



Pharmacognostical Screening and Phytochemical Evaluation of *Albizia lebbeck* Benth. Heartwood

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ABSTRACT

Albizia lebbeck benth, known as *Shirisha* in *Ayurveda* is an important medicinal plant belonging to the family Fabaceae (Formerly Leguminosae) and a member of subfamily Mimosaceae. The plant is useful in many disease conditions and is known for its anti-inflammatory, anti-histaminic, anti-anaphylactic, anti-asthmatic and anti-microbial etc. activities. The pharmacognostical study of flower, leaf, and stem bark of this plant are reported but heartwood is not available in any of the works including API (Ayurvedic Pharmacopoeia of India). Considering this an attempt has been made for first time to evaluate correct and easy identification and authentication of drug. The present study deals with morphology, macro and microscopic studies, preliminary physico-chemical and phytochemical analysis of heartwood of *Shirisha*.

KEY WORDS: *Albizia lebbeck* benth, *Ayurveda*, Heartwood, Pharmacognosy, HPLC, *Shirisha*.

INTRODUCTION

Albizia lebbeck benth. is commonly known as *Shirisha* in Sanskrit, is found throughout India, ranging from Himalayas to Andmans. Bark is dark brown to greenish black, rough, with longitudinal and transverse fissures on outer surface; inner surface whitish with fine longitudinal stations. The sapwood is white or yellowish white and the heartwood is dark brown, streaked with dark and white shades. Leaves are bipinnate with 8-18 leaflets. Flowers greenish yellow in globose heads. Flowering and fruiting season starts from April to June. Pods are yellowish brown with 6-10 seeds. Mature pods remain on the tree for long period and are available till May-July. [Fig.1]^[1] The heartwood contains Melanoxetin, d-pinitol, okanin & leucopelargonidin, a stereoisomer (-) melacacidin (7,8,3',4'- tetrahydroxyflavan-3,4-diol), and lebbeccacidin.^[2] The plant also contains saponins^[3] Macrocylic alkaloids^[4] Phenolic glycosides^[5] Flavonols^[6] The plant has been reported to possess anti-inflammatory^[7], anti-allergic^[8], anti-histaminic^[9], anti-tussive^[10], antioxidant^[11], anticonvulsant effect^[12] and anti-spermatogenic effect^[13]. pharmacognosy of flower, leaf and bark were reported, but was not available for heartwood. Considering these, detailed investigation of powdered heartwood of *Albizia lebbeck* benth. Has been carried out using various pharmacognostical and physico-phytochemical parameters.

MATERIALS AND METHODS:

PLANT MATERIALS:

Fresh heartwood of *Shirisha* was collected from the botanical garden of the Institute for Post Graduate Teaching & Research in *Ayurveda* (IPGT & RA), Gujarat Ayurved University, Jamnagar in the month of august 2011[Figure-1]. Voucher specimen along with crude drug sample is preserved in the Pharmacognosy Lab, IPGT & RA, Gujarat Ayurved University, Jamnagar. Botanical identification was carried out by using various floras.

MACROSCOPIC CHARACTERIZATION:

Macroscopic characters of heartwood were done by naked eye observations like shape, colour, taste and odour.

MICROSCOPIC CHARACTERIZATION:

The heartwood of *Shirisha* was soaked overnight in water. Thin free hand sections were cleared with chloral hydrate by heating and then stained with phloroglucinol and hydrochloric acid. For Powder microscopy; powder of heartwood sieved through # 60 was used. The powder was uniformly spread on glass slides and observed under microscope at different magnifications. For the detection of lignified tissues (stone cell, sclereids, xylem vessel, etc.), the powder was stained with phloroglucinol and hydrochloric acid and to observe the starch grains the powder was stained with iodine solution. Photomicrographs were taken by using Carl zeiss binocular microscope attached with camera^[14].

PHYSICO-CHEMICAL PARAMETERS:

Physicochemical parameters were determined as per Ayurvedic Pharmacopoeia of India. Moisture content, pH, total ash value, acid insoluble ash value, alcohol soluble extractive value and water soluble extractive value were determined.

PRELIMINARY PHYTO-CHEMICAL SCREENING:

The methenolic extract of heartwood was prepared and subjected to relevant tests to detect the presence of various functional groups.

QUANTITATIVE ESTIMATION OF CATECHIN:

The percentage of two catechin derivatives i.e. Epicatechin gallate and Epigallocatechin gallate were carried out by HPLC.

CHROMATOGRAPHIC CONDITIONS:

Model : Shimadzu CLASS-VP V6.10 pump LC-10 ATVP

Sample application: Automatic sampling unit (Auto sampler), SIL-10ADVP

Detector: RID-10A Shimadzu refractive index detector

System controller: SCL-10AVP version 5.40

Data analysis & processing : Shimadzu class VP software version 6.10

Column oven : CTO-10ASVP

Column temperature: 30°C

Column: Merck Lichrospher RP-18 column (5 micrometer, 250 x 4.00 mm ID)

Mobile phase : 0.1% Phosphoric acid in HPLC H₂O: ACN

Flow rate : 1.0 ml/min

Detection : 270 nm UV

Injection volume: 10 µL

RESULTS AND DISCUSSION:

MACROSCOPIC CHARACTERS: The heartwood of *Shirisha* was appeared dark brown in colour streaked with dark and brown shades. On organoleptic examination, very mild characteristic odour with slightly astringent taste was perceived. The surface characteristic of heartwood was appeared to be hard and course in touch, rough texture and splintery in fracture characteristic.[Table.1]

POWDER MICROSCOPY:

The diagnostic characters of the powder are plenty of broken fragments of isolated and groups of thin walled and occasional thick walled fibers with blunt or pointed end associated with ideoblasts embedded with oxalate crystals; longitudinally cut fragments of vessels and tracheids with bordered pitted thickening and beaded walls. Lenticular masses of tangentially cut medullary rays

associated with fibres and fragments of radially cut medullary rays crossing the vessels, parenchyma or fibers, Prismatic crystals of calcium oxalate scattered as such throughout the powder and parenchymatous cells embedded with minute starch grains. Occasional small groups of transversally cut fibers were also found. [Figure-2]

HISTOLOGY:

Transverse sections shows isolated big vessels scattered throughout the section or rarely in radial groups. At places they are often blocked with tyloses impregnated with tannin. In longitudinal section, they exhibit numerous closely arranged minute bordered pits and slit like pores. The fibers are plenty occupying the major area of the section. They are thin walled usually arranged in tangential and radial rows and at places embedded within broad zone of radially running groups of metatracheal parenchyma embedded with prismatic crystals of calcium oxalate. Vesicentric parenchyma encircling the vessel and paratracheal parenchyma with broad radially running bands are also seen at places. Medullary rays are uni to tri seriate. Their cells are pitted and rectangular in shape. In tangential longitudinal section uniseriate medullary rays are seen as a vertically running linear bands while the multiseriate medullary rays as a lenticular areas, embedded with rows of prismatic crystals of calcium oxalate and dark brown colouring matter. In radial longitudinal section, the medullary rays appear as narrow horizontally running bands crossing the vessels and fibres. Parenchymatous cells are pitted and are embedded with starch grains and calcium oxalate crystals. Starch grains being very minute in the vesicentric parenchymatous cells. [Figure-3]

PHYSICO-CHEMICAL PARAMETERS:

The moisture content^[15] was 6.8± 0.456% , total ash^[16] 2.15±0.486 % , acid insoluble ash^[17] 1.0±0.125 %, Water Soluble ash 0.90±0.024 % , alcohol-soluble extractive 16.4± 0.564 %^[18], while the water-soluble extractive^[19] was found to be 7.10.342± %. [Table.2]

PRELIMINARY PHYTO-CHEMICAL SCREENING:

Qualitative analysis for the presence of various functional groups was carried out in methanol solvent extractive^[20]. [Table.3]

QUANTITATIVE ESTIMATION OF CATECHIN:

Quantitative estimation of two catechin derivatives i.e. epigallocatechin gallate and epicatechin gallate was carried out by HPLC. The Epicatechin gallate and Epigallocatechin gallate were found 0.2360 and 0.6014%w/w^[21]. [Figure.4]



(A) (B) (C)
Figure No.1: (A) Whole plant, (B) Bark, Sapwood, Heartwood and Pith, (C) Coarse powder of heartwood

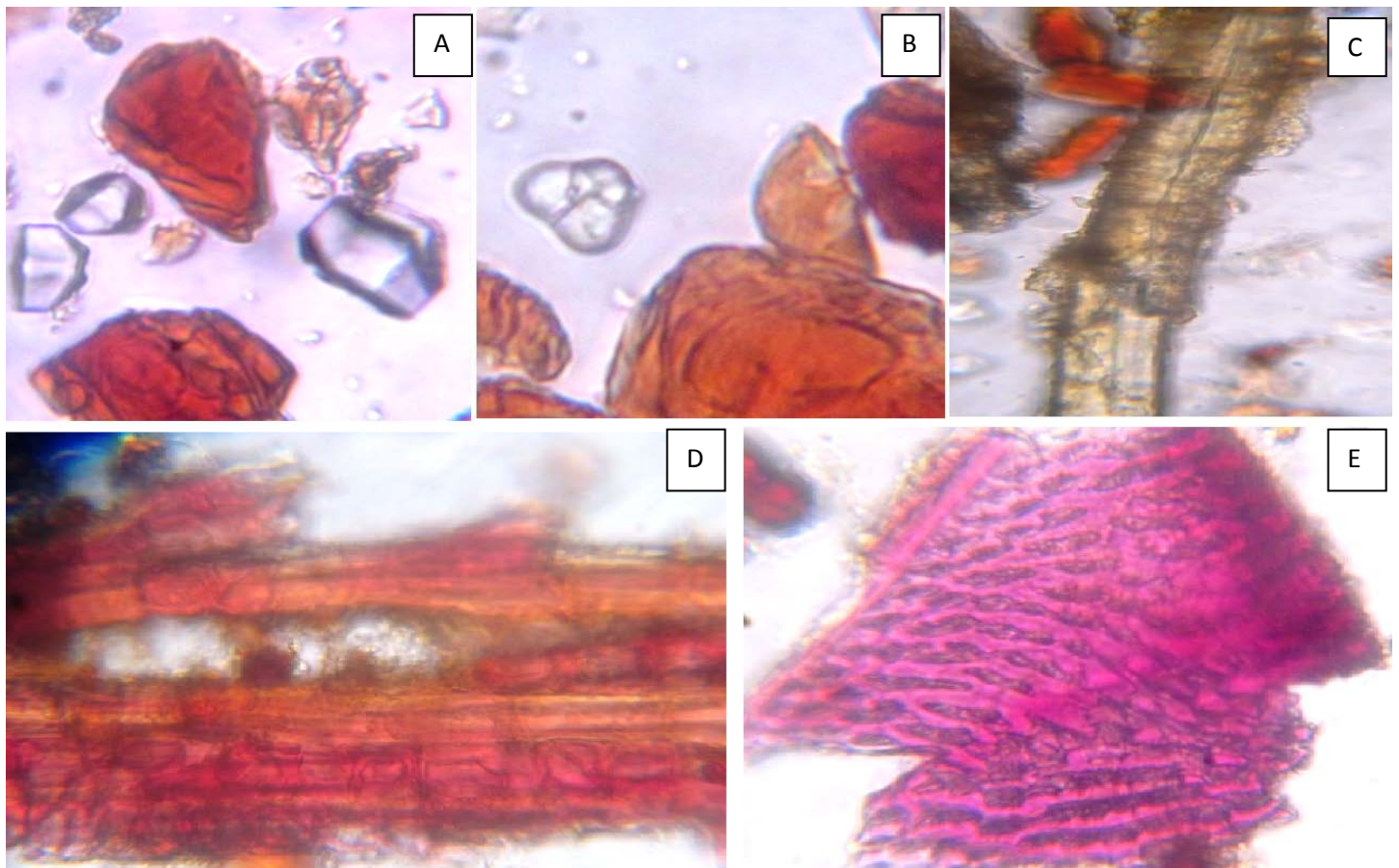


Figure No. 2: Powder Microscopy of Stem Heartwood of *Albizia lebeck benth*
(A) Prismatic crystal with coloring material, (B) Compound starch grain, (C) Lignified fibre, (D) Lignified fiber (stained), (E) Fragment of Border pitted vessels (Stained)

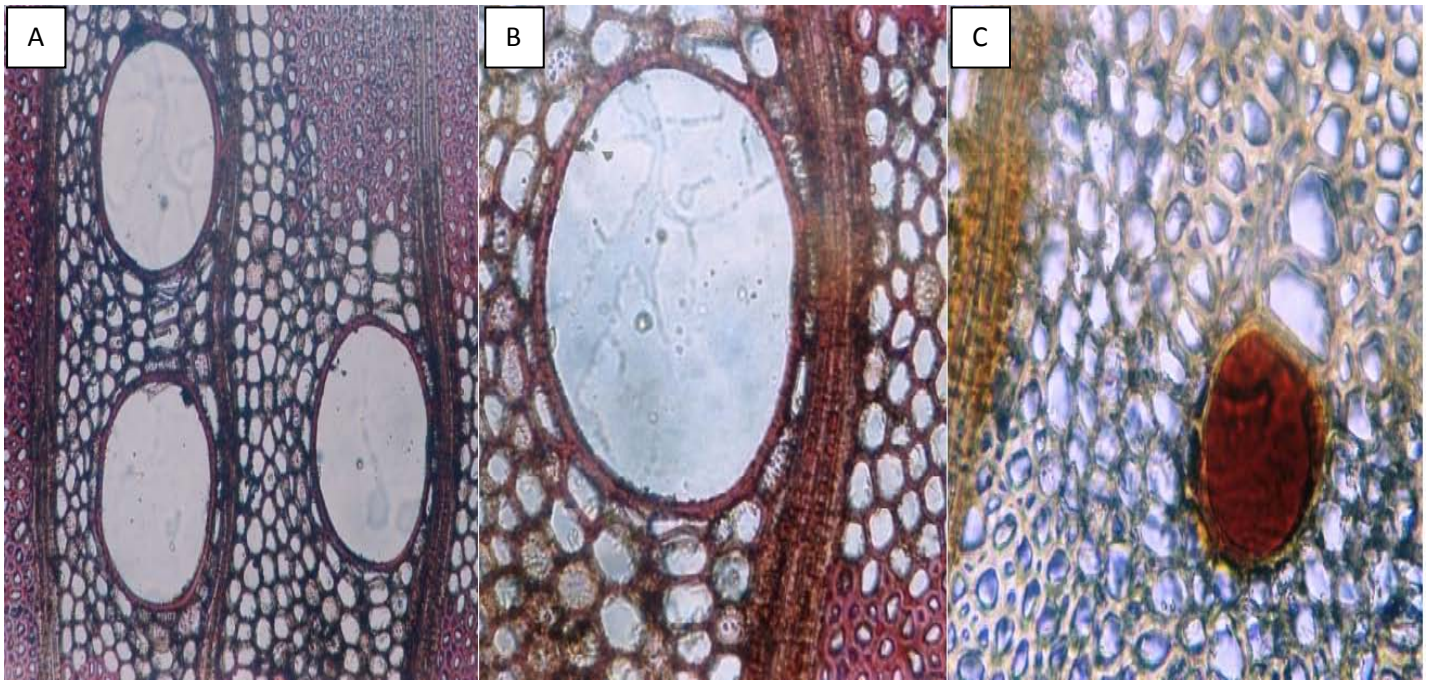


Figure No.3: Transverse section of stem heartwood of *Albizia lebeck benth*

(A) T.S showing large vessels surrounded by wood parenchyma, wood fibers and three biseriate medullary rays, (B) Wood parenchyma and a biseriate medullary rays, (C) Medullary rays and vessel filled with redish brown content, (D) Prismatic crystals along with crystal fibers (E) Tracheids, vessels with simple and bordered pits, (F) Wood parenchyma and wood fibers, (G) Large vessel surrounded by light brown red colouring material, xylem parenchyma, prismatic crystals of Ca-oxalate, medullary rays and wood fibers, (H) Medullary rays crossing through the wood fibers, (I) Tangential rows of medullary rays, wood fibers

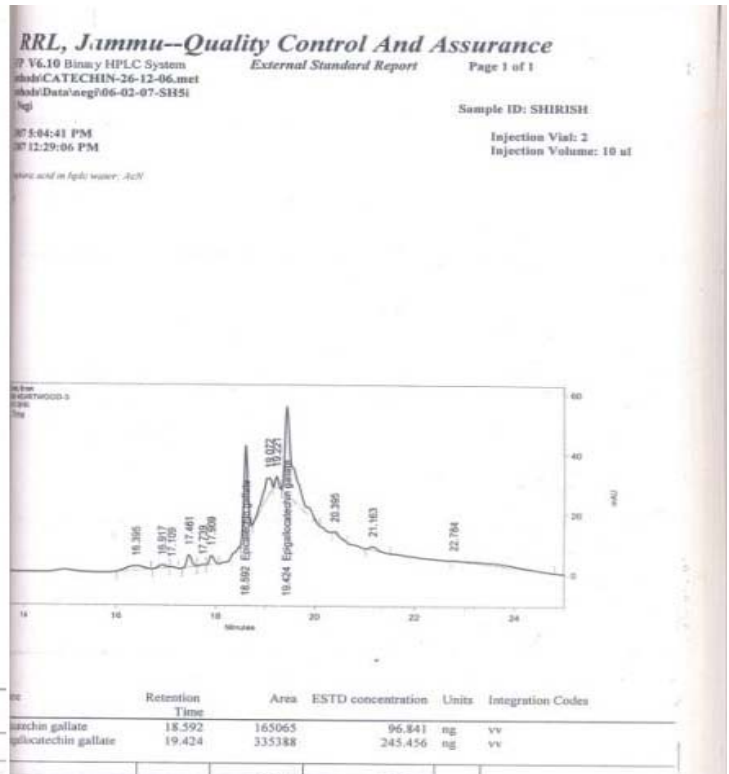
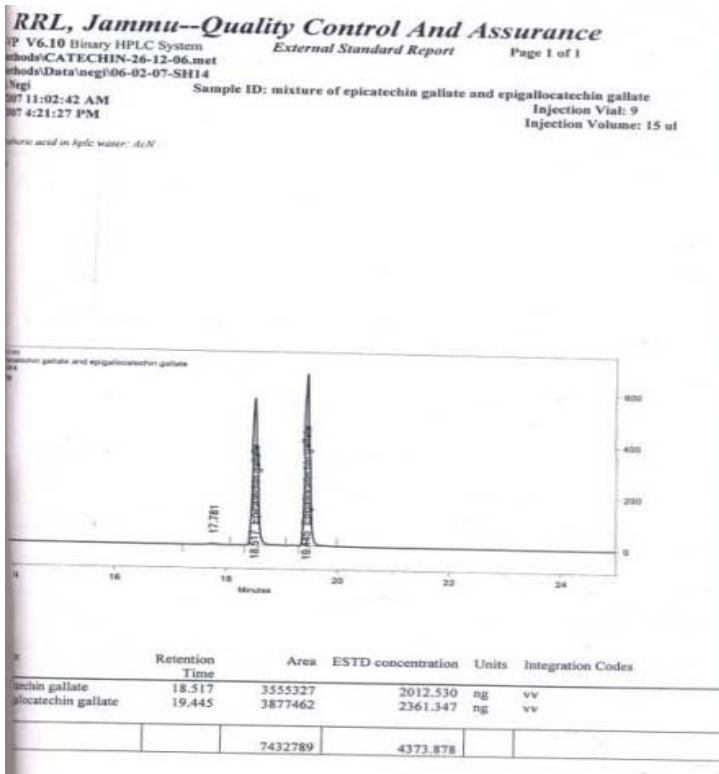


Figure No. 4: HPLC of powder of Stem Heartwood of *Albizia lebeck benth*
 (A) HPLC with marker compound [Epicatechin gallate and Epigallocatechin gallate].
 (B) HPLC with powder of heartwood of *shirish* *Albizia lebeck benth*.

Sr. No.	Character	Observation
1.	Color	Dark reddish brown
2.	Texture	Hard and coarse
3.	Taste	Slight astringent
4.	Odour	Characteristic

Table No.1: Organoleptic characters of heartwood powder of *Albizia lebeck* benth.

Sr. No.	Parameters	Values obtained *
1	Loss on drying	6.8± 0.456%w/w
2	Ash value	2.15±0.486 %w/w
3	Acid insoluble ash	1.0±0.125 %w/w
4	Water Soluble ash	0.90±0.024 %w/w
5	Water Soluble Extractive	7.10.342± %w/w
6	Alcohol soluble extractive	16.4± 0.564 %w/w
7	pH	4.86±0.002

Table No. 2: Physicochemical evaluations of heartwood powder of *Albizia lebeck* benth.

Sr. No.	Material	Reagent	Functional groups	Observation	Result
1	Methanolic extract of heartwood.	Dragendorff's reagent	Alkaloids	No Brown ppt.	Absent
2		Neutral FeCl ₃	Phenols	Violet color	Present
3		Dil. FeCl ₃	Tannins	Blue color	Present
4		Lead acetate	flavonoid	Yellow color	Present
5		Biuret reagent	Proteins	Violet color	Present
6		Benedict's reagent	Carbohydrates	Yellow ppt.	Present
7		Shaking in test-tube	Saponins	NO Frothing with honeycomb appearance	Absent
8		Conc.H ₂ SO ₄	Glycosides	color change	Present

Table No. 3: Preliminary qualitative analysis of *Albizia lebeck* benth. heartwood powder for the presence of various functional groups**CONCLUSION:**

Pharmacognostical and physicochemical evaluation of the heartwood provided specific parameters that will be helpful in proper identification and standardization of the observations can be considered for further researchers.

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