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Original Article

Comparative pharmacognostic investigation of stems and barks of *Albizia lebbeck*

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ABSTRACT

The standardization of *Albizia lebbeck* was carried out as per Ayurvedic Pharmacopoeia of India, Pharmacopoeial standard for Ayuvedic Formulation and as per the W.H.O. parameters of quality control for medicinal plants. *Albizia lebbeck* is wild plant having a number of ethnomedicinal applications. In the present study, the stem and bark of the plant were subjected to comparative pharmacognostical investigation. The study includes macroscopy, microscopy, preliminary phytochemical screening, physicochemical evaluation and fluorescence analysis. Qualitative analysis of plant metabolites like alkaloid, carbohydrates, protein, resins, starch have been carried out.

1. INTRODUCTION

The *Albizia lebbeck*, is commonly known as siris mainly found to the India, Indonesia, Malaysia, Myanmar, Nepal, Pakistan, Thailand. It is a medium to large sized unarmed deciduous tree. The stems are green to brown, fruity odor, taste is bitter, cylindrical shape, rough irregularly cracked surface characteristics. The barks grey to dark brown, fruity odor, taste is bitter, cylindrical shape, and rough irregularly cracked bark in surface characteristics. It mainly contains tannins of condensed type, viz. D-catechin, isomers of leucocyanidin and melacacidin and a new lysine leucoantho-cyanidin, lebbecacidin. It also gives triedelin and t-3-sitosterol. It is a proven drug for leprosy, paralysis, gum inflammation, worm infestation, astringent, cough, treatment of eye, flu, gingivitis, lung problems, pectoral problems, abdominal tumors, psychoactive, antioxidant, antimicrobial, antimalarial, hepatoprotective, antispermatogenic and carcinogenic. It also reduces the release of histamines through a stabilizing effect on mast cells mildly suppresses activity of T-lymphocytes reducing the level of allergy-inducing antibodies [1-3].

2. EXPERIMENTAL

Materials and Methods

Plant Material

The plant material (stem and barks) were collected from (Gangoh) distt. Saharanpur U.P., India. Stem and Barks were then washed to remove adhering material, shade dried and powdered (#60) using a blender. The powders were stored in an airtight container. An exhaustive pharmacognostic work was carried out by using standard methodology [4, 5].

Pharmacognostical Studies

The various pharmacognostical parameters were studied as per standard protocols, which include, macroscopy, microscopy and power examination [6-9].

Macroscopy

Morphological studies of stem and barks such as color, size, odor, taste, surface characteristic and fracture were carried out.

Microscopy

Free hand transverse section of fresh stem and barks were taken, cleaned in 5% KOH solution with gentle warming, stained with phloroglucinol and concentrated hydrochloric

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acid. They were mounted on slide in glycerin and studied under microscope. Microphotographs of sections were documented using microscope with camera.

Powder Examination

Slide for powder microscopy were prepared for determination of powder characteristics of the stem and barks.

Physicochemical Parameters

Physicochemical parameters of the powdered drug such as loss on drying, total ash, acid-insoluble ash, water-soluble ash, alcohol and water soluble extractive values for the stem and barks of *Alibizia lebbeck* were performed according to the standard methods [4, 11, 15, 16].

Successive Extraction with various Solvents

Successive extraction was carried out with ethanol and water, the extracts were dried using rotary evaporator and percentage yield were determined [4, 11, 15, 16].

Phytochemical Screening

The powder of the air dried stem and barks of *Alibizia lebbec*k weighing about 50 gm were successively extracted in Soxhlet apparatus with the solvents of increasing polarity such as petroleum ether, ethanol and water. The extracts were dried using rotary evaporator and percentage extractive values were determined. The dry extracts were screened for the presence of various phytoconstituents [6, 12, 17, 18].

Chromatographic Evaluation

The chromatographic studies were performed using various solvent systems to confirm the phytochemical studies. Prepared silica gel TLC plates were used for the chromatographic evaluation. Finally R_f values were calculated [12-14].

3. RESULTS AND DISCUSSION

Macroscopic and Sensory Characters

Stem: The stem is green to brown, fruity odor, taste is bitter, cylindrical shape, and rough irregularly cracked stem in surface characteristics.

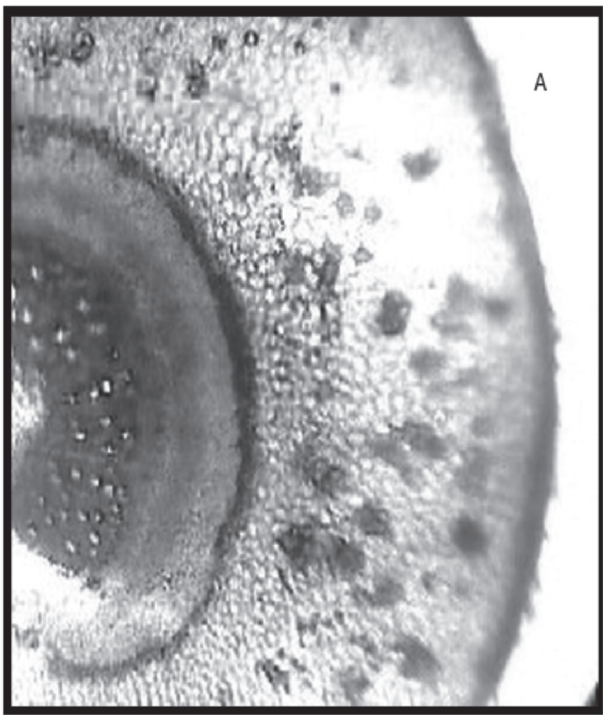
Fig. 1. Photograph showing morphology of stem of *Albizia lebbeck*

Bark: The barks grey to dark brown, fruity odor, taste is bitter, cylindrical shape, and rough irregularly cracked bark in surface characteristics.

Fig. 2. Photograph showing morphology of bark of *Albizia lebbeck*

Microscopic Characters

Stem: The transverse section of stem showed cork cells, vascular bundles, medullary rays, calcium oxalate crystal, cortex region, vascular bundles, pith were present.



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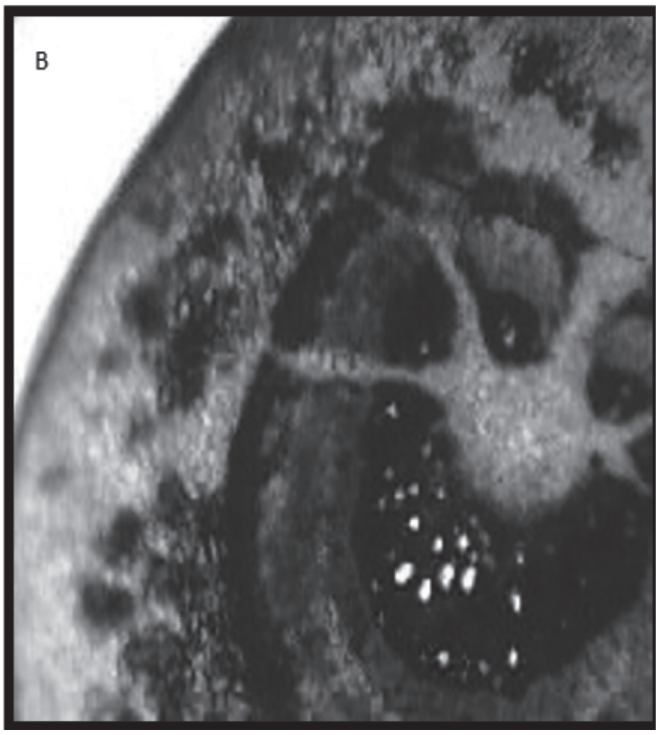


Fig. 3. Microscopic observations of fresh sample of Sem of *albizia lebbeck* (a) Cork cells, Vascular bundles, medullary rays, Calcium oxalate crystal, (b) Cortex region, Vascular bundles, Pith

The powder of stems showed vessels, trichomes, starch grain, lignified cells, fibers, xylem vessels, oil globules were also present.

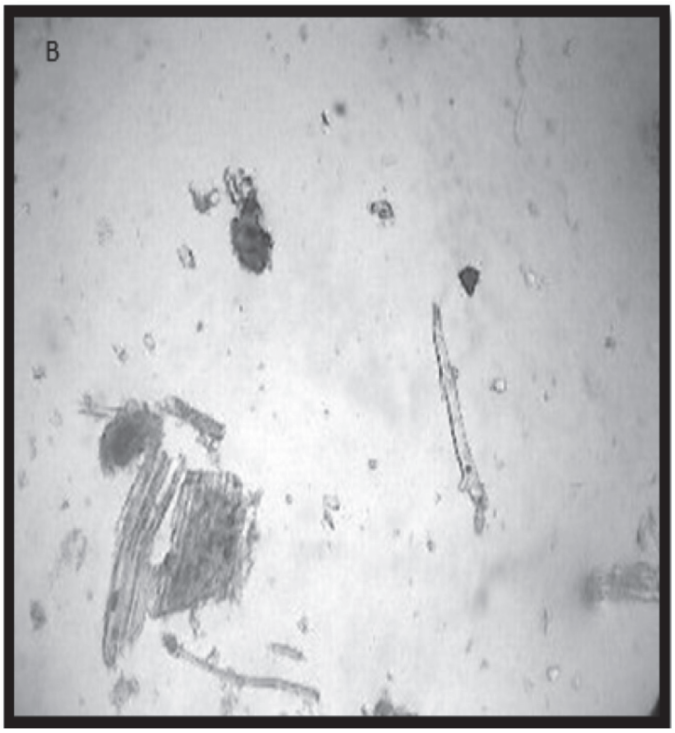
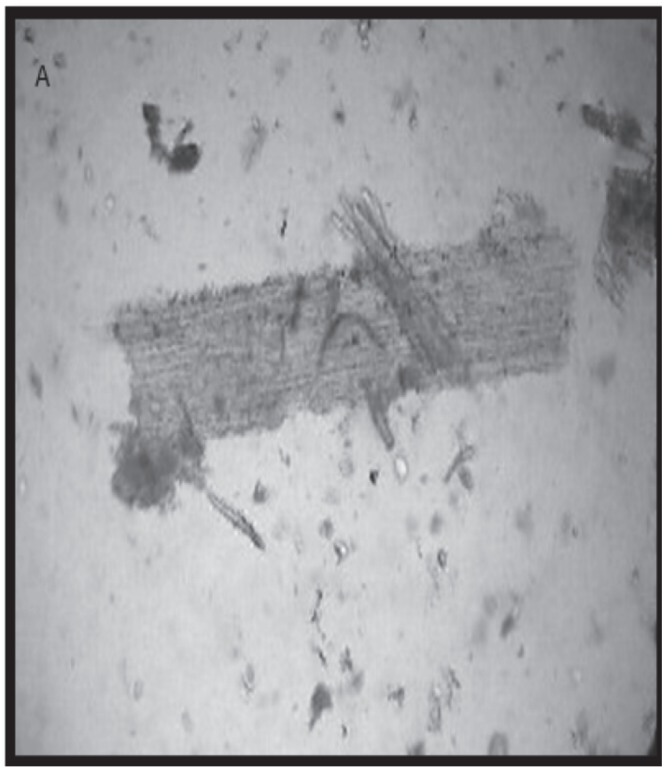


Fig. 4. Microscopic observations of fresh sample of stems of *Albizia lebbeck* (a) Vessels, Trichomes, Starch grain, Lignified cells, (b) Fibers, xylem vessels, Oil globules

Bark: The transverse section of barks showed fibers showing thicked cells of the parenchymatous cells, calcium oxalate crystals, cortex, pigment, starch grain, calcium oxalate crystals were present.



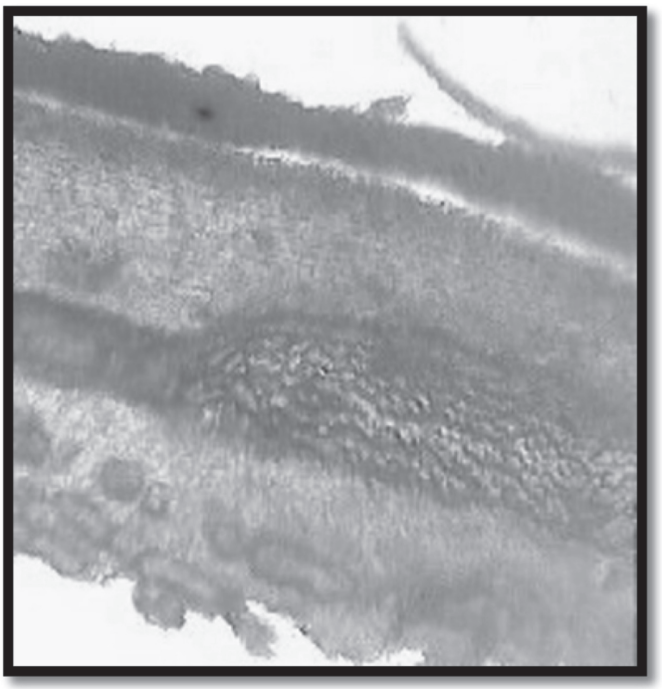


Fig. 5. Microscopic observations of fresh sample of barks of *Albizia lebbeck.* Fibers showing thicked cells of the parenchymatous cells, calcium oxalate crystals, cortex, pigment, starch grain

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The powder of barks showed fibers, starch grain, oil globules were also present.

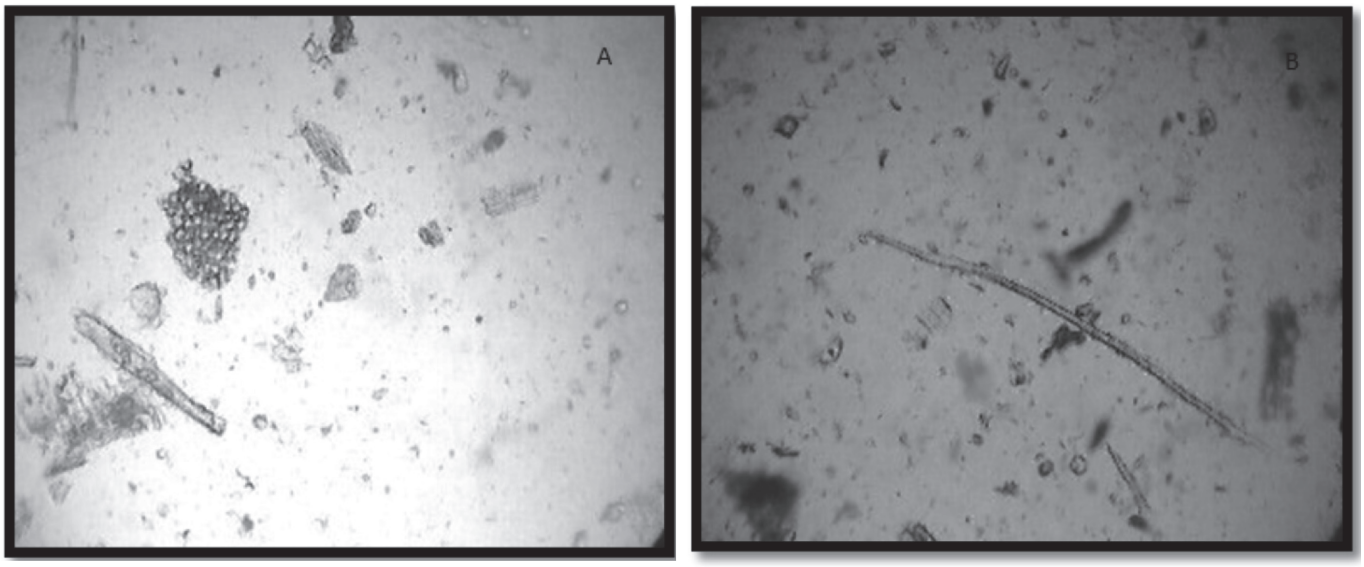

Fig. 6. Microscopic observations of fresh sample of barks of *Albizia lebbeck*
(a) Vessels, Trichomes, (b) Fibers, xylem vessels, Oil globules Fluorescence Analysis

The result are summarized in Table 1.

Table 1. Fluorescence analysis of powdered *Albizia lebbeck* stem and bark

S. No.	Powder Crude Drug + Reagent	Stems			Barks		
		Day Light	**UV (Short) 254 nm**	**UV (Long) 366 nm**	**Day Light**	**UV (Short) 254 nm**	**UV (Long) 366 nm**
1.	Powder Crude Drug as such	Yellowish Green	Dark Green	Light Green	Light yellow green	Dark green	Light yellow green
2.	Drug + 1M NaOH	Dark brown	Blackish green	Dark green	Dark brown	Blackish green	Dark green
3.	Drug + 1M NaOH + Methanol	Yellowish brown	Blackish green	Yellowish green	Dark brown	Blackish green	Dark brown
4.	Drug + 1M NaOH + Water	Yellowish brown	Blackish green	Yellowish green	Dark brown	Blackish green	Yellowish brown
5.	Drug + 1M HCl	Yellowish green	Dark green	Dark green	Light yellow green	Dark green	Yellowish green
6.	Drug + dil. HNO_3	Yellowish green	Blackish green	Dark green	Light yellow green	Dark green	Dark yellowish green
7.	Drug + 5% Iodine	Dark green	Blackish green	Blackish green	Dark green	Blackish green	Blackish green
8.	Drug + 5% $FeCl_3$	Blackish green	Blackish green	Blackish green	Dark green	Blackish green	Blackish green
9.	Drug + dil. Ammonia	Yellowish green	Dark green	Green	Yellowish green	Dark green	Light green
10.	Drug + Methanol	Light green	Dark green	Light yellowish green	Light green	Dark green	Light yellowish green

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11.	Drug + dilute HCl	Light brown	Dark green	Yellowish green	Light yellow green	Blackish green	Dark green
12.	Drug + 1M H_2SO_4	Brownish green	Blackish green	Dark green	Yellowish green	Dark green	Yellowish green
13.	Drug + Conc. HNO_3	Reddish brown	Blackish brown	Blackish brown	Reddish brown	Dark brown	Blackish brown
14.	Drug + $K_2Cr_2O_7$	Dark brown	Dark green	Blackish brown	Dark brown	Blackish brown	Blackish brown
15.	Drug + Ethanol	Yellowish green	Yellowish green	Green	Yellowish green	Dark green	Light yellowish green
16.	Drug + Toluene	Light green	Dark green	Light yellowish green	Light green	Dark green	Light yellowish green

Physicochemical Parameters

The moisture contents were 11.4% (stem) and 11.6% (barks) which were not so high as to facilitate bacterial growth. The other physicochemical parameters which ascertain the quality, purity and also help in evaluating the crude drug, are the ash value, acid insoluble ash value and water soluble ash value which were determined to be not more than 6.5 %w/w, 5.0 %w/w, 3.0 %w/w (stem), and 5.0 %w/w, 1.0 %w/w, 1.0 %w/w (barks) respectively which indicated the presence of the total foreign inorganic matter. While study of extractive values can serve as a valuable source of information and provide suitable standards to determine the quality of plant material in future investigations or application (Table 2, 3 & 4).

Table 2. Physicochemical parameters of *Albizia lebbeck*

Parameter	(Mean)^n %w/w (Stem)	(Mean)^n %w/w (Barks)
Total ash value	6.5	5.0
Acid insoluble ash	5.0	1.0
Water soluble ash	3.0	1.0
Loss on Drying	11.4	11.6

* Where n = 3 on dry weight basis

Table 3. Extractive values of *Albizia lebbeck*

Parameter	(Mean)^n %w/w (Stem)	(Mean)^n %w/w (Barks)
Water soluble extractive values	7.2	9.6
Alcohol soluble extractive values	4.8	0.8

* Where n=3 on dry weight basis

4. PRELIMINARY PHYTOCHEMICAL INVESTIGATION

Preliminary phytochemical screenings were performed to find out the phytoconstituent present in the stem and barks of *Albizia lebbeck.*

Table 4. Preliminary phytochemical screening of stem and barks of *Albizia lebbeck.*

Test	Stem	Barks
Alkaloids	-	+
Glycoside	-	-
Tannin	+	-
Saponins	+	+
Steroids	-	-
Flavonoids	+	-
Carbohydrates	+	-
Amino acids	-	-
Proteins	-	-

(+) = Present (-) = Absent

Chromatographic Studies

After phytochemical screening, chromatographic studies were carried out. The TLC analysis

The TLC analysis (for stems) on using general solvent system for non-polar compounds i.e. Hexane: Ethyl Acetate (8:2) obtaining 2 spots with R_f values 0.74 and 0.70. The solvent system specific for tannins i.e. Ethyl acetate: Benzene (9:11) giving 2 spots with R_f value 0.48 and 0.32. Solvent system specific for Saponins i.e. Chloroform: Carboxytetra chloride: Acetone (2: 2: 1) with 6 spots of R_f value 0.80, 0.78, 0.77, 0.41, 0.42 and 0.41. Solvent specific for Flavonids i.e. Chloroform: Acetone: Formic acid (7.5: 16.5: 13.5) getting 3 spots having R_f value 0.50, 0.55 and 0.63. Similarly the solvent specific for Alkaloids i.e. Chloroform: Diethyl amine (9: 1) obtaining 1 spots with R_f value 0.89.

The TLC analysis (for barks) on using general solvent system for non-polar compounds i.e Hexane: Ethyl Acetate (8:2) obtaining 1 spots with R_f values 0.68. The solvent system specific for tannins i.e. Ethyl acetate: Benzene (9:11) giving 2 spots with R_f value 0.65 and 0.87. Solvent system specific for Saponins i.e. Chloroform: Carboxytetra chloride: Acetone (2: 2: 1) with 6 spots of R_f value 0.77, 0.74, 0.73, 0.44, 0.44 and 0.46. Solvent

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specific for Flavonids i.e. Chloroform: Acetone: Formic Acid (7.5: 16.5: 13.5) getting 6 spots having R_f value 0.56, 0.58, 0.56, 0.60, 0.61 and 0.65. Similarly the solvent specific for Alkaloids i.e. Chloroform: Diethyl amine (9: 1) obtaining 1 spots with R_f value 0.98.

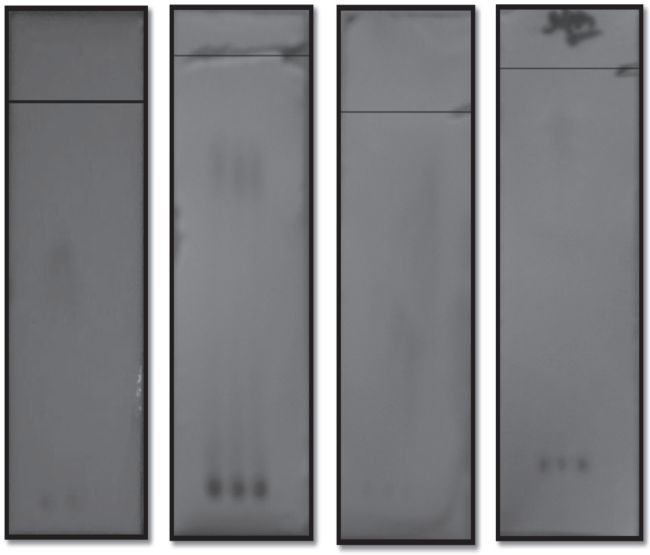

Fig. 7. TLC analysis of stem of *Albizia lebbeck.*

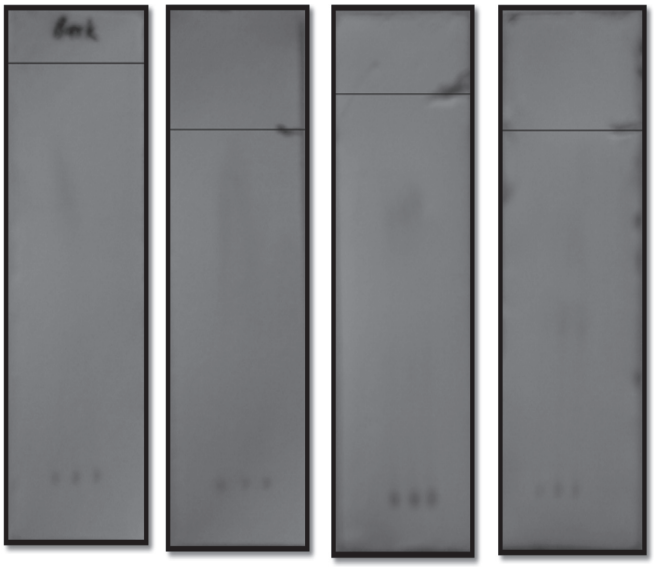

Fig. 8. TLC analysis of bark of *Albizia lebbeck.*

The stem was green to brown in color, with fruity in young leaves odor and immense bitter taste. The shape was cylindrical with rough texture and fibrous fracture. The dried, irregular, broken, shriveled cut pieces of barks were very light varying in size and shape, usually cylindrical, about 7-9 cm in length and 2-4 cm in thickness, bitter in taste. The stem and barks of *Alibizia lebbeck* were subjected to microscopical studies using transverse section and powder microscopy. The transverse section of stems showed cork cells, vascular bundles, medullary rays, calcium oxalate crystal, cortex region, vascular bundles, and pith. Cork was followed by a wide zone of polygonal cortex varying in size and shape. Medullary rays were straight. Calcium oxalate crystals were present. The transverse section of barks showed fibers showing thicked cells of the parenchymatous cells, calcium oxalate crystals, cortex, pigment, starch grain. Calcium oxalate crystals were present. The powder of stem showed vessels, trichomes, fibers, xylem vessels, oil globules were also present. The powder of barks showed fibers, starch grain, oil globules were also present. Physiochemical parameters and extractive value of stem and barks of *Alibizia lebbeck* were studied and results were shown in Table 3, 4 and 5 respectively. Preliminary phytochemical studies on the plant revealed the presence of alkaloids, glycoside, tannins, saponins, steroids, and flavonoids. The preliminary phytochemical test results were rationalized by the thin layer chromatographic studies.

4. CONCLUSION

Detail pharmacognosy of stem and barks of *Albizia lebbeck* has been worked out which helped to resolve ambiguity of species delimitations and proper use values of valued species. Morphological and anatomical studies of the leaves stem and barks will enable to identify the crude drug. The information obtained from preliminary phytochemical screening will be useful in finding out the genuity of the drug. Ash values, extractive values can be used as reliable aid for detecting adulteration.

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