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PHYSICO-CHEMICAL STUDY AND BIOCHEMICAL INVESTIGATION OF BABOOL (*ACACIA NILOTICA* L.) STEM BARK

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ABSTRACT

Acacia nilotica Lam. Family Mimosaceae is known as known as Babool in Hindi. It is a moderate sized tree and found in tropical and subtropical countries. All parts of this plant are used for the treatment of different types of human and animal diseases. The present communication provides a detailed account of the physico-chemical and biochemical study carried out on Babool stem bark. The study includes macroscopy, powder microscopic studies, preliminary phytochemical investigation, physicochemical tests and development of HPTLC (High Performance Thin Layer Chromatography) fingerprints profile. Physicochemical parameters were performed in triplicate and found average values of such as foreign matter 2.63%, loss on drying at 105^0C 4.48%, alcohol soluble extractive 36.18%, water soluble extractive 26.69%, total ash 12.01%, acid in-soluble ash 4.62%. HPTLC (High Performance Thin Layer Chromatography) fingerprints profile of methanolic extract was done by using mobile phase toluene: ethyl acetate (7:3). TLC plate was derivatized by using 5% Methanolic-sulphuric acid derivatizing reagent. Major spots Rf values and colour were recorded at 366nm, after derivatization 366nm and UV light. Florescence studied and biochemical analysis was also done. Various types of phyto-constituents are present such as alkaloids, protein, tannin, resin, saponin and glycosides. Established parameters can be used as standards for quality control and identification of the plant in herbal compound formulations and also preparation of a monograph of the plant.

Keywords: *Acacia nilotica,* Phytochemical, Bio-chemical investigation, Florescence, HPTLC fingerprinting,

INTRODUCTION

Babool Acacia nilotica (L.) family Mimosaceae is known as multipurpose medicinal plant due to is high medicinal values in Indian medical system. It is a moderate sized about 6-22 miter high, branched straight tree. Babool plant is found in tropical and subtropical countries such as Africa, Asia, America and Australia. Stem spherical, bark fissured, outer bark colour black, inner colour is grey-pinkish, and exude a high potential reddish colour gum. Leaves are green, bipinnate, with 2 to 6 pairs, Plant has 4 to 6 cm grey colour long, straight light weight spines in pairs. Flowers are globulous heads in bright yellow or golden colour. Pods are thick, hairy, white-grey colour and strong (Baravker *et. al.*, 2008, Shittu 2010 & Kaur *et. al.*, 2005).

Babool plant various parts like stem bark, root, flowers and seeds are used to treat different types of human diseases such as skin , small pox, teeth ach, dysentery, seminal weakness, bleeding piles, diarrhea, and leprosy (Del, 2009 & Singh *et. al.,* 2009b) and preparation of Ayurvedic compound formulations. Roots are used to treatment of tumor, cancer, liver, spleen (Asres *et.al.,* 2005), gum has working as antipyretic and liver tonic agents, leaves are used to treat diarrhea, and ulcer diseases, similarly seeds are used for treating the arthritis, diabetes (El-Tahir *et. al.,*1999).Whole plants are used as a ethno-medicines and preparation of several Ayurvedic drugs.

Despite the multipurpose medicinal uses attributed to this plant, there are no systematic pharmacognostical studies on the stem bark of this plant have so far been carried out. Hence the present work deals with the morphological, physicochemical tests, preliminary phytochemical screening, florescence study, and High-Performance Thin Layer Chromatography.

MATERIALS AND METHODS

Collection of samples

The fresh plant stem bark of Babool was collected from Rajoula village of Chitrakoot, Satna, Madhya Pradesh in the month of March. The plant was identified and authenticated by Dr. Manoj Tripathi, Senior Research Officer, Deendayal Research Institute Chitrakoot, Satna (M.P.). The voucher specimen (AD/AS/453/2023) prepared as per standard procedure (Anonymous, 1989 & 2017) and maintained in the herbarium of Arogyadham, Deendayal Research Institute, Chitrakoot, Satna (M.P.) for further reference. Fresh material was used for anatomical studies whereas shade dried material was powdered in electric grinder for physico-chemical study, phytochemical investigation and development of High Performance Thin Layer Chromatography fingerprint profile.

Macroscopic study

Macroscopic or organoleptic characters of Babool stem bark like appearance, colour, odour and taste were evaluated (Mukharjee PK, 2002).

Microscopic study

The dried stem bark was powdered and completely passed through 355 μm IS Sieve (old sieve number 44). About 2 gm of stem bark washed with potable water without loss of material. Placed a small quantity of the washed powder in a glass slides and mounted in glycerin were used to all characters of the Babool stem bark, small quantity of sample cleared by heating with chloral hydrate solution, wash and mounted in glycerin, another small quantity of sample stained with sudan red solution and mounted with glycerin, all mounted slide were seen under microscope at 40 x 10x magnification of the Trinocular Research Microscope (Sholapur Hasan Pasha N & Patil Basanagouda M, 2013 & Evans WC, Trease 2003).

Physico-chemical tests

Physico-chemical parameters such as moisture content (loss on drying at 105^0C), water soluble extractive value, Hexane soluble extractive; alcohol soluble extractive value, total ash value, acid insoluble ash value was calculated (Anonymous, 2010 & Kokate C K, 2006).

Preliminary phyto-chemical investigation

Preliminary phyto-chemical tests were carried out on ethanolic and water extract for the presence\absence of phyto-constituents like alkaloids, flavonoids, tannins, resins, carbohydrates, proteins and saponins (Mitra R and Mehrotra S, 1980, Tripathi M and Sikarwar R L S, 2014, 2015).

Florescence study

Fluorescence study was carried out of studies samples power separately in various mounts through prescribed standard methods (Choudhary *et. al.*, 2014 & Tiwari *et. al*., 2015).

High Performance Thin Layer Chromatography (HPTLC) fingerprint profile

For High performance thin layer chromatography, the powdered 5 gm of the sample was extracted with 100 ml of ethanol overnight, filtered and concentrated. It was applied by spotting extracted sample on pre-coated silica-gel aluminium plate 60 F_{254} (5x10 cm with 0.2 mm layer thickness Merk Germany) using Camag Linomat -5 sample applicator and a 100 µl Hamilton syringe. The sample, in the form of bands of length 6 mm, were spotted 15 mm from the bottom, 15 mm from left margin the plate and 10 mm part. Plates was developed using mobile phase consisting of toluene*: Toluene: ethyl acetate* (7: 3 v/v). Linear ascending development was carried out in 10x10cm twin through glass chamber equilibrated with mobile phase. The optimized chamber saturation time for mobile phase was 30 min. at room temperature. The length of chromatogram run was 8 cm. 20 ml of the mobile phase. Subsequent to the development, Thin Layer Chromatography plate was dried with the help of Hot Air Oven. The peak area for samples and standard were recorded with Camera photo documentation system Camag Reprostar 3. Visualization of spot was made before and after derivatization (with 5% Methanolic - sulphuric acid reagent) at UV light with Win cat software and R_f values noted (Venugopal *et. al*, 2015, Tripathi *et. al*., 2015 & Anonymous 2007).

RESULT & DISCUSSION

Macroscopic characters

Babool Stem spherical, bark fissured, outer bark colour black, inner colour is grey-pinkish texture- fibrous, taste slightly bitter and odour characteristics. Images are given in (Fig.1a &1b).

Powder microscopic characters

Babool stem bark powder colour is brownish red, taste slightly bitter and odour characteristics. Under microscope powder shows cork cells in surface view, cork cells in sectional view, parenchymatous cells, group of stone cells, tangential-longitudinal section showing medullary rays, cortical parenchyma, prismatic crystals of calcium oxalate, fibres, and reddish brown contents, structure are given (Fig.2)

Florescence study

Fluorescence study was carried out of studies sample power separately in various mounts and colour was observed at UV light, 254nm and 366nm. Fluorescence study results are given in (Table -1).

Physico-chemical analysis

The physico-chemical tests like extractive values are applied for the determination of adulteration in the drug. Ash values of the drug gave an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. Physicochemical parameters were performed in triplicate and found average values of such as foreign matter 2.63%, loss on drying at 105^0C 4.48%, alcohol soluble extractive 36.18%, water soluble extractive 26.69%, total ash 12.01%, acid in-soluble ash 4.62%.

Preliminary phyto-chemical investigation

Qualitative phyto-constituents were screened in the extracts taken in water and ethyl alcohol. The screening exhibited presence of alkaloids, protein, tannin, resin, saponin and glycosides.

HPTLC finger print profile

High performance thin layer chromatography (HPTLC) study of the ethanolic extract three spots of the Babool stem bark samples extract applied in precoated TLC plate. Applied 6 µl of the test solution as 8 mm bands and develop the plate in a solvent system toluene*: ethyl acetate* (7: 3 v/v) to a distance of 8 cm. Dry the developed plate in room temperature and examined. Derivatized the plate using 5% *Methanolic-sulphuric acid* reagent and heating at 105^0C till the bands are clearly visible. Major spots R_f values with colour were recorded before derivatization at 366nm, after derivatization at 366nm and at UV light. Chromatogram profile and R_f values are given (Fig. 3 & Table 2).

The macroscopic, microscopic and powder microscopic distinguished characters have been established to identify Babool stem bark. The pharmacognostic and physicochemical parameters can be used for checking the adulteration and purity of this drug. HPTLC finger print profile helps in identification of various phytochemical constituents present in the crude drug thereby substantiating and authenticating of crude drug. The TLC profile also helps to identify and isolate's important phyto-constituents. All findings are indicating samples are genuine and free from any adulterations. These finding could be helpful in identification and authentication of Babool stem bark.

CONCLUSION

Due to the harmful effects of modern medicines on human health, the importance and uses of herbal drugs are increasing day to day. Because the plants have natural chemicals which do not have any adverse side effects on human health. But herbal drugs are suffering due to lack standardized data. Hence the evaluation of biochemical and phytochemical of Babool stem bark is necessary. Babool plant various parts like stem bark, root, flowers and seeds are used to treat different types of human diseases such as skin, small pox, teeth ach, dysentery, seminal weakness, bleeding piles, diarrhea, leprosy, roots are used to treatment of tumor, cancer, liver, spleen, gum has working as antipyretic and liver tonic agents, leaves are used to treat diarrhea, and ulcer diseases, similarly seeds are used for treating the arthritis, diabetes and preparation of Ayurvedic compound formulations. Whole plants are used as ethno-medicines and preparation of several Ayurvedic drugs.

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Table-1: Florescence study of Babool stem bark powder

S. No.	Drug powder+ Chemical	Observation in UV light	Observation in 254nm	Observation in 366nm
1	Powder	Brown	Green	Dark green
2	Drug powder + Distilled water	Light yellow	Green	Brown
3	Drug powder + Methanol	Yellow	Green	Brown
4	Powder + Acetic acid	Dark black	Brownish white	Blackish brown
5	Powder + 50% KOH	Dark brown	Green	Black
6	Powder + 1N HCL	Yellow	Green	Brown
7	Powder + 1N NaOH water	Pale yellow	Greenish yellow	Pale yellow
8	Powder + H2SO4	Dark brown	Dark green	Dark brown
9	Powder + Iodine water	Light green	Green	Black
10	Powder + 1N NaOH methyl	Dark brown	Dark green	Black
11	Powder + 50%H2SO4	Light green	Green	Dark green
12	Powder + 50%HNO3	Brownish black	Dark brown	Dark yellow

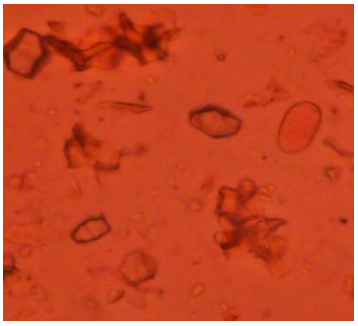
Table-2: Rf values of HPTLC Fingerprints profile of Babool stem bark

R_f value	Before derivatization	After derivatization	
	366nm	**366nm**	**UV light**
$R_{f\,1}$	0.06 (red)	0.06 (brown)	0.06 (brown)
$R_{f\,2}$	0.12 (pink)	10. (sky blue)	0.70 (sky blue)
$R_{f\,3}$	0.20 (blue)	0.22 (sky blue)	0. 80 (brown)
$R_{f\,4}$	0.28 (blue)	0.28 (blue)	-
$R_{f\,5}$	0.40 (pink)	0.40 (brown)	-
$R_{f\,6}$	0.50(pink)	0.50 (brown)	-
$R_{f\,7}$	0.60 (pink)	0.56 (brown)	-
$R_{f\,8}$	0.70 (pink)	0.58 (blue)	-
$R_{f\,9}$	0.78 (blue)	0.60 (brown)	-
$R_{f\,10}$	0.80 (red)	0.70 (white)	-
$R_{f\,11}$	0.90 (red)	0.90 (brown)	-

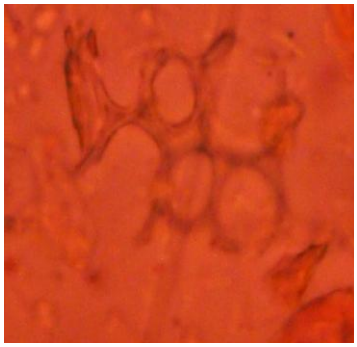
Fig.1a: Babool plant

Fig.1b: Babool stem bark

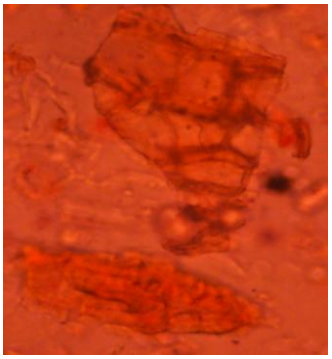
Fig.-2 : Powder microscopic characters of Babool stem bark



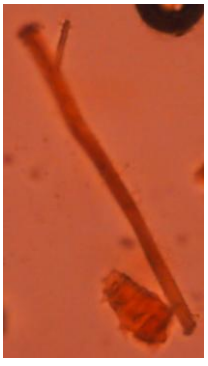
Prismatic crystals of calcium oxalate



Cortical parenchyma

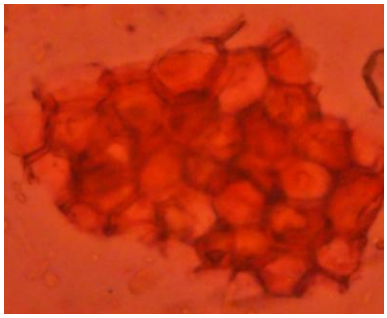


Group of stone cells

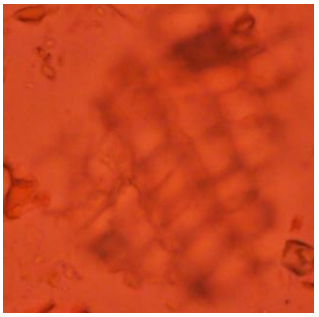


Reddish contents

Reddish contents



Cork cells in surface view



Cork cells in sectional view

Fig.-3: HPTLC Fingerprints profile of Babool stem bark

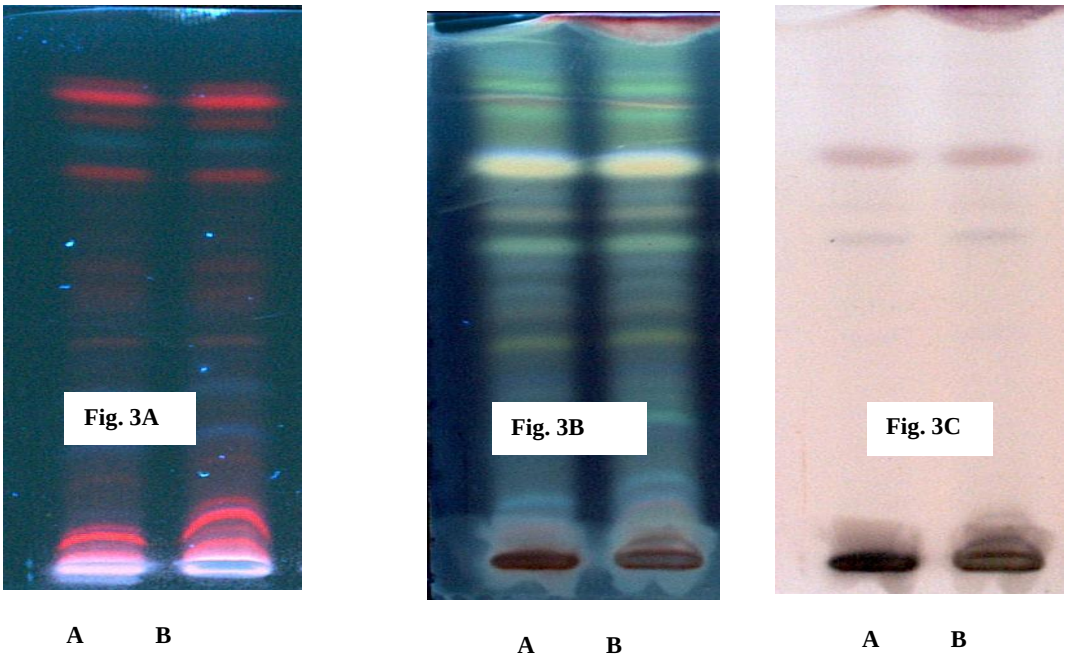


Fig. 3- HPTLC fingerprints profile of Babool stem bark, where **Fig. 3A** at 366nm before derivatization; **Fig. 3B** at 366nm after derivatization; **Fig. 3C** at UV light after derivatization. Where Tracks A =Sample Babool stem bark , track B = Babool stem bark