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Pharmacognositical and phytochemical analysis of stem bark of *Albizzia lebbeck* Benth

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Abstract
Albizzia lebbeck Benth. is one of the significant plant of Ayurveda, belongs to the family Fabaceae. Ethno medicinally *A. lebbeck* is claimed to be used in the management of leprosy, wound, asthama, urticaria, migraine, worm infection and others. The present study has been carried out to establish the stem bark of the plant for its morphological, microscopical and physicochemical characters along with different phytochemical qualitative tests, as per Ayurvedic Pharmacopoeia of India. Microscopy of the stem bark showed dead tissue of rhytidoma, prismatic crystals of calcium oxalate, and tangential bands of ceratenchyma. Physicochemical parameters showed loss on drying (7.15%w/w), ash value (4.05%w/w), acid insoluble ash (0.33%w/w), water soluble extractive (15.46%w/v) and alcohol soluble extractive (14.24%w/v). Preliminary phytochemical analysis for the presence of various functional groups revealed the presence of flavonoids, proteins, saponins, tannins and free amino acids. The observed data can be useful to identify and standardize of stem bark of *Albizzia lebbeck* Benth.

Keywords: *Albizzia lebbeck*, ayurveda, shirisha, stem bark, flavonoids, proteins, fabaceae

Introduction
The knowledge about medicinally important plants has been scientifically documented, and systematically presented in Ayurvedic Samhita, Nighantu and other texts. During the last decade, use of traditional medicine like Ayurveda has expanded worldwide and has achieved recognition. Majority of the world population still relies on herbal medicines to meet its health desires. It has not only continued to be used for primary health care of the poor in developing countries, but has also been used in countries where conventional medicine is predominant in the national health care systems. Accurate identification and certification of purity through Pharmacognosy and pharmaceutical chemistry measures is first step for the quality assurance and standardization of any of the herbal medicine. *Albizzia lebbeck*, Fabaceae, one of the important plants is known as Shiris in Hindi and Lebbeck Tree in English. It is large erect unarmed deciduous, spreading tree common all over India, from the plains up to 900m in the Himalayas, and also in the Andamans [1]. Bark is expectorant [2], aphrodisiac, anti-inflammatory, anti-allergic [3], anti-anaphylactic [4], anti-asthmatic [5], anti-histaminic [6], analgesic [7], antioxidant [8], immunomodulatory [9], anticonvulsant [10] and anti spermatogenic [11]. Inspite of its ethno medicinal claims, scientific assessment is also necessary to set standards of pharmacognositical and preliminary phytochemical analysis for its proper identification. Hence, this present study has been carried out to establish its identification and standardization characters through pharmacopoeial parameters.

Materials and Methods
Stem bark of *A. lebbeck* was authenticated by Dr. Jasmeet Singh, Curator, Department of Dravyaguna, Faculty of Ayurveda, IMS, BHU. The sample was authenticated with Ref. No. DG/17-18/155. The plant material was ground into coarse powder, filtered by sieve 80# so obtained was used for further analysis. The standardization parameters were determined according to the methods detailed in the Ayurvedic Pharmacopoeia of India. For morphological study, Living whole plant, Dried stem bark and Powder of stem bark was examined. For microscopic study, transverse section of stem bark was prepared after staining with safranine and fast green. Then transverse section mounted in dpx and studied under microscope by focusing different power of lenses. Preliminary phytochemical analysis of different extracts was performed using specific reagents by employing standard procedures. Physicochemical analysis for loss on drying at 105°C, alcohol soluble extractive value, water-soluble extractive value, total ash value and acid insoluble ash values was carried out in triplicate in the studied sample [12].

Morphology
Morphological characters of dried stem bark were done by visual observations, following standard procedure of taxonomy and verified with existing floras for authentication [13].

Microscopic evaluation
For microscopical studies, thin free hand transverse section of stem bark was taken and after staining with safranine and fast green, section was fixed with cover slip and studied under microscope by focusing different power of lenses [14].

Physicochemical parameters
Assessment of the parameters such as foreign matter, loss on drying, ash value, acid insoluble ash, water soluble extractive and alcohol soluble extractive were carried out by following standard procedures recommended by Ayurvedic Pharmacopoeia of India and other standard texts [12, 15, 16].

Preliminary Phyto-chemical screening of stem bark
Macerate 5 g of the coarsely powdered air dried stem bark of *A.lebbeck*, with 100 ml of Methanol in a closed flask for 24 hours, shaking frequently during six hours and allowing to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry on water bath, to constant weight and weigh. The percentage of alcohol-soluble extract was calculated with reference to the coarse powder of drug. This extract was used for preliminary phytochemical screening by using relevant reagents [12].

Thin layer chromatography
For Thin Layer Chromatography (HPTLC), hydro-alcoholic extract was prepared, it was shaken for some time; mild heat was provided to it for half an hour and then filtered on cooling. The filtrate is evaporated on water bath to approximately 20 ml and used. Pre-coated silica gel GF 254 plate was used as stationary phase [17]. Ethyl acetate: Toluene: Acetic acid (7: 2: 1) v/v was used as mobile phase. After 30 minutes of chamber saturation, plate was developed, and then scanned under normal light and UV spectrum (254 nm).

Results
Morphological Characters of stem bark
Morphologically, Bark pieces were 1.5 -2.5 cm thick, external surface was dark brown, rough due to longitudinal fissures and transverse cracks, rhytidoma forming major part of bark and peeling off in flakes exposing buff coloured surface, middle bark was brown, inner bark was much fibrous, light yellow to gray; fracture, laminated in outer region and fibrous in inner region; taste was very astringent (Fig.1).

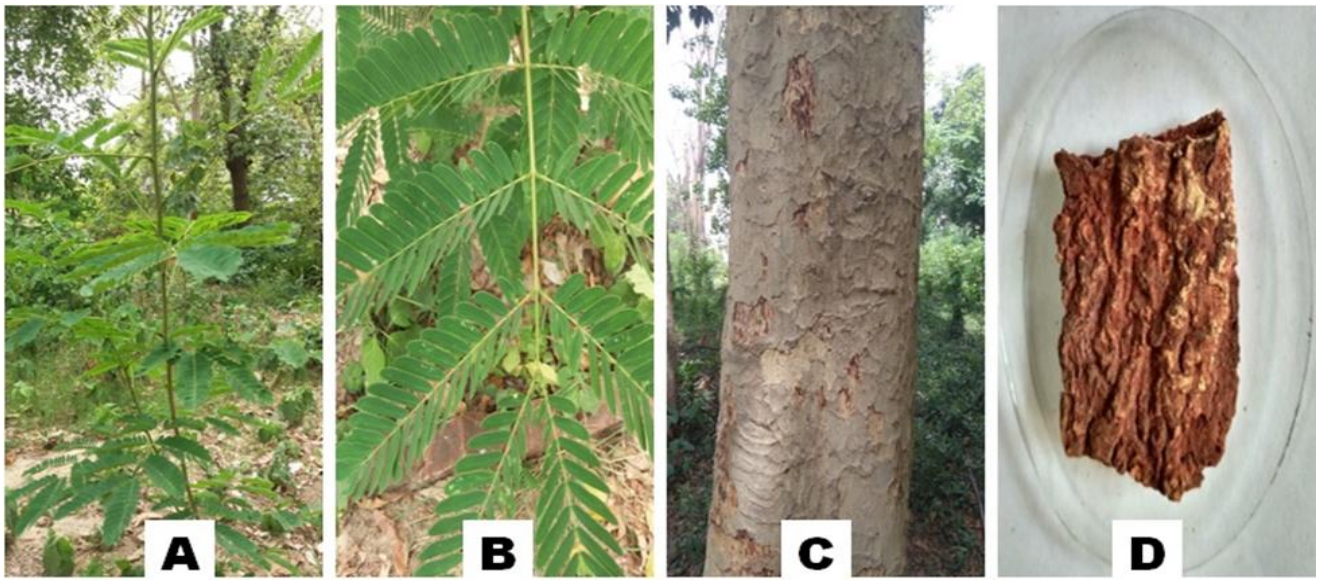

Fig 1: Morphology of *Albizzia Lebbeck* Benth.

Microscopic Characters
The transverse section of the mature bark is made, Mature bark about 2 cm thick, shown dead tissue of rhytidoma; cork consisted of a few layers of thin-walled, transversely elongated and radially arranged cells; secondary cortex wide, composed of radially elongated to squarish, moderately thick walled cells containg orange to reddish brown contents; a few of the cells contain prismatic crystals of calcium oxalate; stone cells, variable in shape and size, present in singles or in groups throughout the region; secondary phloem consisted of sieve elements, phloem parenchyma, phloem fibres and crystal fibres, transversed by phloem rays; prismatic crystals of calcium oxalate present in most of the phloem phloem parenchyma cells; tangential bands of ceratenchyma present in middle and outer phloem region; phloem fibres. Elongated, thick-walled, lignified, present in many concentric strips, mostly enclosed by crystals sheath throughout the middle and inner regions of phloem; crystal fibers having a number of septa, each chamber containing a single prismatic crystal of calcium oxalate; phloem rays numerous, radially elongated, somewhat wavy in outer phloem region and bi to multiseriate in the inner phloem region being 2-5 cells wide and 7-25 cells high (Fig. 2).

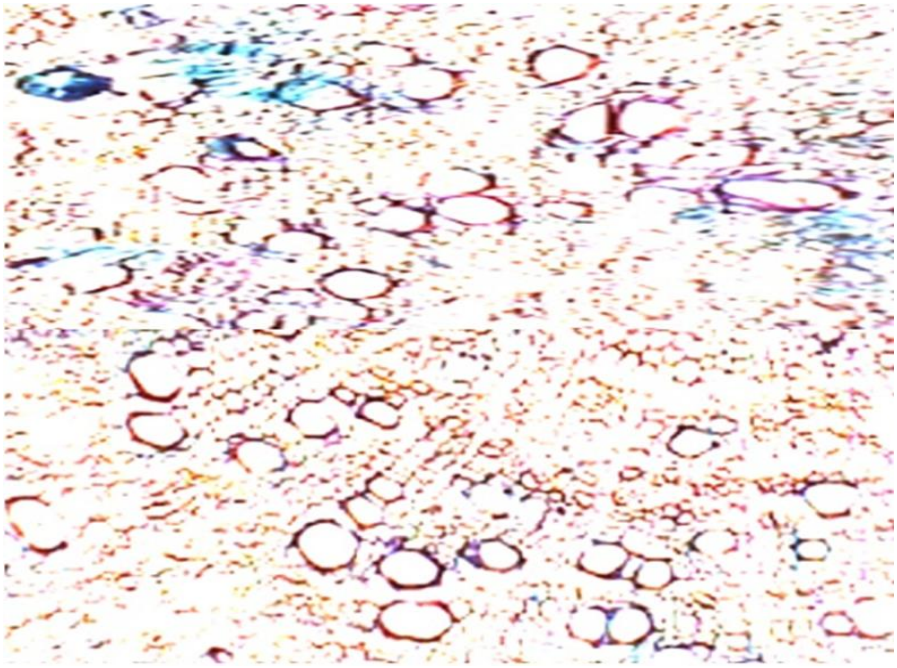

Fig 2: Microscopic Characters of Stem Bark of *Albizzia Lebbeck* Benth.

Physicochemical Parameters of stem bark:
Detailed outcomes of physicochemical parameters are given in Table 1. Loss on drying has been found (7.15% w/w). Foreign matter has not been found in the sample, which may be due to the good harvesting practice followed during the collection of the drug. Total ash value (4.05% w/w) and acid insoluble ash have been found (0.33% w/w), which may signify low level of inorganic matter, sand and silica content in the sample. Water soluble extractive in the sample has been found (15.46% w/w) more in comparison to alcohol soluble extractive value (14.24% w/w) which indicates the probability of the presence of high water soluble constituents than the alcohol soluble in the sample.

Table 1: Values Of Physicochemical Parameters Of Stem Bark Of *Albizzia Lebbeck* Benth.

Physicochemical parameters	Results
Foreign matter	Nil
Loss on drying (%w/w)	7.15
Ash value (%w/w)	4.05
Acid insoluble ash (%w/w)	0.33
Water soluble extractive value (%w/w)	15.46
Alcohol soluble extractive value (%w/w)	14.24

Preliminary Phytochemical analysis of stem bark:
Phytochemical analysis showed the presence of flavonoids, proteins, saponins, tannins and free amino acids in the sample of stem bark of *Albizzia lebbeck*. Results also showed that alkaloid and glycoside were absent or may be present in very negligible amount in sample (Table 2).

Table 2: Result of Preliminary Qualitative Tests of Stem Bark Extract of *Albizzia Lebbeck*

Plant Metabolites	Results
Alkaloids	-ve
Flavonoids	+ve
Proteins	+ve
Saponins	+ve
Tanins	+ve
Glycosides	-ve
Free Amino acids	+ve

Table 3: TLC of Shirisha Stem Bark

Chromatogram	No. of spots	Rf Value
Normal light	3	0.10, 0.2, 0.36
254 nm	4	0.10, 0.2, 0.36, 0.67

Discussion
Pharmacognosy is the methodical study of crude drugs from natural sources. The standardization is a very important step in establishing the accurate identity, purity, safety and quality of crude drugs and it should be established before it can be successfully incorporated in pharmacopoeia [18]. Recently, there has been an emphasis in standardization of medicinal plants of therapeutic potential. In spite of modern techniques, pharmacognositical evaluation is still more reliable for identification and evaluation of plants. World Health Organization recommends that the morphological and microscopic evaluation is most important in establishing the identity and purity of the plants [18]. In the present study, pharmacognositical standardization of stem bark of *Albizzia lebbeck* was done which included morphological, microscopic, physicochemical and phytochemical analysis. This analysis provides the easiest, speedy and economical means to establish the identity and purity of drug and also acts as a reliable implement for detecting adulteration. Adulteration of the original plant material is the main cause of decrepitude of original therapeutic effect of plants used in traditional systems of medicine [19, 20]. In physicochemical parameters, ash values like total ash, acid insoluble ash and extractive values were estimated which serve as a reliable aid for identifying adulteration and identification of plant. Ash values give an idea about inorganic composition, other impurities present along with drug while extractive values are helpful for the determination of exhausted and adulterated drugs. The chemical constituents of crude drug that are soluble in particular solvents can be known by extractive values [21, 22]. Loss on drying of drug should be at minimal level so that bacteria yeast or fungi will not grow during storage [23, 24]. The preliminary phytochemical analysis showed the presence of flavonoids, proteins, saponins, tannins and free amino acids. Pharmacognostical studies are important in herbal technology as it ensures plant identity lays

down standardization parameter which will help and prevent adulterations. Such studies will assist in authentication of the plants and ensures reproducible quality of herbal products which will lead to safety and efficacy of natural products.

Conclusion

Standardization of a crude drug is very essential for its accurate and proper identification. The data specified in the present study regarding morphology and microscopical characters will help for easy identification of the plant *Albizzia lebbeck*. Physicochemical and phytochemical test reports will help in establishing standards in identity, degree of purity and quality of the plant material as per pharmacopoeial necessities.

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