiouronic acids and some hexoses.

For the study of foliar epidermis, leaf samples were cleared following the Bokhari's method (1970). The cleared leaf samples were then mounted on the slide with a drop of 10% glycerine and 1% aqueous safranin solution and observed under compound light microscope. For wood elements study, the stem pieces were macerated following the standard method (Johansen, 1940); washed several times, teased with needles, stained in safranin, mounted on the slides with 10% glycerine and observed under microscope. The drawings of the foliar micromorphological characters and stem xylem elements were made with the help of camera lucida and measurements were taken with standardized ocular micrometer in each case. Finally, the leaf powder was extracted (Soxhlet extraction) with 90% methanol and these extracts were used for different chemical colour reaction tests for identification of different phytochemical groups. Physical constants and the UV fluorescence nature of the powder were studied following the standard methods (Trease and Evans, 1985).

III. Results and Discussion

Micromorphology

General description and measurement of the epidermal cells, stomata, trichomes and crystals are given below.
Epidermis: Cells are irregular in shape and cell walls are wavy. Epidermal cell size of the upper surface is 32.76 µm x12.01µm and on the lower surface the size is 36.80 µm x 11.20 µm. Frequency is 664.46 /mm^2 on the upper surface and 609.02 /mm^2 on the lower surface. Palisade ratio is 2.16 **(Table- 1; Fig. -I: A).**

Stomatal Complex: Leaves are amphistomatic, stomata mostly diacytic with few anaisocytic types. Stomatal size is 21.02 µm x 11.41 µm on the upper surface and 21.11 µm x 10.55 µm on the lower surface. Stomatal frequency is 165.60 /mm^2 on the upper surface and 259.49 /mm^2 on the lower surface. Stomatal index is 29.79 on the upper surface and 30.08 on the lower surface **(Table- 2; Fig.-I; A).**

Trichomes: Non-glandular, unicellular with pointed apex are present on both surfaces. Size of the trichomes are 114.83 µm x 12.60 µm on the upper surface and 106.28 µm x 11.28 µm on the lower surface. Frequency of the trichome is 16.70 /mm^2 on the upper surface and 14.74 /mm^2 on the lower surface. Trichome index is 2.08 on the upper surface and 1.92 on the lower surface **(Table- 3; Fig. -I: B).**

Wood elements

General description and measurement of the type and size, pits, perforation plates of vessel elements, side wall thickening of tracheids, fibres size and nature, etc. of the investigated plant have been represented in **Table- 4**.

Perforation plates are simple and obliquely placed. Pits are bordered. Tails are present in the vessel elements. Size of the vessel element is 38.94 µm x 15.65 µm. Frequency is 39.31 /mm^2 (**Fig.- II**: **A**). Tracheids

are very long with spiral sidewall thickening. Diameter and frequency are 16.01 µm and 39.31 /mm^2, respectively **(Fig.- II: C).** Fibres are long, typically libriform type; ends narrow, pointed or sometimes blunted. Pits and septa are absent. Fibre length is 251.88 µm and diameter is 15.65 µm and frequency is 46.19 /mm^2 **(Fig.- II: B).**

Stem anatomy

The T.S. of a stem is more or less circular in outline **(Fig.- III: a)**. Cuticularized epidermis, multicellular, uniseriate trichomes emerged from the epidermis. The cortex consisting of 1- layer, thick walled collenchymatous cells, lying below the epidermis. 3-4 layers of parenchyma cells are present beneath the collenchymatous layer. A band of sclerenchymatous zone is present. Vascular bundles are collateral, conjoint and open type. They forming a continuous cylinder of vascular tissue i.e. xylem and phloem. The cambium is indistinct. Pith consists of thin walled, isodiametric parenchyma cells with profuse intercellular spaces which constitute the central portion of the stem.

Petiole anatomy

The transverse section of the petiole is horse shoe shaped in outline **(Fig.- IV: a)**. Epidermis consisting of single layered, compactly arranged, barrel-shaped cells. Cuticle is present on the outer side of the epidermal layer. The cortex consists of thin walled parenchymatous cells of 4-6 layers. A distinct sclerenchymatous zone is present bellow the cortex forming a continuous ring. 3 vascular bundles are present of which one larger, centrally located and two smaller vascular bundles are present in the periphery. Each bundle consists of xylem and phloem. Pith consists of parencymatous cells.

Organoleptic features of the crude drug

Colour: Greenish-black**; Odour:** Characteristic; **Taste:** Acrid**; Texture:** Fibrous in fresh form.

Microchemical evaluation of the powdered drug

Through the microchemical colour reaction tests of the methanolic extract of plant, the important phytochemical groups like steroids, reducing sugars, proteins, gums, tannins, flavonoids, etc. have been detected **(Table- 5).**

Histochemical study

Histochemical study has been detected various phytochemicals localized in different tissue zones of the stem. Different histochemical localizations have been identified in the stem which contains some specific phytochemical groups like- lignins, gum, proteins, alkaloids and tannins **(Table- 6).**

Physical evaluation

a) Total ash - 9.17 %
b) Water soluble ash- 02.24 %
c) Acid insoluble ash- 3.70 %
d) Moisture content- 67.09 % (in fresh form).

Fluorescence analysis

Here in this study it is observed that drug powder treated with different chemical reagents gives characteristic colourations when seen under UV light and it is compared with the colourations observed under ordinary light. In some cases there are marked differences in colour **(Table- 7).**

The present study reveals that foliar epidermal features stem xylem element characters, primary phytochemical screenings and physical evaluation are of some importance in identification of this investigated plant species in its fresh as well as dried form. Some of the general anatomical characters of the investigated plant conformed to the features identified in the other members of the family Mimosaceae earlier by different workers (Metcalfe and Chalk, 1950; 1979). Studies in stomata can have a great taxonomic as well as pharmacognostic value in proper identification of different plant taxa including medicinal plants (Inamdar, 1970; Kothari and Shah, 1975). Trichome features are also very important in proper identification of the plants and considered as one of the valuable taxonomic markers now (Leelavathi and Ramayya, 1983). Vessel

elements of root of the investigated plant show a characteristic feature in respect of their size. The arrangement and number of vascular bundles in the petiole may sometimes provide the diagnostic feature for identification of the plant species. Here in the petiole, 3 vascular bundles are present which can be used as a marker for identification of this species of Mimosaceae. Chemical analysis and biological assays are considered as important aspects in pharmacognostic evaluation of the medicinal plants (Trease and Evans, 1985; Harbone and Williams, 1994). The physical constants like ash value (9.17 %), moisture content (67.09 %) and UV fluorescence characters of the powder drug of this plant can also be used as important characters for proper identification of crude drug of it. Finally some diagnostic features have been given below which can be employed for easy identification of *Acacia nilotica* (Lam.) Willd. ex Del. in its fresh as well as dried form.

Table: 1. Foliar epidermal cell characters

Leaf surface	Cell shape	Cell length (µm)	Cell width (µm)	Cell frequency (No./mm^2)	Cell wall outline	Palisade ratio
Upper	Irregular	32.76	12.01	664.46	Wavy	2.16
Lower	Irregular	36.80	11.20	609.02	Wavy	

Table: 2. Stomatal features

Leaf surface	Stomatal type	Stomatal length (µm)	Stomatal width (µm)	Stomatal frequency (No./mm^2)	Stomatal Index (%)
Upper	Diacytic with few anaisocytic	21.02	11.41	165.60	29.79
Lower	Diacytic with few anaisocytic	21.11	10.55	259.49	30.08

Table: 3. Trichome features

Leaf surface	Types	Trichome length (µm)	Trichome width (µm)	Trichome frequency (No./mm^2)	Trichome Index(%)
Upper	Non-glandular, unicellular with pointed apex	114.83	12.60	16.70	2.08
Lower	Non-glandular, unicellular with pointed apex	106.28	11.28	14.74	1.92

Table: 4. Wood elements characters

Structure	Type	Measurement
Vessel elements	Perforation plate	Simple
	Arrangement	Oblique
	Pits	Bordered
	Tail	Sometimes short tail present
	Length (µm)	38.94
	Breadth (µm)	15.65
	Frequency (No./mm^2)	39.31

Tracheids	Wall thickening	Spiral
	Length (µm)	Very long
	Diameter (µm)	16.01
	Frequency (No./mm^2)	39.31
Fibres	End	Pointed, sometimes blunt
	Pits	Absent
	Septa	Absent
	Length (µm)	251.88
	Breadth (µm)	15.65
	Frequency (No./mm^2)	46.19

Table -5. Microchemical tests of the plant extract

Tests/ Reagents	Tests for	Nature of changes	Degree of changes
Dragendroff's reagent	Alkaloids	Orange brown ppt.	++
Wagner's reagent	Alkaloids	Orange brown ppt.	+++
Mayer's reagent	Alkaloids	Crème colour	++
Millon's reagent	Proteins	Yellow to brown	++
Benedict's test	Reducing sugars	Brick red ppt.	-
Fehling's test	Reducing sugars	Brick red ppt.	-
10% aq. $K_2Cr_2O_7$ Solution	Tannins	Yellowish-brown ppt.	+++
10% aq. Lead acetate solution	Tannins	Yellow ppt.	+
5% aq. Ferric Chloride solution	Tannins	Greenish - black	+++
Lugol's reagents	Proteins	Yellowish- brown	++
Kedde reagent	Glycosides	Violet- blue	-
Molish's test	Gum	Red- violet ring	+
1% Lead acetate solution	Saponins	White ppt.	+

- = Absent; + = Present

Table-6. Histochemical localization test

Reagents	Colour change	Tissue zones	Compounds detected
Dragendroff's reagent	Orange- brown	Phloem zone, some cortical cells	Alkaloids
5% $FeCl_3$	Greenish- black	Pith zone	Tannins
Lugol's reagent	Yellowish- brown	Phloem zone, middle cortex zone	Protein
Phloroglucinol + HCl	Reddish brown to rose red	Pith, sclerenchyma patch, some phloem cells	Lignin
Wagner's reagent	Orange- brown	Phloem zone, some cortical cells	Alkaloids
Millon's reagent	Yellow to brown	Vascular bundle	Protein
Molish's test	Red-violet	Few cells of pith	Gum

Table -7: UV- Fluorescence nature of the powdered drug

Materials and treatment	In UV light	In ordinary light
Powder as such	Greenish-brown	Blackish-green
Treated with dilute nitric acid	Brownish-green	Orange-brown
Treated with dilute sodium hydroxide	Greenish-brown	Brownish black
Treated with dilute hydrochloric acid	Greenish-brown	Greenish-black
Treated with dilute sulphuric acid	Brownish-green	Black
Treated with antimony trichloride	Deep green	Deep brown
Treated with concentrate methanol	Light green	Greenish-black
Treated with concentrate ethanol	Light green	Greenish-black
Treated with concentrate acetone	Deep green	Brownish-green

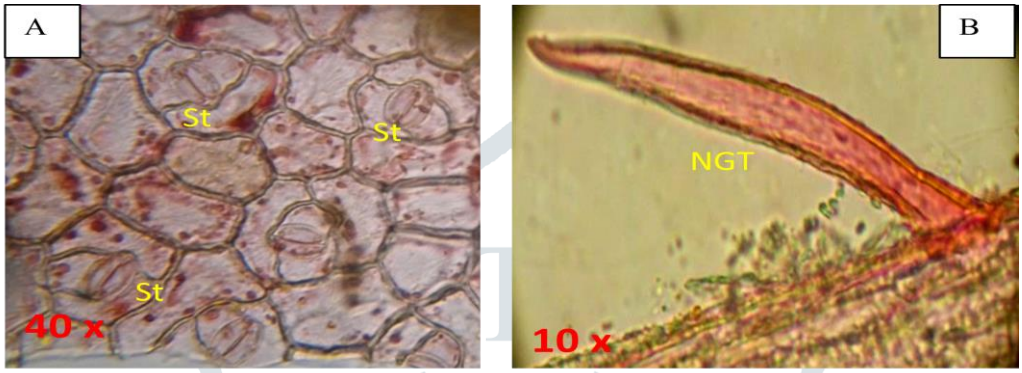

Fig. I: **Epidermal micromorphology: A -** Epidermal cells with stomata**; B –** Non-glandular, unicellular trichome**;** [St – Stomata; NGT – Nonglandular trichome]

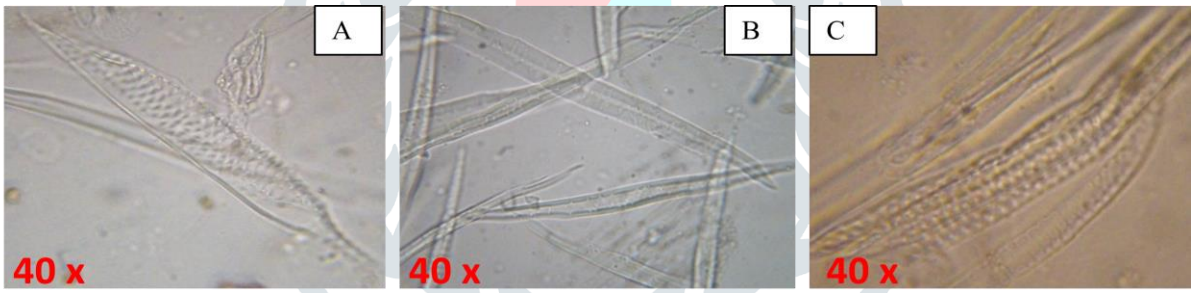

Fig. II: **Wood elements: A -**Vessel elements; **B –** Fibres (Portion); **C** - A portion of tracheid

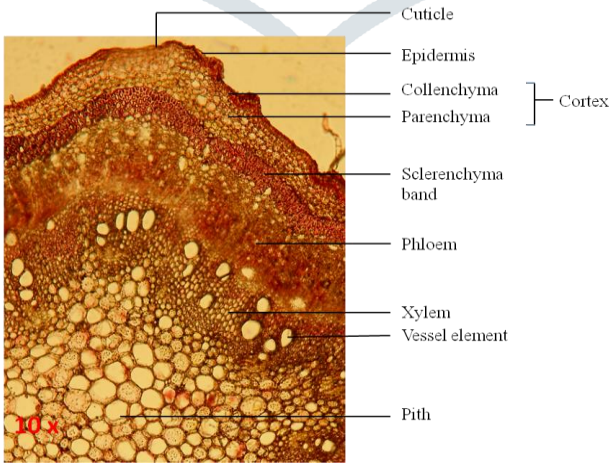

Fig. III: **a- T. S. of Stem** (Snap taken by Light Microscope, ZEISS, AXIOSTAR plus, 176045)

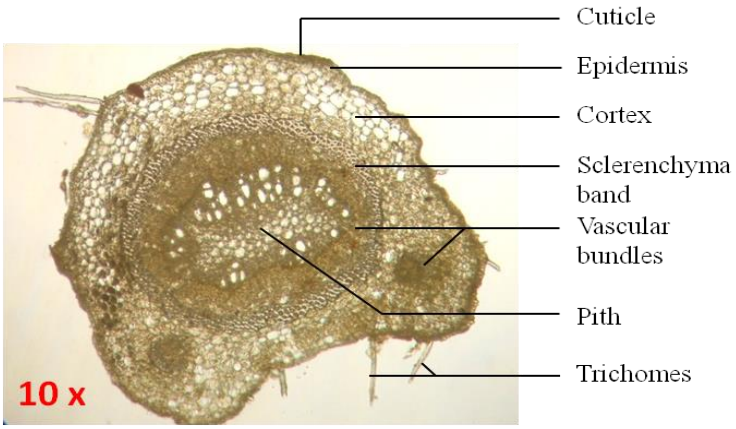

Fig. IV: **a- T. S. of Petiole** (Snap taken by Light Microscope, ZEISS, AXIOSTAR plus, 176045)

IV. Diagnostic features

- Mostly diacytic with few anaisocytic types of stomata are present.
- Stomatal indices of upper and lower surfaces are 29.79 and 30.08 respectively.
- Strictly non-glandular, unicellular trichomes with pointed apex are present on both surfaces.
- In petiole, number of vascular bundles is 3 of which one larger, centrally located and two smaller vascular bundles are present in the periphery.
- Ash Value-total ash- 4.17 %

V. Conclusions

The present work was undertaken with an aim of pharmacognostic and phytochemical investigation of *Acacia nilotica* (Lam.) Willd. ex Del. of the Mimosaceae providing useful information, which could be useful to detect the authenticity of this medicinal plant. Pharmacognostic evaluation can be useful to substantiate and authenticate the drug.

VI. Acknowledgement

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