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Pharmacognostical standardization of *Albizia lebbeck* (L.) BENTH (Fabaceae)

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ABSTRACT

Albizia lebbeck (L.) BENTH (Fabaceae), commonly known as *Sirisha*, traditionally used as astringent, to treat boils, cough, pain, swelling and it is also used against diarrhea. In the present investigation, various pharmacognostical standards for *A. lebbeck* have been established. Collection of plant material was carried out and its morphological, physico-chemical and phytochemical studies including HPTLC of leaf, root and stem of plant were investigated. Microscopically, leaf of *A. lebbeck* showed presence of anomocytic stomata, arc shaped and cortical parenchyma. The roots of the plant showed cork cambium, secondary phloem, and calcium oxalate crystals and stem exhibited fibres, parenchyma cells, vessels, stomata, palisade cells and trichomes. Phytochemically, the ethyl acetate and methanol extracts of *A. lebbeck* showed variety of phytochemicals such as alkaloids, glycosides, steroids, flavonoids, saponin, tannin and phenolic compounds. HPTLC profile of ethyl acetate extract revealed up to six phytoconstituents amongst which gallic acid was most prominent. The study concludes that leaves, roots and stem can be differentiated on the basis of macro and microscopic characters, physico-chemical values, HPTLC fingerprint profile and gallic acid detection as marker component. These studies provide referential information for correct identification and standardization of the plant and also be helpful to differentiate *A. lebbeck* from the closely related other species of *Albizia.*

Keywords: HPTLC, Gallic acid, Pharmacognostical standardization, Phytochemical analysis.

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INTRODUCTION

The genus *Albizia* also called silk tree or silk plant, genus of trees or shrubs in the pea family (Fabaceae). The genus is pantropical, though most species are native to warm regions of the old world. The plants are widely used for fodder and timber, and many are important in traditional medicine[1]. *Albizia lebbeck* is a deciduous Tree with an open, large, spreading crown; it usually reaches a height of 15-20 meters, with exceptional specimens growing up to 30 meters. In India plantation-grown siris yields a high quality hardwood that is traded in Europe as 'Indian walnut' or 'koko'. The tree has been widely planted in agro-forestry schemes in the drier parts of the Tropics. Plants are used to provide shade for coffee and cocoa plantations as well as to provide a valuable timber and fuel. It is a popular amenity tree throughout the dry tropics because of its shady spreading habit, although the copious litter produced beneath it is often regarded as a disadvantage[2-4]. *Albizia lebbeck* has many medicinal properties like Antiseptic, antibacterial, anti-allergic, antidermatosis, antidysenteric etc. Used in the treatment of Bronchitis, piles, hemicranias, cough, tropical pulmonary eosinophilia, asthma etc. *Albizia lebbeck* is used as an astringent, to treat boils, cough, to treat the eye, flu, gingivitis, lung problems, pectoral problems, to treat abdominal tumors and it is also used as a tonic. The bark is used medicinally to treat inflammation[5-7]. Phytochemically the plant is less explored. Flavone, 3', 5-dihydroxy-4', 7-dimethoxyflavone and a nitrogenous compound, N-benzoyl-L-phenylalaninol, friedelan-3-one and g-sitosterol; Hexaglycosylated saponins, quercetin, unsaturated carboxylic acid methyl ester, a triterpene saponin, albigenic, albigenin, two tri-O glycoside flavonols i.e, kaempferol, quercetin; albizziahexoside (a hexaglycosylated saponins) and cardiac glycoside[8-12]. The biological activities which are attributed to this species are antibacterial, diuretic, analgesic & anti-inflammatory, anti-tumor, *in vitro* antioxidant activity, antimicrobial, anti-larvae, antiulcer, antiviral and ecbolic activities[13-26]. Despite the numerous medicinal uses attributed to this plant, there are no pharmacognostical report on leaf, root & stem of this plant. Hence the present

investigation deals with the morphological and anatomical evaluation, determination of physicochemical constants, preliminary Phytochemical screening, and HPTLC profile of the different extracts. The profile presented in this paper may be proposed as parameters to establish the authenticity of *Albizia lebbeck* and can possibly help to differentiate the drug form its other species.

METHODOLOGY

Morphological studies

Whole plant of *Albizia lebbeck* was collected from the Dausa, Rajasthan and after due identification with the help of floras and previous works deposited at Botanical Survey of India, Jodhpur, India herbarium vide voucher specimen number BSI/JODHPUR/AL/1.

Morphological studies of the plant drug were performed at macro- and microscopic levels. For microscopic studies, fresh formaldehyde–acetic acid and alcohol (FAA) preserved materials were used. Microtome sections of leaf, stem & root were cut and stained with phloroglucinol, safranin & toluidine blue and photographed with electronic digital microscope Motic DMBA 300, New Delhi. Quality preparations for microscopic studies were mounted on Canada balsam and rendered transparent with gelatin–glycerin.

Physico-chemical studies

Physicochemical studies were carried out on shade dried powdered material of leaf, root and stem of *Albizia lebbeck*. Physico-chemical values such as the percentage of total ash, acid-insoluble ash, water-soluble ash, sulphated ash, water and alcohol soluble extractive values, foreign matter, volatile oil, swelling index, foaming index, crude fiber and loss on drying were calculated as per the WHO guideline and Indian Pharmacopoeia[27-30].

Phytochemical studies

For phytochemical studies 1.2 kg powdered material of leaf, stem & root was extracted in a Soxhlet apparatus with petroleum ether, chloroform, ethyl acetate, methanol and water successively. The extracts were dried and weighed. The presence or absence of different phytoconstituents viz., alkaloid, steroid, fixed oil, glycoside, tannin, phenolic compound and flavonoid etc. were detected by usual prescribed methods[31]. TLC studies of the petroleum ether, chloroform, ethyl acetate, methanol and aqueous extracts of leaf, root & stem were carried in various solvents by using silica gel G as adsorbent and the same phase were used for HPTLC profile of plant extracts.

RESULTS

Morphological study

Microscopy

The transverse section of the leaf shows a dorsiventral condition as shown in Fig. 1. The tissues represented in the lamina and the midrib regions are as follows:

The lamina of the leaf shows the upper epidermis, mesophyll and lower epidermis. The upper epidermis is single-layered with a thick cuticle, rectangular cells with trichomes. The Mesophyll being a dorsiventral leaf is differentiated into palisade and spongy parenchyma. Palisade cells are single layered, cells are elongated and compact. Spongy Parenchyma is 5 to 8 layered, loosely arranged with intercellular spaces. Big cluster crystals are seen frequently. Vascular strands are seen at times. The lower epidermis resembles the upper epidermis. The lower and upper epidermal layers are continuous over the midrib. The midrib is occupied by the cortical parenchyma with the vascular bundle embedded in the middle. Vascular bundle is arc shaped, collateral with xylem towards upper epidermis and phloem towards the lower epidermis. Pericyclic fibers are seen as a ring around the vascular bundles. Surface preparation shows beaded epidermal cells and anomocytic stomata.

The sample of powdered leaves of *Albizia lebbeck* was mounted in chloral hydrate and in phloroglucinol: concentrated hydrochloric acid respectively, the following diagnostic characters were observed: fragments of unicellular trichomes, phloem fibres, parenchyma cells, numerous xylem vessels of spiral type and epidermal cells with anomocytic stomata and rosette type ca-oxalate crystals (Fig. 1)

T. S. of root showed a circular out line with stratified cork and other secondary features. The following structure could be seen from the periphery (Fig. 2). Periderm: Cork consisting of several layered cells with dark coloration. Phellogen is indistinct. Phelloderm is 3-5 layered of parenchymatous cells below the cork with abundance of calcium oxalate crystals are seen. Phloem consists of sieve tubes, companion cells, phloem parenchyma, and cambium 4-5 rows of tangentially elongated cells. Starch grains and calcium oxalate crystals were in abundance and present throughout the phloem tissue. Xylem is traversed by well developed medullary rays. The xylem consists of vessels with bordered pits and horizontal perforations, wood fibers and parenchyma. Starch grains abundant, simple, mostly spherical, oval or rounded without any striation. Microcrystals are present in parenchyma cells.

Microscopic study of powdered root revealed the presence of lignified and non-lignified fibres, long, slender and cylindrical shaped, xylem vessels are lignified with bordered pits, vessel elements, xylem fibres and xylem parenchyma, calcium oxalate crystals are present in parenchymatous cells and scattered, starch grains which are simple, oval or rounded without any striation (Fig. 2).

The T.S. of the young stem is circular in outline with uneven surface and show different regions (Fig. 3). The epidermal cells are radially oblong with the outer anticlinal walls projecting as papillate hemispherical bodies. Cortex is consisted of 4-5 layers of tangentially oblong thick walled cells. Inner cortex is thin, continuous cylinder of sclerenchyma elements sclereids with thick lignified walls. Vascular bundles consist of secondary xylem and secondary phloem. The peripheral zone of secondary xylem is uneven with furrows and ridges. Secondary xylem consists of fibres with thick lignified walls and narrow thick walled vessels which are either solitary or in short radial multiples. There are 2 types of phloem one occurred outer to the secondary xylem cylinder which is normal type of inter-xylary phloem. The second type of phloem occur inner to the xylem and on the periphery of the pith called as medullary phloem or intra-xylary phloem. The phloem comprises of sieve tubes, companion cells and parenchyma cells. The pith consisted of mass of sclereids which are circular, wide-lumened and lignified. Some sclereids have dark amorphous inclusions.

The various diagnostic characters of the stem powder are depicted in Fig 3. Coarse powder of stem show different types of vascular elements, sclereids and parenchyma cells. Xylem elements consist of vessel elements, fibres and xylem parenchyma. Xylem vessels are long, narrow and cylindrical with simple perforation plate, long narrow tails with bordered lateral wall pits. Some xylem fibres have no lateral wall pits while others have a vertical row of well developed

pits. Xylem parenchyma is rectangular and thin walled. Starch grains are scattered in pith cells.

Physicochemical studies

The percentage of total ash, acid-insoluble ash, water-soluble ash, sulphated ash, water and alcohol soluble extractive values, foreign matter, volatile oil, swelling index, foaming index, loss on drying and crude fiber are presented in Table 1.

Phytochemical studies

The percentage of successive Soxhlet extractives was calculated and results are presented in histogram (Fig. 4). Presence and absence of different phyto-constituents was detected as Table 2. Preliminary chromatographic profiling using TLC and HPTLC methods revealed the presence of various compounds, among which gallic acid was the most prominent in ethyl acetate fraction of *Albizia lebbeck* (Table 3, Fig. 5, Fig. 6 & Fig. 7). Ethyl acetate fraction revealed the presence of six phytoconstituents with R_f 0.24, 0.33, 0.42, 0.63, 0.72, and 0.86 (Fig. 5 & Fig. 6). However, as per existing literature bands that corresponded to R_f 0.33 in ethyl acetate fraction was the same as that of gallic acid (R_f 0.33) (Fig. 7). Gallic acid is an important, natural compound with confirmed pharmacological activity. It is common constituent of many medicinal herbs and plants. Thin-layer chromatography is an important tool of phytochemical investigations.

Table: 1 Physico-chemical values of whole plant of *Albizia lebbeck*.

S. no.	Parameters	
1	Total ash	3.03 ± 0.09
2	Acid-insoluble ash	0.24 ± 0.04
3	Water-soluble ash	0.89 ± 0.11
4	Sulphated ash	5.16 ± 0.08
5	Alcohol-soluble extractive (Hot)	14.46 ± 0.23
6	Alcohol-soluble extractive (Cold)	11.85 ± 0.22
7	Water-soluble extractive (Hot)	7.32 ± 0.21
8	Water-soluble extractive (Cold)	5.59 ± 0.18
9	Foreign matter	0.82 ± 0.02
10	Volatile oil	1.16 ± 0.11
11	Swelling index	14.12 ± 0.06
12	Crude fibres	53.65 ± 0.47
13	Loss on Drying	0.87 ± 0.03
14	Foaming index	Less than 100

[a] Mean value of six readings

Table: 2 Preliminary phytochemical screening of *Albizia lebbeck*.

S. no.	Chemical constituents	Fractions				
		PE	CF	EA	NB	AQ
1	Alkaloids	-ve	+ve	-ve	-ve	+ve
2	Fixed Oil	+ve	-ve	-ve	-ve	-ve
3	Flavonoids	-ve	-ve	+ve	+ve	+ve
4	Glycosides	-ve	-ve	+ve	+ve	+ve
5	Phenolic compounds	-ve	-ve	+ve	+ve	+ve
6	Saponins	-ve	-ve	+ve	+ve	+ve
7	Steroids	+ve	+ve	-ve	-ve	-ve
8	Tannins	-ve	-ve	+ve	+ve	+ve

(+ve, present; -ve,absent; PE, petroleum ether; CF, chloroform; EA, ethyl acetate; NB, n-butanol; AQ, aqueous)

Table: 3 R_f values of different fractions obtained from *Albizia lebbeck*.

Fractions	Solvent Systems	No. of Spot	R_f Value
Petroleum ether	**Hexane : Ethyl acetate (9:1)**	1	0.21
		2	0.32
		3	0.39
		4	0.78
Chloroform	**Hexane : Ethyl acetate (2:1)**	1	0.53
		2	0.62
		3	0.78
		4	0.89
Ethyl acetate	**Toluene : Ethyl acetate: Formic acid: Methanol (3:3:0.8:0.2)**	1	0.24
		2	0.33
		3	0.42
		4	0.63
		5	0.72
		6	0.86
n-butanol	**Chloroform : Methanol (4:1)**	1	0.36
		2	0.41
		3	0.52
		4	0.83
		5	0.88
Aqueous	**Water: n-butanol: dichloromethane (1:3:5)**	1	0.28
		3	0.35
		3	0.41
		4	0.56
		5	0.79

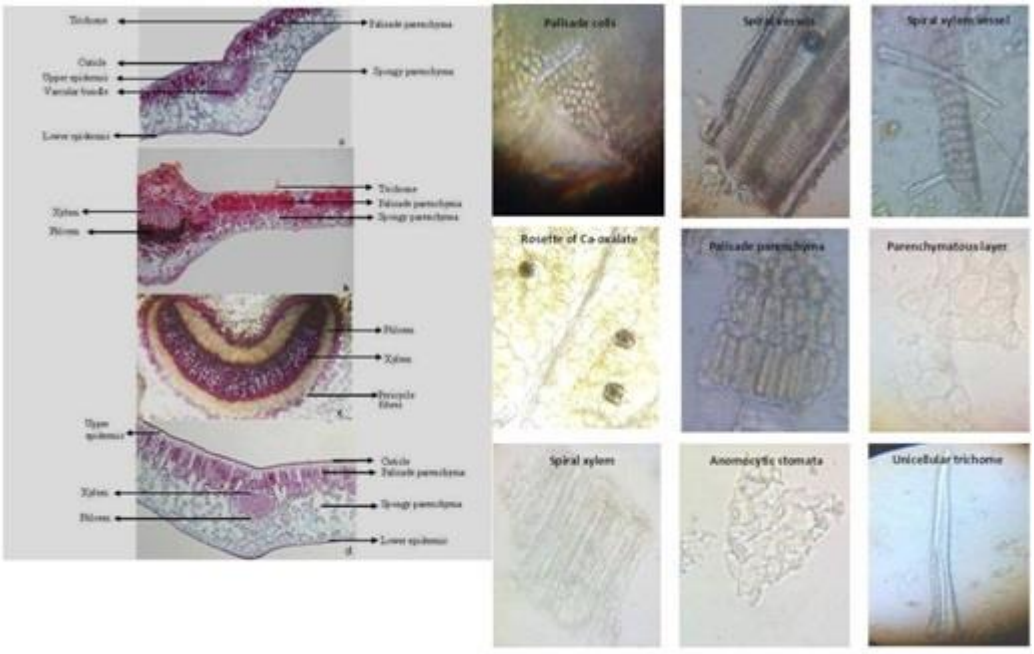

Fig. 1. Microscopical characters of the leaf of Albizia lebbeck

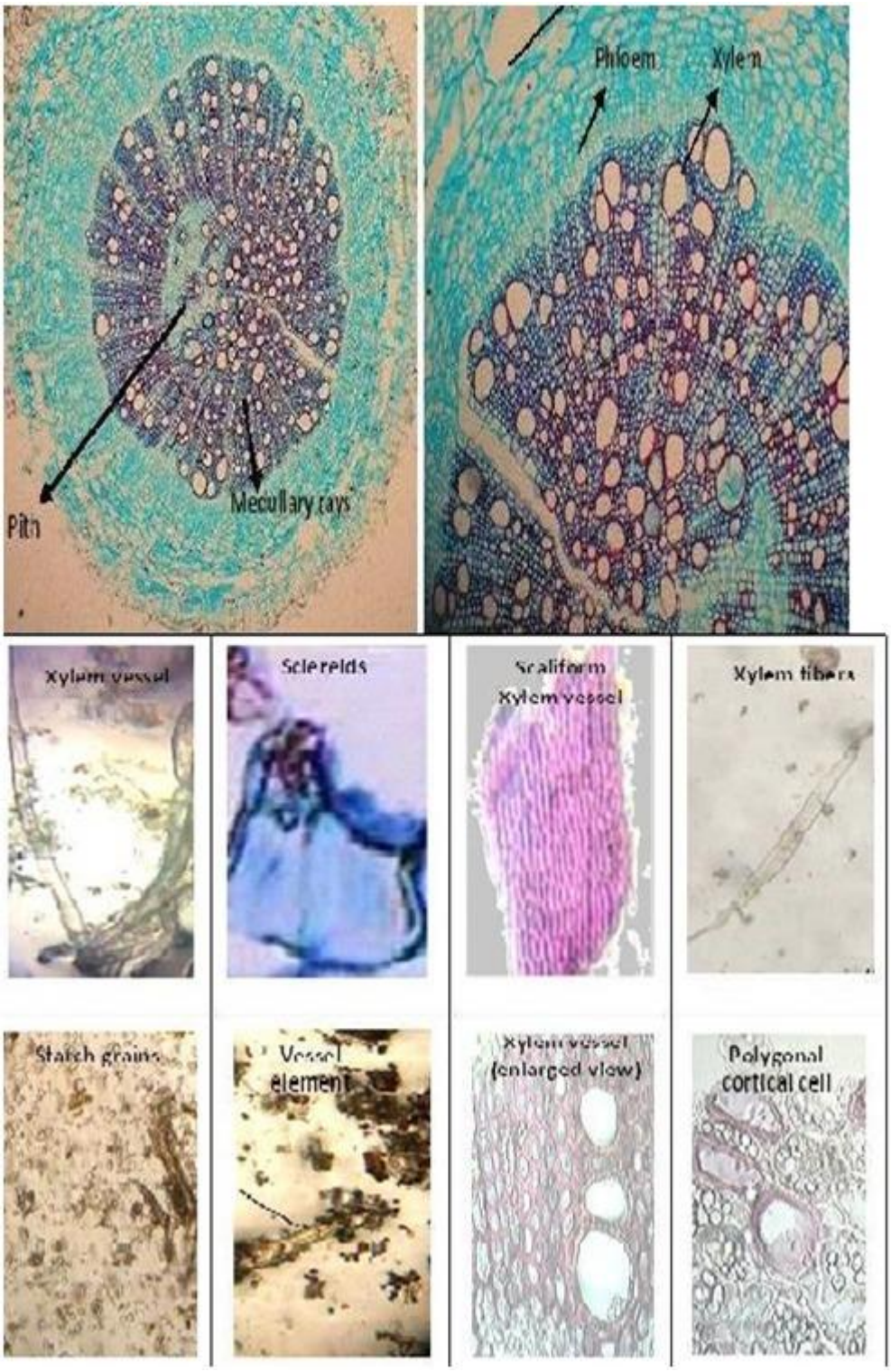

Fig. 2. Microscopical characters of the stem of Albizia lebbeck

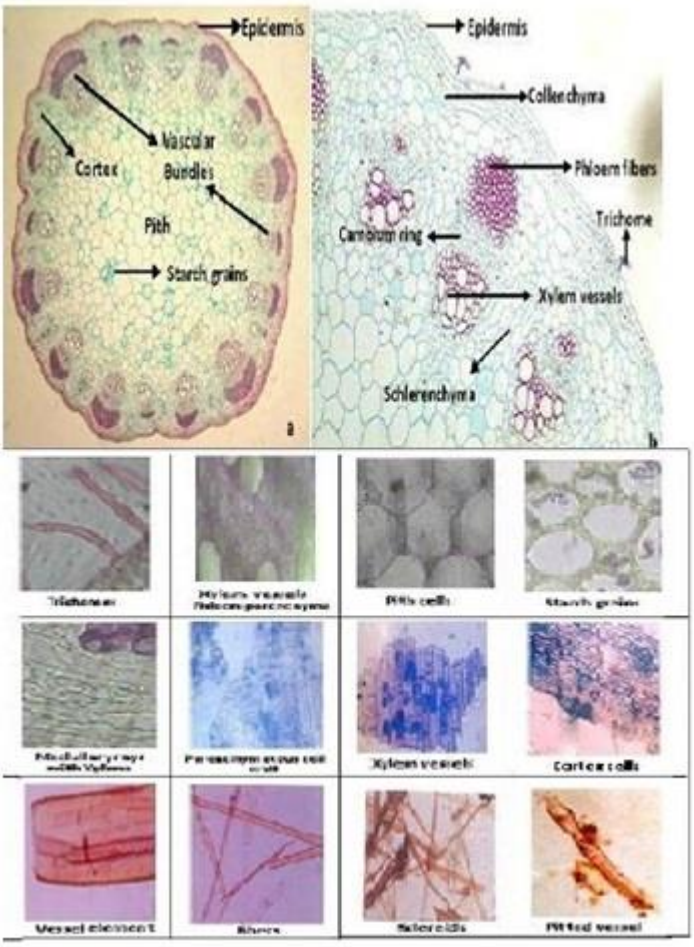

Fig. 3. Microscopical characters of the root of Albizia lebbeck

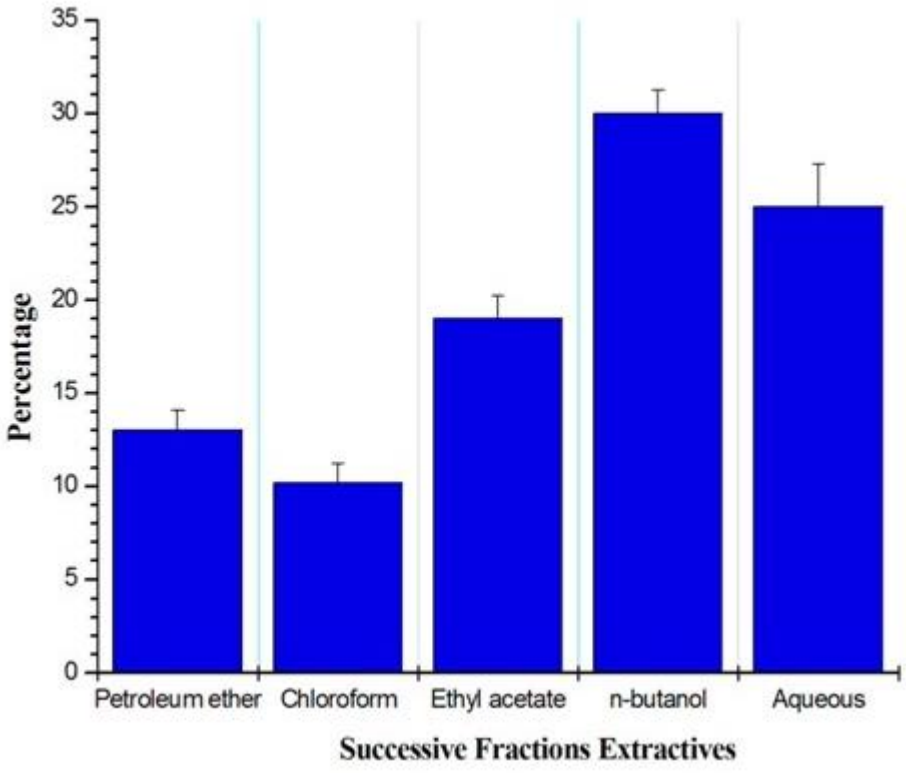

Fig. 4. Successive fractions extractive values of Albizia lebbeck.

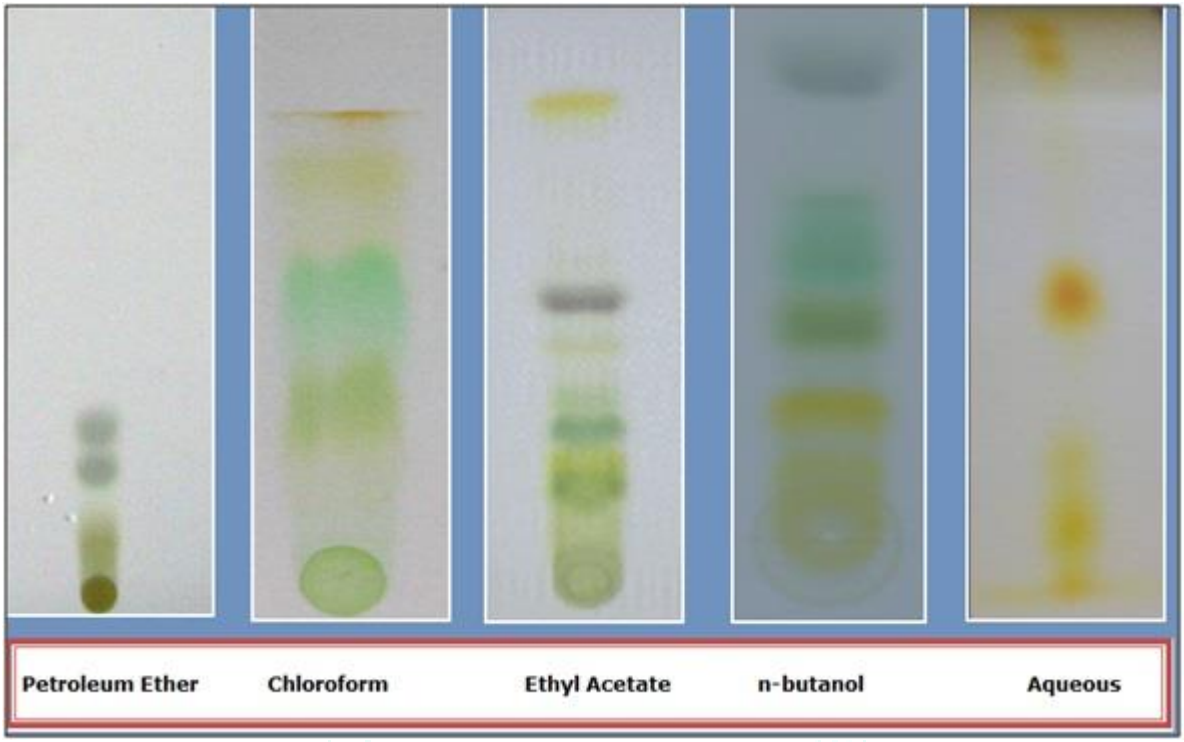

Fig. 5. Thin layer chromatography of Albizia lebbeck.

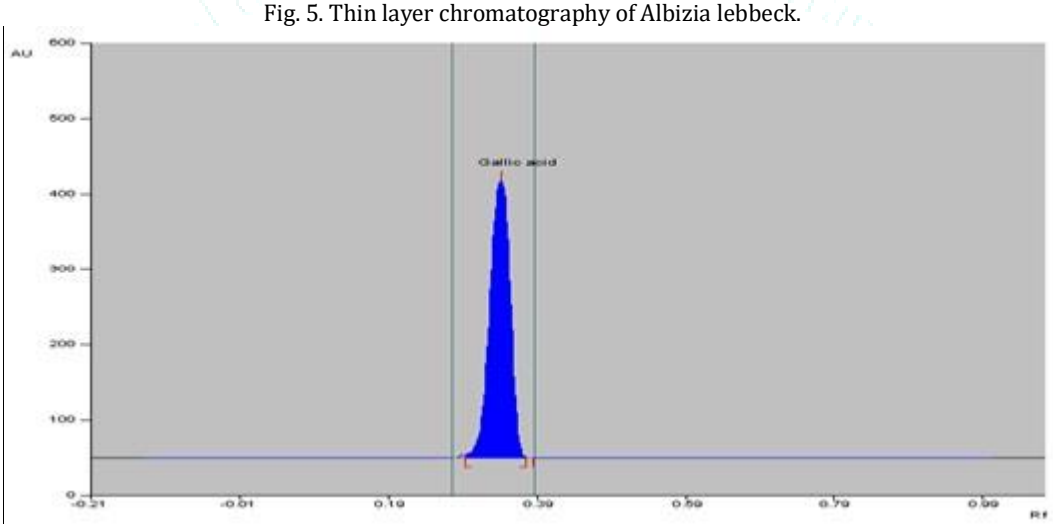

Fig. 6. HPTLC chromatogram of ethyl acetate fraction of Albizia lebbeck

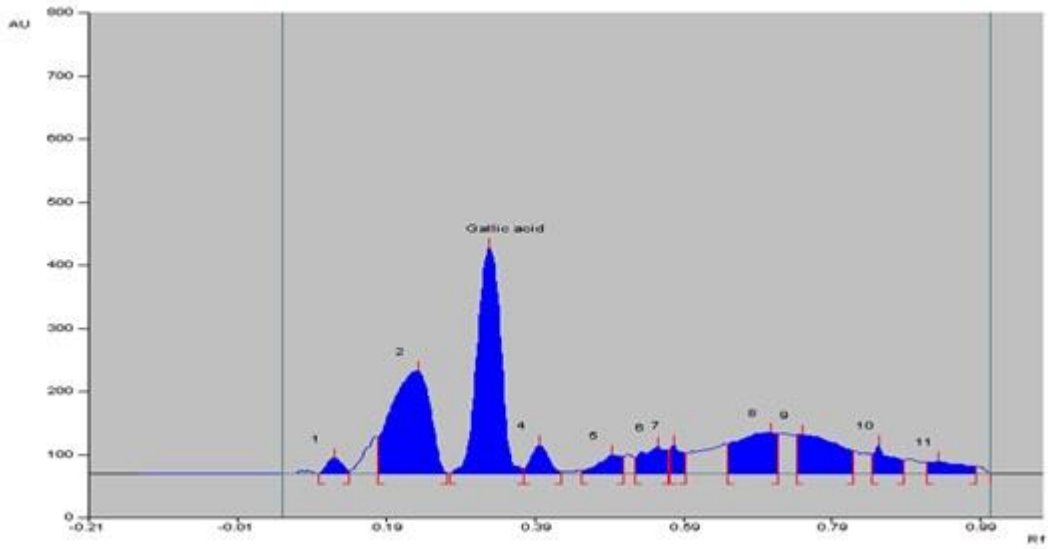

Fig. 7. HPTLC chromatogram of peak of standard Gallic acid.

DISCUSSION

The use of plants in medicine is booming up. Now in the developed countries also people are returning to nature. Use of traditional medicine is the mainstay of primary healthcare, virtually in all developing countries. The reasons for the frequent use of traditional medicine being (i) the strong association of people with local flora and their belief on traditional knowledge regarding plants as medicine, (ii) easy availability of local medicinal plants, (iii) relatively poor access to allopathic drugs and their high cost and (iv) lower economic profile of the rural people [32].

Use of traditional medicine particularly in developing countries is the mainstay of primary healthcare. The major raw materials for such products are different medicinal plants. Therefore, the first step towards the quality herbal drug is the authenticated raw material for which pharmacognostic parameters are very important. *Albizia lebbeck* can easily be distinguished on the basis of morpho-microscopic characters, but it becomes very difficult when the drug is in dried and matted condition. In this context, some reliable characters which can be used for identification of crude drug are arc shaped vascular bundle and anomocytic type stomata seen in T.S. of leaf midrib, while T.S. of root shows starch grains and calcium oxalate crystals present throughout the phloem tissue in abundance. In T.S. of stem the phloem comprises of sieve tubes, companion cells and parenchyma cells. The pith consists of mass of sclereids which are circular, wide-lumened and lignified.

Microscopic analysis and physicochemical parameters are carried out on plant samples in order to establish appropriate data that may be utilized not only for identification but also to establish the purity and standard of crude drug, particularly those supplied in powder form. The phytochemical screening of the drug is a very sensitive aspect in the process of standardization and quality control because the constituents vary quantitatively and qualitatively not only from plant to plant but also in different samples of the same species depending upon various atmospheric factors and storage condition[32]. These standard pharmacognostic parameters can be used to differentiate closely related plant species or varieties with similar constituents or pharmacological activity. Chromatography is used for the isolation and identification of various substances present in the drug. In the present study thin layer chromatography (TLC) has been conducted for the separation of different components and Rf value of developed spots in different solvent system have been noted. Rf value can also be used as a tool for the standardization of drug[32]. These parameters may be utilized not only for the identification of the drug but also to establish its purity and standards.

CONCLUSION

It can be concluded that the **s**tandardization of herbal drugs is very important as they are derived from heterogeneous sources. The pharmacognostic characters and phytochemical values reported in this paper could be used as a diagnostic tool for the standardization of this medicinal plant. Adulterants if any can be easily identified using these parameters. The microscopic features could help in laying down micromorphological standards as per WHO guideline for authentication of the drug. HPTLC profile helps in establishing marker component. One marker component with Rf 0.46 was identified as Gallic acid. The occurance of Gallic acid, an anti-cancer, anti-inflammatory and antioxidant is significant[33-34]. The Pharmacognostical study carried out with a focus on bringing out diagnostic character will be of immense help in the proper identification and standardization of *Albizia lebbeck*. So, the efforts were made to provide the scientific data to standardize the plant material.

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