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Pharmacognostic Studies on the stem bark of Nimba (*Azadirachta indica*)

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Abstract

Azadirachta indica A. Juss of family Meliaceae, is an important plant employed in various indigenous systems of medicine against several diseases and almost every part of the plant such as stem bark, leaf, flower, fruit and seed has diverse medicinal properties. The current communication provides a detailed account of the pharmacognostical investigation carried out on *Azadirachta indica* A. Juss. The study includes macro and micro morphological characters including powder characters, physicochemical studies, HPTLC fingerprinting, microbiological studies and preliminary phyto-chemical aspects. The results of the study could be useful for the identification and preparation of a monograph of the plant.

Key-Words: Microbiology, Pharmacognosy, HPTLC fingerprinting, Phyto-chemical analysis, *Azadirachta indica*

Introduction

Azadirachta indica A. Juss of family Meliaceae is none as Nimba and occurs throughout the greater part of India and commonly cultivated in gardens, road sides and by the side of irrigation wells as a shade tree. Nimba is a moderate sized to fairly large, evergreen tree with large spreading branches and stout trunk. The trunk and older branches are covered with moderately thick, dark brown, rough, longitudinally and obliquely furrowed bark with exfoliating woody rind. The plant flowering during the month of February to May and fruiting are July to Aug[1].

The stem bark is traditionally, used in different ailments and diseases such antiperiodic, insecticidal, liver tonic, urinary astringent, anthelmintic, pectoral and tonic. It is useful in hyperdipsia, skin diseases, leprosy, eczema, malarial fever, wounds, ulcers, tumour, intestinal worms, cough, bronchitis, diabetes and inflammation[2].

In view of its diverse medicinal applications and in odour to ensure the quality of its supply, especially at a time in which adulteration on the crude drug markets of India, the present communication deals with a detailed pharmacognostical evaluation of the Nimba stem bark.

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Material and Methods

Plant material

The fresh plant stem bark of *Azadirachta indica* was collected from the Bagdara Ghati, Chitrakoot forest of Satna district in the month of October. Voucher specimens were collected and placed in the herbarium of Department of Pharmacognosy, Ayurveda Sadan Research Laboratory, Deendayal Research Institute Chitrakoot. Fresh material was used for anatomical studies whereas shade dried material was powdered in electric grinder for physico-chemical, phytochemical, microbiologial screening and HPTLC studies.

Macroscopy

Macroscopic (morphological and anatomical) or organoleptic characters like appearance, colour, odour and taste were evaluated.

Microscopy

Bark section were cut by free hand sectioning and numerous sections examined microscopically[3-7]. Photographs of the microscopical sections were captured with the help of Olympus trinocular research microscope CX- 21*I* with Dig eye camera using Caliper plus version 4.2 software.

Powder Characteristics

The dried bark was subjected to powdered and completely passes through 355 µm IS Sieve (old sieve number 44) and not less than 50% pass on through 180 µm IS Sieve (old sieve number 85). About 2 g of powder washed thoroughly with potable water, pour

out the water without loss of material. Mounted a small portion in Glycerine, warmed a few mg with chloral hydrate solution, wash and mounted in Glycerine, treat a few mg with iodine solution and mount in Glycerine, about 1 g of powder warmed over water bath with 50% con. Nitric acid till brown fumes appear, cool and wash with water thoroughly and mount a small portion in glycerin and seen under microscope at 40 X 10X magnification of the trinocular research microscope[8,9].

Physico-chemical parameters

Physico-chemical parameters such as moisture content (loss on drying at 105^0C), water soluble extractive value, alcohol soluble extractive value, total ash value and acid insoluble ash value[10] .

Preliminary phyto-chemical studies

Preliminary phyto-chemicak tests were carried out on Ethanolic and water extract for the presence\absence of phyto-constituents like alkaloids, flavanoids, tannins, resins, carbohydrates, proteins and saponins [11-12].

High Performance Thin Layer Chromatography (HPTLC)

For HPTLC, the powdered bark 5 gm of 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 samples, 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 were developed using mobile phase consisting of *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, TLC plates 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 254nm, 366nm and day light with Win cat software and R_f values noted[13].

Microbiological test

Mac-concy broth, XLD, PDA, Plate count agar, Citrimide agar and Staphylococcus aureus enriched agar media were used for the isolation of yeast & moulds, *E. coli, Salminella* sp, TBC, *Staphylococcus* sp. & *Pseudomonas* sp. To perform this test different proportion of different media were dissolved in different flasks containing distilled water. All the media were prepared & autoclaved at 15lb/in^2 pressure at 121^0c for 15-20 minutes. After autoclaving media were poured into Petri plates and allowed to solidify. Plates were prepared & marked for different isolates. Culture suspension was prepared in 1gm of test sample in 100 ml distilled water. 0.1 ml suspension was spreaded on the plates with the help of spreader. All plates were prepared, inoculated & incubated at 26-28 ^{0}c for 3-4 days. After incubation period, the observations were taken for presence or absence of the colonies. The concentration of different microorganisms and media used were given[14-16].

Results and Discussion

Morphological (Macroscopy)

The outer bark consists of a thick hard "woody" outer rind exfoliating in large pieces comparatively thin corky inner portion. And entire portion is fairly externally dark brown, thick with oblique furrows but the fresh bark is fairly thick with its peripheral region purplish red, middle region lustrous starchy white and inner part tangentially lamellated in transverse section and dry stem bark inner region easily separates into thin papery fibrous flakes. (**Fig.1**).

B. Colour, odour and taste

Bark varies much in thickeness according to age and parts of tree from where it is take, external surface rough, fissured and rusty grey, laminated inner surface yellowish and foliaceous, fracture, fibrous, odour characteristic, taste-bitter (**Fig.2**).

Microscopy

Diagrammatic TS of the stem bark shows outer rhytidoma dark reddish brown, very narrow band of phelloderm with tangentially running tannin cells and very wide phloem with discontinuous groups of crystal fibres alternating with medullary rays.

The transverse section of the stem bark showed cells of the dark brown coloured rhytidoma consisting of 12-15 rows of cork cells, and narrow band of stone cells. Narrow zone of phelloderm embedded with tangentially running bands of tannin cells and small groups of stone cells. Wide phloem consists of row of tangentially running groups of thin –walled fibres associated with idioblast containing prismatic crystals of calcium oxalate, alternating with vertical rows of uniseriate, biseriate and triseriate medullary rays running almost parallel to each other. A few large secretory cavities also occur in phloem. Most of phloem parenchyma contains starch grains and prismatic crystals of calcium oxalate; starch grains simple, round and measuring 2.5-6 µ**. (Fig.3&4)**.

Powder microscopy

The powder colour is reddish- brown, odour characteristic and taste-bitter. Under microscope examined powder showed Cork cells in sectional view and surface view, numerous prismatic crystals of calcium oxalate, tannin cells, phloem fibres with narrow lumen and pointed ends, stone cells mostly in groups, lignified rectangular to polygonal, having wide lumen and distinct striations, starch grains simple, rounded to oval measuring 2.5-6 µ, crystal fibres, sclereids associated with parenchyma containing prismatic crystals of calcium –oxalate, tangential-longitudinal cut medullary rays associated with idioblasts and phloem parenchyma and radially-longitudinally cut medullary rays crossing the fibres. **(Fig.5).**

Physico-chemical analysis

The physico-chemical parameters such as extractive values are useful for the determination of exhausted or adulterated 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. Physico-chemical results of the drug are given in (**Table-1).**

Preliminary phytochemical studies

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

HPTLC finger print profile

High performance thin layer chromatography (HPTLC) study of the methanolic extract two spots of the sample extracts applied in the TLC plate. Major spots R_f values with colour were recorded under 366nm, after derivatization 366nm and UV light. Chromatogram profile and R_f values are given **(Fig 6 & Table-2).**

Determination of microbial load

Microbial tests were carried out to determine the microbes in *Azadirachta indica* stem bark powder. The results are given in **(Fig. 7 & Table 3).**

The macroscopic, microscopic and powder microscopic diagnostic features have been established to identify Nimba stem bark. The pharmacognostic and physico-chemical parameters can be used for checking the adulteration and purity of this drug. HPTLC finger print profile helps in identification of various phyto-chemical constituents present in the crude drug thereby substantiating and authenticating of crude drug. The TLC profile also helps to identify and isolate important phyto-constituents. These finding could be helpful in identification and authentication.

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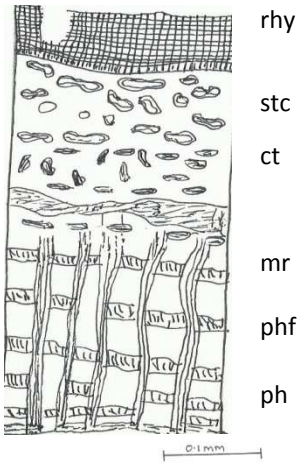

Fig.1: Nimba tree Fig. 2: Nimba dry stem bark Fig. 3: TS st. bk.(diagrammatic)

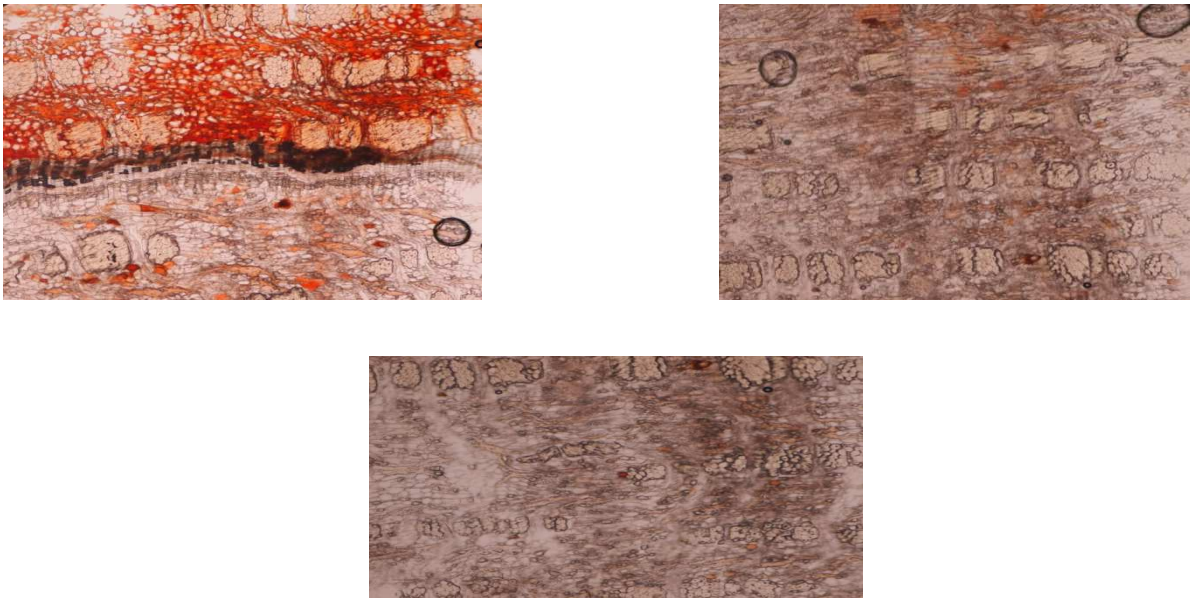

Fig. 4: TS Stem bark

Abbreviations: rhy, rhytidoma; crt, ceratenchyma; ck, cork; ct, cortex; stc, stone cells; ph, phloem; mr, medullary rays; lat, laticiferous tubes; rd, resin duct; sg, starch grains; prc, prismatic crystals of calcium oxalate; phf, phloem fibres.

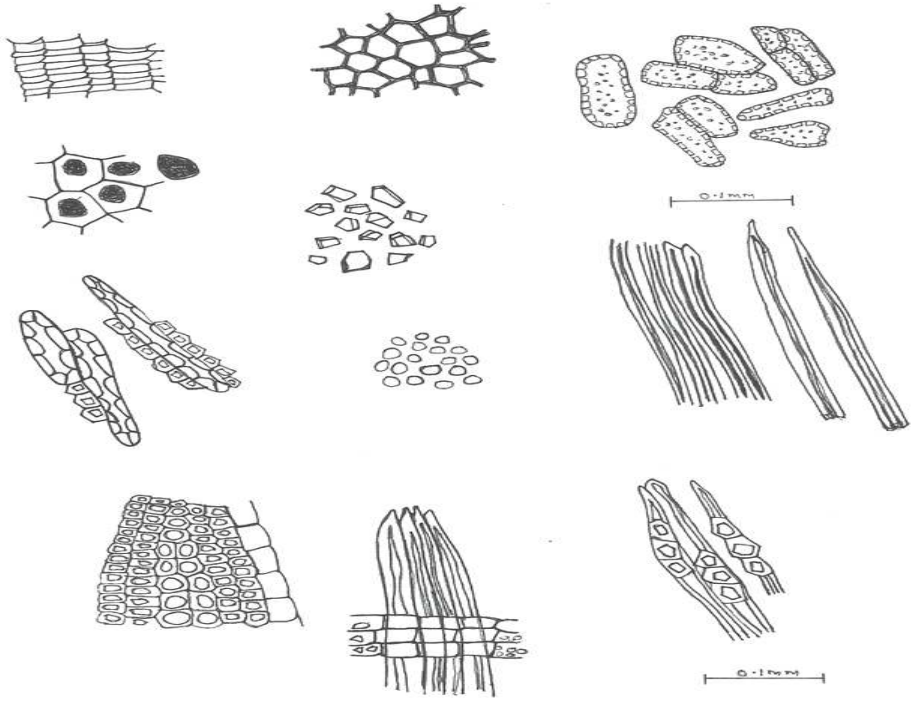

Fig.5. Powder microscopy

[Tripathi *et al.*, 5(2): Feb., 2014:3338-3344]
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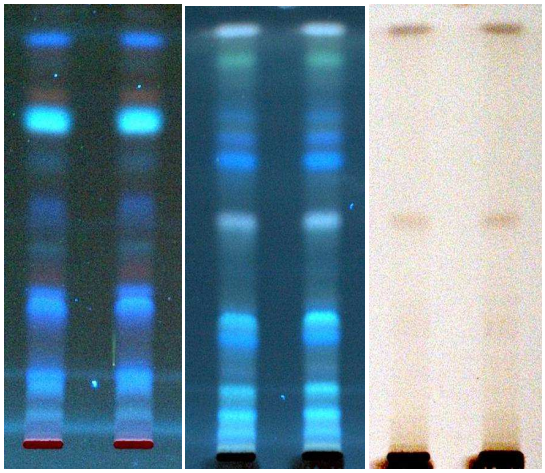

Fig. 6: HPTLC Finger Print Profile

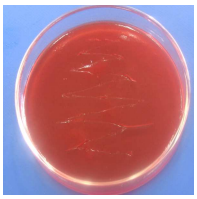

Fig.6.2. Showing negative results for *Pseudomonas aeruniosa*

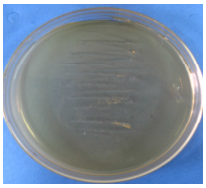

Fig.6.1. Showing negative results for *Staphylococcus aureus*

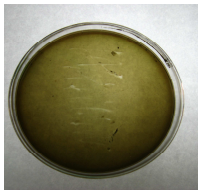

Fig.6.3: Showing negative results for *E.coli*

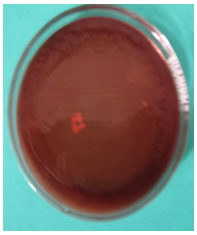

Fig.6.4: Showing negative results for *Salmonella*

Fig. 7: Microbiological limit test

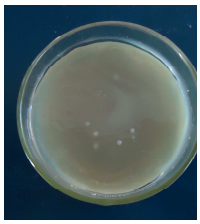

Fig.6.5. Showing TBC

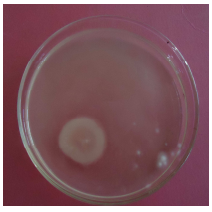

Fig.6.6. Showing Showing Yeast & Moulds

Table 1: Physico-chemical analysis theNimba stem bark

Parameter	Values
Loss on drying	5.8%
Ethanol-soluble extractive	12.5%
Water- soluble extractive	15.6%
Total ash	6.8%
Acid-insoluble ash	1.4%

Table 2: R_f Values in test solution of Nimba stem bark

R_f values	**stem bark test solution of *Azadirachta indica***		
	366nm(before derivatization)	**366nm (after derivatization)**	**UV light (after derivatization)**
R_f1	0.11(sky blue)	0.10(sky blue)	-
R_f2	0.18 (sky blue)	0.16 (sky blue)	-
R_f3)	0.36 (sky blue)	0.36 (florescent blue)	-
R_f4	0.40 (brownish red)	0. 50 (light brown)	-
R_f5	0.54 (sky blue)	0.60(sky blue)	-
R_f6	0.68 (florescent blue)	0.66 (sky blue)	0.60 (brown)
R_f7	0.74(deep brown)	0.76(light yellow)	-
R_f8	0.80 (Sky blue)	0.80(white)	0.80 (brown)

Table 3: Determination of Microbial screening

S.No	**Parameters**	**Result**	**Permissible Limits WHO**
1.	*Staphylococcus aureus* /g	Absent	Absent
2.	*Salmonella spp.* /g	Absent	Absent
3.	*Pseudomonas aeruginosa* /g	Absent	Absent
4.	*E.coli*	Absent	Absent
5.	Total Aerobic microbial count. (AMC)	134cfu/g	10^5/gm
6.	Total Yeast & mould.	56 cfu/g	10^3/gm

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