Original Article

Pharmacognostical, Physicochemical & Phytochemical Studies On Roots Of Bombax Ceiba Linn

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Abstract

Context: Bombax ceiba Linn. (Bombacaceae) is a well-known plant for its antihypertensive, antioxidant, antidiabetic, aphrodisiac and uterine tonicity properties.
Aims: To study pharmacognostical, physicochemical and phytochemistry of the roots of this plant.
Methods: Pharmacognostical study included the macroscopic characters like size, color, surface characteristics, texture, fracture characteristics and odor of the roots. The intact roots as well as powdered drug were studied under a microscope to analyze the cellular characteristics of the drug. Powder drug also study for microbial count & heavy metal contents. Physicochemical parameter like extractive values, loss on drying (LOD), total ash, water-soluble and acid insoluble ash, foaming index and hemolytic index of Bombax ceiba root powder were determined as per WHO guidelines. Preliminary phytochemical screening and qualitative chemical examination studies have been carried out for the various phytoconstituents. HPTLC have also carried out using cyclohexane: diethyl ether: ethyl acetate as mobile phase. Quantitative estimation like Total phenolic contain, Total flavonoid contain, Total saponin contents are also determined.
Results: The microscopic characters have shown presence of cork, cambium, xylem vessels, stone cells, starch grains, calcium oxalate crystals and phloem fibers. Microscopy analysis of the powder included the cork cells, fibers, calcium oxalate crystals and vessel. Chemical evaluation and TLC studies shown presence of alkaloids, glycosides, flavonoids, steroids, saponins and tannins. The presence of steroids was confirmed in HPTLC fingerprinting studies.
Conclusions: Pharmacognostical and preliminary phytochemical screening of Bombax ceiba roots will be useful in order to authenticate standardize and avoid any adulteration in the raw material. The diagnostic microscopic characters and physicochemical data will be helpful in the development of a monograph. The chromatographic fingerprinting profile can be used to standardize extracts and formulations containing Bombax ceiba roots.

Keywords: phytochemical, studies, pharmacognostical.

Introduction

Standardization of herbal drugs is very much important to establish their products in the global markets. Standardization starting from production of quality materials, analysis of raw materials for authentication, presence of foreign matter, organoleptic evaluation, microscopic examination, extractive values, chromatographic profiles, pesticides residue, presence of heavy metals, etc., is necessary for standardization of drugs. Similarly, the standardization methods of medicinal plants and its extracts have great importance in the fields of cosmetics and nutraceuticals, which are emerging as the two most important segments in the global markets1.

The present study has been performed on Bombax ceiba Linn or Bombax malabaricum D.C. or Salmalia malabarica (DC.) Schott & Endl is belonging to family Bombacaceae2. The therapeutic effects have been reported in roots, gums, stem bark, flowers, seeds, prickles and young fruits. The family Bombacaceae consists of 22 genera and 150 species. Genera Bombax consists of 60 species, Ceiba 15 species, Durio 15 species, Salmalia 10 species and Adansonia 10 species3. This tropical tree has a straight tall trunk and its leaves are deciduous in winter. Red flowers with five petals appear in the spring before the new foliage. It produces a capsule which, when ripe, contains white fibers like cotton. Its trunk bears spikes to deter attacks by animals4. Reports have shown the presence of glycosides and tannins in roots, stem and leaf. In the stem some alkaloids and in roots, proteins are identified. The stem bark and root contains mangiferin, lupeol and β-sitosterol (Fig. 1). The root bark has 3 naphthalene derivatives related to gossypol (toxic principle of cotton seed) and called as 'semigossypol'. Flowers contain β- sitosterol, traces of essential oil, kaempferol and quercetin. On hydrolysis gums yields arabinose, galactose, galacturonic acid and rhamnose5. It has been claimed in Ayurveda that Bombax ceiba possesses proven medicinal properties and is the ingredient of many

formulations.

The roots are sweet, cooling, stimulant, restorative, astringent, alternative, aphrodisiac, demulcent, emetic and tonic. It is used in the treatment of diarrhea, dysentery, menorrhagia, styptic and for wounds. The gum is cooling, astringent, stimulant, aphrodisiac, tonic and demulcent in nature. It is useful in dysentery, hemoptysis, and pulmonary tuberculosis, influenza, burning sensation, menorrhagia and enteritis. Bark is mucilaginous, demulcent, emetic and tonic. Flowers are astringent and good for skin troubles and hemorrhoids. Seeds are useful in treating gonorrhea and chronic cystitis. A paste made out of prickles is good for restoring skin color especially on the face. Young fruits are useful in calculus affections, chronic inflammations and ulceration of bladder and kidney4, 5.

Numbers of reports are available on pharmacognostic and phytochemical studies of stem6, 7 and leaf8, 9, 10, 11, 12 of Bombax ceiba Linn. The presence of certain secondary metabolites in Bombax ceiba has been reported, in the present study an attempt has been made to perform the pharmacognostic studies and proximate analysis of Bombax ceiba roots. The preliminary phytochemical screening of the roots was also be done by extraction in different extracts and then performing chemical tests and TLC studies of the same.

Materials And Methods

Plant material

Roots of Bombax ceiba were collected from Govt. Vidharbha Institute of Science & Humanities localities, Amravati (Maharashtra) in summer season (March-May) of

about 8 to 10 year old plant. The plant was identified and authenticated by Dr. Indrapratap S. Thakare Department of Agriculture Botany, P. R. Pote Patil College of Agriculture, Amravati (MS) (Ref. No. PRPPCA/Certificate/576/2020-21) and dried in the shade at room temperature. Dried roots were powdered in grinder and powder material was kept in air tight container for further study.

Macroscopic evaluation

The size, color, surface characteristics, texture, fracture characteristics and odor of the roots were studied 13.

Microscopic evaluation

The intact root, as well as powdered drug, was studied under a microscope to analyze the cellular characteristics of the drug.

Study of transverse section

The roots were taken in a test tube and 5% potassium hydroxide in methanol was added so that the sample remained submerged. The samples were boiled for few minutes. Transverse sections of the drugs were taken in a watch glass containing water with the help of a brush. The sections were then transferred to a watch glass containing phloroglucinol-hydrochloric acid (1:1) and allowed to stain for 2-3 minutes. The sections were again transferred to watch glasses containing water, so as the excess stain was washed away. The sections were then placed on clean glass micro-slides, with the help of a brush. Few drops of water were added, and a clean cover-slip was placed on the slide. The slides were mounted for study on the microscope13. The transverse sections were also stained using iodine, methylene blue, Sudan red to identify the starch, mucilage, and fats and fixed oils.

Study of powder characteristics

The microscopic structures of powdered drugs were also studied using the slides prepared by above method using powdered drugs in place of sections. The fiber length of the root powder was also determined using the reported methods13. The representative photos of sections were taken with the help of Digital Olympus microscope.

Physicochemical evaluation

Physicochemical parameter like extractive values, loss on drying (LOD), total ash, water soluble and acid insoluble ash, foaming index, hemolytic index, pesticide residues & heavy metal contents of Bombax ceiba root powder were determined as per WHO guidelines14.

Qualitative phytochemical evaluation

Extraction

The coarse powder of shade-dried plant were successively extracted with petroleum ether, chloroform, methanol, water:ethanol (30:70) and water. The extracts obtained were concentrated and dried under vacuum.

Determination of Microbial Count and Heavy Metal Content15

Contamination of medicinal plant materials with heavy metals like arsenic, cadmium and lead can be attributed to many causes including environmental pollution and traces of pesticides. The limit of this heavy metal can be determined by using atomic absorption spectrophotometer. To determine microbial level of aerobic bacteria like Escherichia coli, Staphyllococus aureus, Pseudomonas aeroginosa and heavy metal contents, the sample of plant extract has been sent to Nishka Research Pvt. Ltd. Hyderabad.

Pesticide Residue16

Medicinal plant materials are liable to contain pesticide residues which accumulate from agricultural practices, such as spraying, treatment of soils during cultivation, and administration of fumigants during storage. Pesticide residues were determined by performing simple TLC using mobile phase Dimethyl formamide: Ether (4:6) with detecting agent tetrabromophenolethalein for organochloride and 0.5% silver nitrate in water & Acetone (50:50) for organophosphorus as per AOAC (Association of Official Agricultural Chemists) international guideline.

Chemical tests

All plant extracts were subjected to chemical tests for the presence of following phytochemical classes like carbohydrates, alkaloids, anthraquinones, saponin glycosides, phytosterols, phenolics tannins, flavonoids, proteins and amino acids using reported methods13.

Thin layer chromatographic studies

The hydro-alcoholic extract obtained was subjected to thin layer chromatographic studies using reported methods to determine the presence of various phyto-constituents17. The results were compared with the results obtained in the qualitative tests. The mobile phases and detecting reagents of different classes of compounds were used18.

High-performance thin layer chromatography

A Camag microlitre sample (Hamilton, Bonaduz, Switzerland) syringe was used for a sample application on

pre-coated silica gel aluminium plate 60F-254 (5 cm x 10 cm with 0.2 mm thickness (E. Merck, Darmstadt, Germany) using a Camag Linomat-V (Switzerland). Samples were then separated using solvent systems cyclohexane: diethyl ether:ethyl acetate of different compositions. Densitometric scanning was performed on Camag TLC scanner III in the reflectance-absorbance mode for all measurements and operated by CATS software (V1.4.3 Camag). The plate was scanned at 254 and 366 nm before and after sparing. The detecting reagent used was anisaldehyde sulphuric acid reagent18. Reference standard lupeol was also applied on pre-coated HPTLC plates, and its presence in Bombax ceiba root extracts was confirmed. The samples were prepared by dissolving 500 mg hydro-alcoholic extract of Bombax ceiba root powder in 5 ml distilled water. All the studies were performed three times.

Results And Discussion

The morphological studies of roots of Bombax ceiba show the shape of the root is more or less cylindrical, slightly tapering. Color of the unpeeled root is yellowish brown to dark brown while a peeled root is pale yellow with a rough surface. All the organoleptic features studied have been summarized in Table 1.

In transverse section (Fig. 2), roots are characterized by the presence of thick brownish continuous eight to ten layers of tubular cells. Outer layers contain reddish brown amorphous cells, and inner layers show thick-walled cells. Single flattened layer of endodermis were converted with distinct medullary rays of multiseriate parenchymatous cells. The phloem was presence of thick walled oval shape cells of lignified parenchymatous sheath. Cambium was observed by double walled polygonal cells of xylem in horizontal tracks. Crystals of calcium oxalate also present with simple or compound starch grains. Small circular pith was seen with dark brown colored outer covering with intercellular spaces at center. Table 2 shows the results of micro-chemical tests on transverse sections of Bombax ceiba. Powder analysis of Bombax ceiba root observed that polygonal cells of parenchyma pitted type of vessels, unicellular, bunches of fibers with yellow thick mass of tissues along with mesh-like structure. Fig. 3 shows the presence of the cork cells, fibers, calcium oxalate crystals and vascular bundles.

The fiber length was also determined and found to be 2.7 x 33.75 µm. This value can be used as an identifying characteristic for Bombax ceiba roots. The proximate analyses revealed are summarized in Table 3. The phytochemical evaluation showed the presence of saponins, thus foaming index and hemolytic index were determined. The results are summarizing in table 3 along the results for pesticide residues and heavy metal contents.

Hydroalcoholic extract of Bombax ceiba roots shows the antibacterial activity against Escherichia coli, Staphyllococus aureus, Pseudomonas aeroginosa, Bacillus subtilis and Salmonella typhi.

Successive solvent extraction values in various organic solvents were observed as with petroleum ether 1.1%, chloroform 2.2%, methanol 5.3% and water 15.0% in percentage yield. The qualitative phytochemical evaluation by chemical tests of all the extracts obtained after successive extraction showed the presence of alkaloids, glycosides, flavonoids, saponins and tannins. Result is summarized in Table 4. TLC studies of the crude hydroalcoholic extract also showed the presence of similar constituents as shown in Table 5.

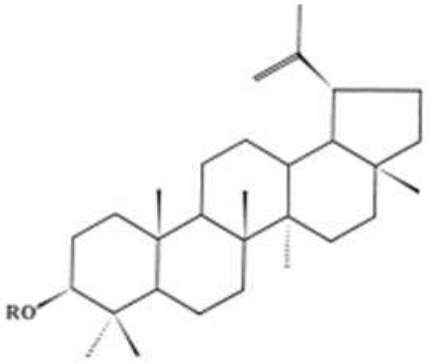
HPTLC fingerprinting studies was performed on the hydro-alcoholic extract of Bombax ceiba roots. The results obtained are depicted in Fig. 4. The presence of steroids was confirmed by use of detecting agent, anisaldehyde-sulphuric acid. Table 6 shows the comparative Rf values observed. The presence of lupeol in the hydroalcoholic extracts was confirmed by HPTLC at Rf value ~ 0.26 (Fig. 5).

Through screening of the literature on B. ceiba shows that it is a traditional plant by ethnobotanical way. The crude extract and aqueous extracts of stem bark, root and leaf of B. ceiba have be screened for some pharmacological activities like hepatoprotective, antiangiogenic, analgesic

and antioxidant, hypoglycemic, antimicrobial activity and cholinesterase activity. Other parts of plants such as gum, seed and seed oil which are well documents to possess valuable medicinal properties are not explored for their biological potential12. In future study the isolated principle from B. ceiba needs to be evaluated in animal model and clinic trial to understand the molecular mechanism of action, in search of lead molecules from natural resources.

Conclusions

The present study gives an account on the preliminary pharmacognostic and phytochemical screening of Bombax ceiba roots which may useful in order to authenticate, standardize and avoid any adulteration. The diagnostic microscopical characters and physicochemical data reported in this paper will be helpful in the development of a monograph. TLC & HPTLC studies help to verify adulteration in quality control of crude extract. Further due to the presence of various phytochemicals which may be therapeutically active, pharmacological screening of roots of Bombax ceiba extracts can also be performed.



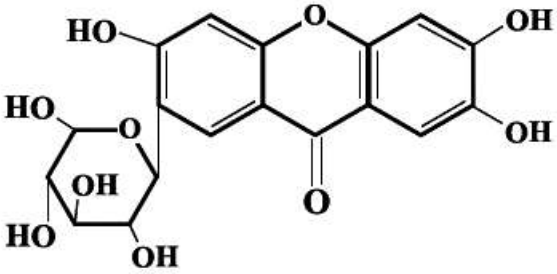


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Figure 1. Chemical structure of (A) lupeol, (B) mangiferin present in B. ceiba.

Table 1. Organoleptic features of Bombax ceiba roots.

Features	*Observations*
Shape	Cylindrical
Width	1-5 cm
Length	20-50 cm
Color	Peeled-pale yellow, unpeeled yellowish brown to dark brown
Odor	Faint and characteristic
Taste	Characteristic, free from bitterness

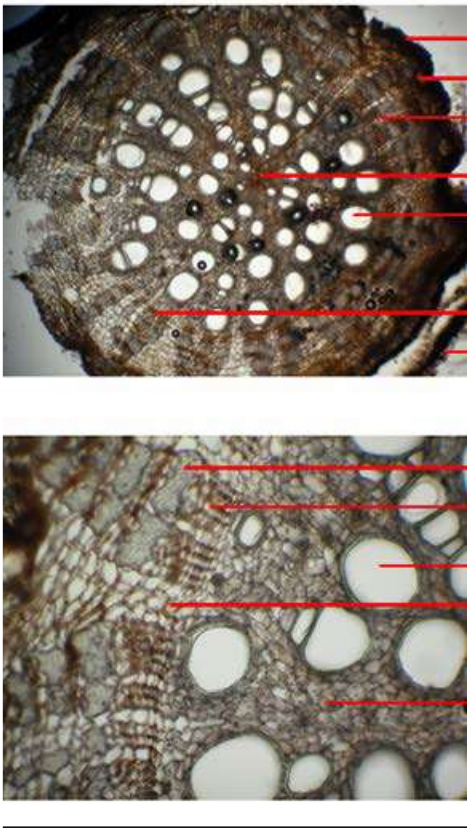


Figure 2: Transverse section of Bombax ceiba roots. Cork with eight to ten layers of tabular cells. Phelloderm: One to three layers of radially arranged parenchymatous cells containing stone cells. Phloem fibers: Thickened walls, Lignified parenchymatous sheath. Medullary rays: Distinct, multiseriate parenchymatous cells. Xylem fibers: Thickened walls, parenchymatous sheath containing starch grains and calcium oxalate crystals. Pith: Parenchymatous cells with intercellular spaces.

Table 2. Micro-chemical tests performed on transverse section (TS) of *Bombax ceiba* roots

Test	***Observation***	***Inference***
TS + phlouroglucinol + conc. HCl	Pink colour observed	Fibres present
TS + iodine	Blue coloured granules	Starch granules present
TS + methylene blue	Blue colored bundle of cells absent	Mucilage absent
TS + methyl red	Distinguished cork cells and cork cambium	Differentiate Cork, cambium
Sudan red III	Red coloured droplet absent	Fixed oil, fats absent

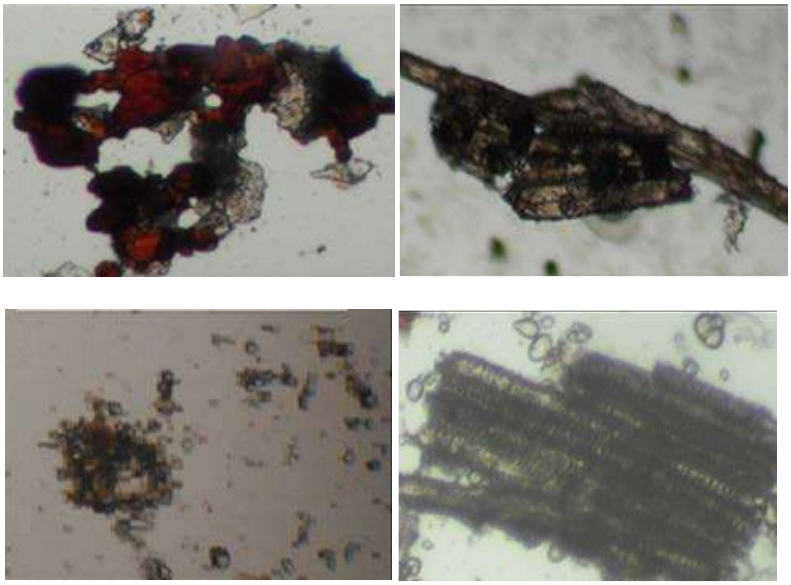


Figure 3: Microscopy of powdered Bombax ceiba Linn. roots. (A) Cork cell: Brownish, thin-walled, wavy cells are present. (B) Fibres: Occur in groups and be associated with the vessels. (C) Calcium Oxalate crystal: Minute prism shaped crystal present as cell content and scattered. (D) Vessels: Fragments of lignified reticulately thickened, annual and spiral vessels.

Table 3: Physicochemical parameters

S.No.	*Physicochemical parameters*	*Percentage values*
1	Loss on drying	6.82
2	Total ash value	4.83
3	Acid insoluble ash	0.475
4	Water soluble ash	4.355
5	Water soluble extractive value	16.8
6	Alcohol soluble extractive value	19.2
7	Pet. Ether soluble extractive value	9.6
8	Heamolytic index	500
9	Foaming index	142.86
10	Pesticide residues	
	a) Organochloride	Absent
	b) Organophosphorus	Absent
11	Heavy metal	
	a) Limit of Arsenic & Lead	Below detectable
	b) Limit of Cadmium	level
		1.2 ppm

Table 4. Preliminary phytochemical screening of *Bombax ceiba* root extracts.

Name of chemical test	Petroleum ether extract	Chloroform extract	Methanol extract	Water extract	
Carbohydrates	-	-	+	+	
Protein and amino acid	-	-	+	+	
Fat and oils	+	-	-	-	
Steroid	+	+	-	-	
Glycosides					
Cardiac glycosides	-	-	-	-	
Anthraquinone glycosides	-	-	+	+	
Saponin glycosides	-	-	+	+	
Flavonoids glycosides	-	-	+	+	
Alkaloids	-	-	+	+	
Tannins and phenolic compounds	-	-	+	+	

Table 5. TLC profile for hydro-alcoholic extract of *Bombax ceiba* roots powder.

Groups	Mobile Phase	Detection	Rf Value
Anthraglycosides	Ethyl acetate: methanol:water (100:13.5:10)	Bornträger reagent	Absent
Bitter Principles	Ethyl acetate: methanol:water (77:15:8)	Vanillin-Sulphuric acid reagent	0.62
Alkaloids	Toluene: ethyl acetate:diethylamine(70:20:10)	Dragendorff reagent	0.78
Flavonoids	Ethyl acetate: formic acid:glacial acetic acid: water(100:11:11:26)	Aluminium chloride reagent	0.91
Saponins	Chloroform: glacial acetic acid: methanol: water(64:32:12:8)	Vanillin-sulphuric acid reagent	0.42
Essential oils	Toluene: ethyl acetate(93:7)	Vanillin-sulphuric acid reagent	Absent
Coumarins	Diethyl ether: toluene(1:1) saturated with 10% acetic acid	UV 365 nm	Absent
Steroid	Cyclohexane: diethylether: ethyl acetate (4:6:2.5)	Anisaldehyde sulphuric acid	0.3,0.85, 0.88

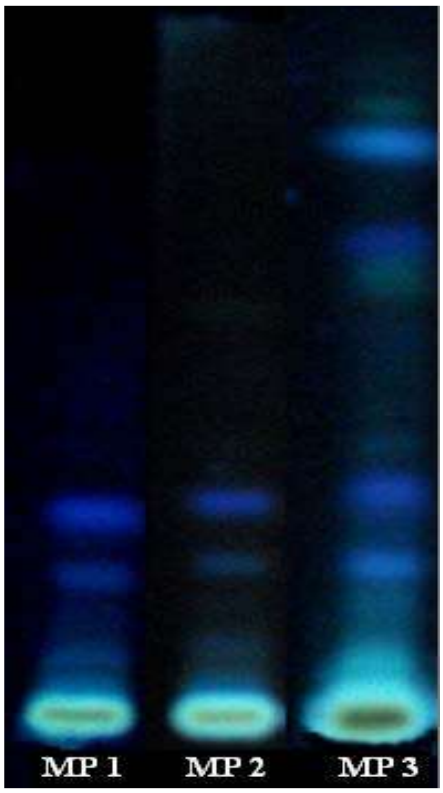


Figure 4: HPTLC fingerprinting profile of hydroalcoholic extracts of Bombax ceiba root powder.

MP 1 (Mobile Phase 1): Cyclohexane: diethyl ether: ethyl acetate (4:3:2.5)

MP 2 (Mobile Phase 2): Cyclohexane: diethyl ether: ethyl acetate (4:4:2.5)

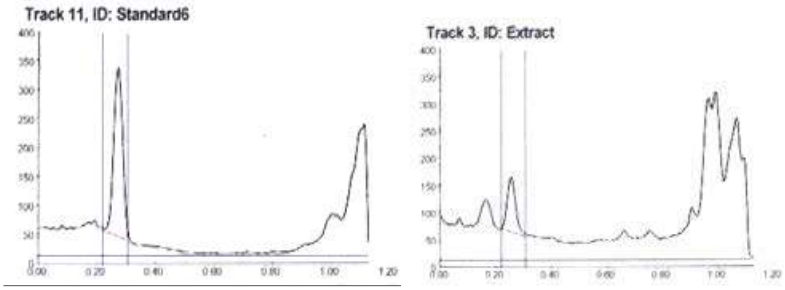
MP 3 (Mobile Phase 3): Cyclohexane: diethyl ether: ethyl acetate (4:6:2.5)

Table 6. HPTLC fingerprinting results of hydro-alcoholic extract of *Bombax ceiba* root powder.

Spot No.	*Rf values at 254 nm before spraying*			*Rf values at 366 nm before spraying*		
	MP 1	MP 2	MP 3	MP 1	MP 2	MP 3
01	0.05	0.05	0.05	0.05	0.05	0.05
02	0.09	0.09	0.24	0.36	0.12	0.10
03	0.34	0.21	0.36	0.60	0.35	0.24
04	0.48	0.38	0.67	0.62	0.39	0.37
05	0.60	0.54	0.76	0.70	0.97	0.67
06	0.62	--	0.86	0.75	--	0.87
07	0.72	--	--	0.82	--	0.94
08	0.75	--	--	0.85	--	--
09	0.82	--	--	0.93	--	--
Spot No.	*Rf values at 254 nm after spraying*			*Rf values at 366 nm after spraying*		
	MP 1	MP 2	MP 3	MP 1	MP 2	MP 3
01	0.12	0.21	0.10	0.11	0.10	0.10
02	0.20	0.36	0.18	0.34	0.15	0.14
03	0.27	0.42	0.26	0.45	0.20	0.16
04	0.30	0.50	0.31	0.50	0.27	0.19
05	0.36	0.67	0.36	0.59	0.30	0.22
06	0.40	0.70	0.40	0.61	0.35	0.25
07	0.45	0.80	0.44	0.63	0.40	0.29
08	0.53	0.86	0.52	0.67	0.43	0.31
09	0.61	0.90	0.57	0.70	0.59	0.34
10	0.66	0.96	0.62	0.75	0.70	0.37

11	0.75	--	0.68	0.82	0.93	0.67
12	0.85	--	0.78	0.85	--	0.84
13	0.92	--	0.85	0.87	--	0.97

Mobile Phase 1 (MP 1): Cyclohexane: diethyl ether: ethyl acetate (4:3:2.5)
Mobile Phase 2 (MP 2): Cyclohexane: diethyl ether: ethyl acetate (4:4:2.5)
Mobile Phase 3 (MP 3): Cyclohexane: diethyl ether: ethyl acetate (4:6:2.5)



(B)

Figure 5. (A) HPTLC chromatograms of lupeol standard (Rf value ~ 0.26) and (B) Hydroalcoholic extracts of Bombax ceiba root.

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