QUALITY CONTROL PARAMETERS OF PRICKLES OF *BOMBAX CEIBA* LINN.

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Keywords: *Bombax ceiba* Linn.; pharmacognostic; phytochemical; prickle; quality control.

Bombax ceiba Linn. is a deciduous tree known as Red silk cotton tree. *Bombax ceiba* stem, stem bark and prickle are used for the treatment of rheumatism, swellings, asthma and inflammation of the legs. The stem bark of *Bombax ceiba* is official in the ayurvedic Pharmacopoeia of India recommends its use in acne. Prickles are also used and recommended in anti-acne marketed formulation. However, limited work was carried out on the prickle toward establishing quality control parameters. Studies were therefore carried out to determine the phytochemical and pharmacognostic profile of prickle of *Bombax ceiba* Linn. The study, including determination of macroscopic, microscopic characters, ash values, extractive values, loss on drying, phytochemical screening, total phenolic content, TLC and HPTLC fingerprinting were carried out. Successive extracts were prepared using soxhlet extraction. The phytochemical analysis of extracts revealed the presence of tannin, saponin, phenol, carbohydrate, steroids, triterpenoids and flavonoids. Total phenolic and tannin content of methanol extract was found 327.78±1.09mg GAE/gm equivalent and 4.98±0.22%w/w, respectively. The mobile phase showing distinct spots in TLC was found in Toluene: Ethyl acetate (9.5:0.5) and (9:1) for petroleum ether and methanol extracts, respectively. HPTLC fingerprinting shows 8 & 3 peaks at 366nm for petroleum ether and methanol extract, respectively.

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INTRODUCTION

Bombax ceiba L. is a tall deciduous tree, buttressed at base, belongs to the Bombacaceae family.[1] *Bombax ceiba* scientific synonyms are *Bombax malabaricum* and *Salmalia malabarica*. The plant is also known as a silk-cotton tree, shalmali and shemlo. The plant is used for the treatment of gastrointestinal, skin diseases, gynecological, urinogenital disorders, general debility, diabetes and impotence. The plant parts like root, stem, stem bark and prickle are used for the treatment of rheumatism, swellings, bone fracture, asthma, snake bite, edema, hotness and inflammation of legs in several parts of Indian continent.[2] The bark of *Bombax ceiba* (*B. ceiba*) is official in The Ayurvedic Pharmacopoeia of India and it recommends its use in the treatment of acne.[3] Prickles of *B. ceiba* have been employed to treat acne face in many tribal communities.[3] According to *Charak Samhita*, the plant is among the top ten drugs to treat sepsis, bowel regulator and tissue regenerator.[4] According to *Sushrut Samhita* stem bark is useful in removing acne, hemorrhagic disorders, wound healing and in burns.[5] Patnakar has studied the *in-vitro* and *in-vivo* anti-acne potential of bark and thorns and results shown that an alcoholic extract of bark and thorns possess anti-acne potential.[6] It is interesting to note that prickles of *B. ceiba* are now an important ingredient of Himalaya's acne-n-pimple cream.[7] However, limited work has been carried out on the prickle toward establishing its physio-chemical & pharmacognostic quality control parameters like total tannin, total phenolic, total ash, loss on drying, TLC and HPTLC fingerprinting.[8,9] Therefore, the present research of *Bombax ceiba* prickle was taken up to establish quality control parameters of prickle, which will help in the identification of crude drug as well as in the standardization of the formulation. In our present investigation, we have carried out macroscopic, microscopic, ash values, extractive values, fluorescence analysis, phytochemical screening, total tannin, phenolic content, qualitative TLC as well as HPTLC analysis of prickle of *Bombax ceiba* plant.

MATERIALS AND METHODS

Chemical and reagents

Gallic acid, Folin Ciocalteu reagent, and methanol were purchased from S.D. Fine Chemicals ltd. Mumbai, India. Whatman (Florham Park, NJ) No. 1 filter paper was used for the filtration of the samples. All other chemicals and solvents used were purchased from Merck Chemicals, Mumbai, India. 10 X 10 cms HPTLC aluminum plate precoated with silica gel 60 F_{254} (0.2mm thickness) of Merck Pvt. Ltd. (India) were used for HPTLC analysis.

Collection of plant material, extraction and sample preparation

Prickles of *Bombax ceiba* (*B. ceiba)* were collected in the month of August-September 2007, from Gir Forest, Junagadh, Gujarat. The identity of the plant was also confirmed by the Department of Botany, Christ College, Saurashtra University, Rajkot. And Herbarium voucher specimen no. SU/DPS/Herb/07-08/2 was deposited at the Department of Pharmaceutical Sciences, Saurashtra University, Rajkot.

Sun-dried Prickle of *Bombax ceiba* was powdered by pulverizer and passed through 40 # sieve and stored in an airtight bottle. The powdered materials were used for further investigation. The powder of the drug, weighing about 150 g, was successively extracted in soxhlet apparatus with solvents of increasing polarity as follows, petroleum ether, benzene, chloroform, acetone, methanol and water. All extracts were concentrated by distilling the solvent and the extracts were dried on a water bath at 50 °C. Dried extracts were used further for fluorescence analysis, phytochemical screening, total tannin, total phenolic, TLC and HPTLC analysis.

Figure 1. Prickles of *Bombax ceiba*

Macroscopy and microscopy

In organoleptic evaluation, various sensory parameters of the prickle, such as color, odor, taste, shape and texture of the prickle were recorded. Fresh prickles were taken for microscopic studies. Sun-dried Prickles of *Bombax ceiba* were powdered by a pulverizer. Powder passed through 40 # sieve was used to study microscopical characters. For the microscopical studies, transverse sections of prickle were prepared and stained as per the standard procedure.[10,11] The powder microscopy was performed according to the method described by Khandelwal.[12]

Physico-chemical investigations

The dried prickle powder was used for the determination of loss on drying, ash values, extractive values, foaming index and swelling index.[13]

Fluorescence analysis

Then consistency, percentage yield, fluorescence examination of all successive extracts by color in daylight and ultraviolet light 366 nm were also studied which may help to confirm the purity of the drug.[14]

Preliminary phytochemical investigation

For the preliminary phytochemical investigation of prickle, extracts were done according to methods described in Harborne (1973) and Kokate (1997).[15,16] The different extracts obtained by successive solvent extraction were tested separately for the presence of various phytoconstituents, viz. alkaloids, glycosides, carbohydrates, Phenolics and tannins, phytosterols, fixed oils and fats, proteins and amino acids, flavonoids, saponins, gums and mucilage.

Determination of total tannin content

Total tannin content was estimated for methanol extract of prickle of *Bombax ceiba* by the method described in Indian Pharmacopoeia, 1996.[17,18] Accurately weighed 0.08 g of methanol extract of *Bombax ceiba* prickle powder were heated gently with 100 ml of water. From this 100 ml solution, 10 ml was taken into another conical flask. 10 ml of indigo carmine solution and 300 ml water were added to the flask. This solution was heated at 60-70 °C and then titrated with 0.1 N $KMnO_4$ until royal blue color changes to bottle green and then titrated dropwise until the solution becomes bottle green to golden yellow in color. Similarly, Blank reading was taken by using 10 ml of the indigo carmine solution alone with 300 ml of water. Total tannin content was calculated using the following formula.

$$\varphi = \frac{(A-B)\times N \times 4.157}{0.1\times m_{\text{sample}}} \qquad (1)$$

where,

φ=% of total tannin content,

A = blank reading,

B = test reading,

m_{sample}= sample weight in g,

N=normality of $KMnO_4$ solution

Determination of total phenolic content

The total phenolic content of methanol extract of prickle was determined by the Folin-Ciocalteu method.[19] 10 mg of methanol extract of prickle was dissolved in 10 ml of methanol to get 1 mg/ml sample solution for the test. 500 µL of the sample was taken in a 25 ml volumetric flask. To this, 10 ml of water and 1.5 ml of Folin-Ciocalteu reagent were added. The above mixture was kept for 5 min. and then 4ml of 20 % sodium carbonate solution was added and the volume was made up to 25 ml with distilled water. The mixture was kept for 30 min. And the absorbance of the blue color developed was recorded at 765 nm in UV- visible spectrophotometer Shimadzu, UV-1700, Japan. The total phenolic content was calculated from the calibration curve of Gallic acid plotted by using the above procedure. The total phenolic content was expressed in terms of mg GAE/g of dry extract equivalent to Gallic acid.

Statistical analysis All the experiments were carried out in triplicate, and the results were expressed as mean ± SEM.

Thin layer chromatography analysis

TLC analysis was carried out according to the method described by Harborne.[20] Methanol and petroleum ether extract of prickle of *Bombax ceiba* were selected for the

development of the mobile phase for thin-layer chromatography analysis. Glass plates were coated with silica gel G and allowed to dry followed by activation of plates in hot air oven at 110℃ for 10min. Pilot TLC was developed for methanol extract and petroleum ether extract by preparing various mobile phases using various solvents like toluene, chloroform, n-butanol, ethyl acetate, methanol and distilled water in different proportion. 1mg/ml of extracts were prepared and spotted onto the TLC plates. After the development of TLC with the mobile phase, plates were air-dried. Detection of R_f values was done in UV light 366 nm, and after spraying with anisaldehyde sulphuric acid followed by heating at 110 °C for 10 min. The movement of the active compound was expressed by its retention factor (R_f) values, calculated as:

$$R_f = \frac{D_{solute}}{D_{solvent}} \quad (2)$$

where

D_{solute} = distance traveled by solute

$D_{solvent}$=distance traveled by solvent front

HPTLC Fingerprinting profile

HPTLC fingerprint profile was developed for methanol and petroleum ether extract of prickle of *Bombax ceiba.* Samples were prepared by dissolving dried methanol and petroleum ether extract of prickle in methanol and petroleum ether to obtained a concentration of 5 µg/ml. 10µl of sample solution was applied on 10 X 10Cms HPTLC aluminum plate precoated with silica gel 60 F_{254} (0.2 mm thickness) of Merck Pvt. Ltd. (India). The most recent automatic device, 'CAMAG LINOMAT-5' was used for the present analysis. The plates were developed with mobile phase Toluene: Ethyl acetate (9:1) and Toluene: Ethyl acetate (9.5:0.5) for Methanolic extract and Petroleum ether extract of prickle, respectively. The plate was scanned at UV 366 nm (CAMAG TLC SCANNER 3). R_f values of each compound that were separated on the plate were recorded.[21]

RESULTS AND DISCUSSION

Macroscopy and microscopy

Prickles were present surrounding the stem and stem branches. Prickles were a conical shape, sharp and hard. Prickle color was reddish-brown to grey. The size of the prickle was found 0.5 cm to 2 cm bottom diameter and 1cm to 1.5 cm in height. (Figure 1) It does not have any characteristic odor.

Microscopy study of prickle showed the presence of stone cells, numerous clusters of prism shape calcium oxalate crystals, epidermal cells and no. of brown-colored, double-walled, polygonal cork cells with the size of 30 ± 1.5 µm and cells with orange content. (Figure 2)

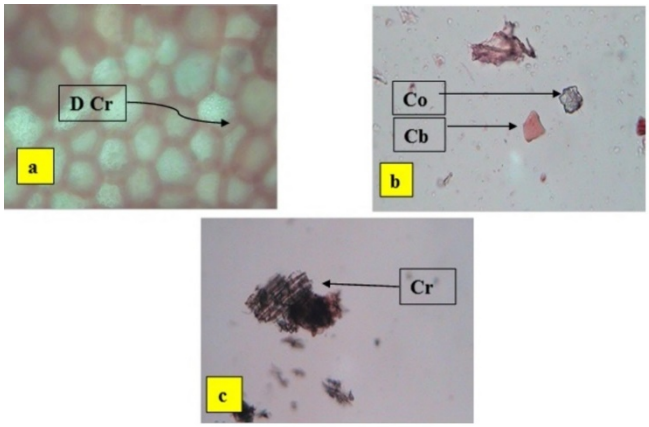

Figure 2. Microscopy of prickle of *Bombax ceiba*. a) Transverse section of prickle, b) & c) Powder microscopy, where D Cr- Double-wall polygonal cork cell, Co- Cluster of prism shape calcium oxalate crystal, Cb - Cells with orange content, Cr- Cork cell in powder study.

Physico-chemical investigation

Physio-chemical investigation of *Bombax ceiba* prickle powder was carried out according to WHO guidelines[13]. Results of parameters such as loss on drying, ash values, extractive values, foaming index and swelling index are as depicted in Table 1.

Table 1. Physico-chemical investigation of prickles of *Bombax ceiba.*

No.	Parameters	Average values *
		Prickle
1	Loss on drying	4.60±0.23 %w/w
2	Total ash	3.50±0.11 %w/w
3	Acid insoluble ash	0.35±0.05 %w/w
4	Water-soluble ash	1.60±0.10 %w/w
5	Alcohol extractive value	19.20±0.22 %w/w
6	Hot water extractability	16.50±0.16 %w/w
7	Coldwater extractability	11.40±0.19 %w/w
8	Foaming index	111
9	Swelling index	4.64±0.14 mL

Results are presented in Mean±SEM (n=3). Values given here are expressed as % w/w of air-dried material.

Fluorescence analysis

A powdered prickle of *Bombax ceiba* was subjected to successive solvent extraction and the consistency, percentage yield, fluorescence examination of all successive extracts by color in daylight and ultraviolet light at 366 nm were recorded. The results of fluorescence analysis are presented in Table 2. Methanol successive solvent extract of prickle revealed the highest extractive value of 12.16 %w/w followed by acetone and petroleum ether extract, whereas water, chloroform and benzene extracts were of low % yield value. Characteristic colors and fluorescent properties recorded can be used for identification and authentication of different extracts prepared from prickle of *Bombax ceiba.*

Table 2. Fluorescence analysis for prickle of *Bombax ceiba*.

No.	Extract	Colour		Consistency	Yield, %w/w
		Under daylight	Under UV light (366 nm)		
1	PEP	Light yellow	Yellowish green	Dry non-sticky	6.30
2	BEP	Yellow	Yellow	Dry non-sticky	2.48
3	CEP	Dark yellow	Dark yellow	Sticky	1.00
4	AEP	Brown	Light brown	Sticky	12.00
5	MEP	Dark brown	Magenta	Sticky	12.16
6	WEP	Orange-brown	Dark brown	Dry non-sticky	2.82

PEP- Petroleum ether (60-80ºC) extract of *Bombax ceiba* prickle, BEP-Benzene extract of *Bombax ceiba* prickle, CEP- Chloroform extract of *Bombax ceiba* prickle, AEP-Acetone extract of *Bombax ceiba* prickle, MEP- Methanol extract of *Bombax ceiba* prickle, WEP-Water extract of *Bombax ceiba* prickle

Table 3. Preliminary phytochemical analysis of prickle of *Bombax ceiba*.

No.	Class of compound	PEP	BEP	CEP	AEP	MEP	WEP	MPBC
1	Alkaloids	-	-	-	-	-	-	-
2	Cardiac Glycoside	-	-	-	-	-	-	-
3	Phenolics/Tannins	-	-	-	+	+	+	+
4	Flavonoids	-	-	-	+	+	+	+
5	Saponins	-	-	-	-	+	+	+
6	Fixed oils & Fats	-	-	-	-	-	-	-
7	Proteins & Amino acids	-	-	-	-	-	-	-
8	Gums/mucilage	-	-	-	-	-	-	-
9	Volatile oil	-	-	-	-	-	-	-
10	Carbohydrates	-	-	-	-	+	+	+
11	Phytosterols/ Triterpenoids	+	+	+	-	-	-	+

- Negative, + - Positive, PEP- Petroleum ether (60-80ºC) extract of *Bombax ceiba* prickle, BEP-Benzene extract of *Bombax ceiba* prickle, CEP- Chloroform extract of *Bombax ceiba* prickle, AEP-Acetone extract of *Bombax ceiba* prickle, MEP- Methanol extract of *Bombax ceiba* prickle, WEP-Water extract of *Bombax ceiba* prickle, MPBC- Crude methanol extract of *Bombax ceiba* prickle

Preliminary phytochemical investigation

Successive extracts of prickle of *Bombax ceiba* were then subjected to phytochemical screening by various qualitative chemical tests. Results of phytochemical screening of successive extracts of prickle powder were depicted in Table 3.

Preliminary qualitative chemical tests of prickle have shown the presence of phenolics, tannins, flavonoids, saponins, carbohydrates and phytosterols/ triterpenoids. The methanol extract of prickle of *Bombax ceiba* revealed the presence of the highest number of phytoconstituents e.g., phenolics, tannins, flavonoids, saponins, carbohydrates and phytosterols/ triterpenoids.

Total tannin content

The total tannin content of *Bombax ceiba* methanol extract of prickle was determined by the Lowenthal Permanganate titration method[17,18]. The method relies on the oxidation of phenolics by potassium permanganate solution in the presence of indigo carmine as a 'redox indicator' to show the endpoint. The total tannin content of prickle was found 4.98±0.22 %w/w, as shown in Table 4.

Table 4. The total tannin content of the prickle of *Bombax ceiba*.

No.	Sample	Mean±SEM % w/w
1	MPBC	4.98±0.22

The values given here are expressed as % w/w of dry weight. MPBC – methanol extract of prickle of *Bombax ceiba*, values are expressed as Mean±SEM, where, n=3

Table 5. Estimation of total phenolic content for methanol extract of prickle of *Bombax ceiba in* µg mg^{-1} gallic acid equivalent

Sample	Total phenolics)			Mean ± SEM
	I	II	III	
MPBC	325.60	328.87	328.87	327.78±1.09

MPBC–Methanol extract of prickle of *Bombax ceiba*, values are expressed as Mean **(**µg mg^{-1} gallic acid equivalent**)**±SEM, where *n*=3

Determination of total phenolic content

The total phenolic content of the methanol extract of prickle was determined by Folin-Ciocalteu method[19]. Table 5 showed the total amount of phenolic content present in the methanol extract of *Bombax ceiba* prickle. 1mg of methanol extract of prickle of *Bombax ceiba* contains 327.78±1.09 µg gallic acid equivalent of phenols.

Table 6. Thin-layer chromatography of petroleum ether and methanol extract of *Bombax ceiba.*

Extract and solvent system	Detection under UV 366 nm light			Detection after spraying of anisaldehyde/sulphuric acid reagent		
	Spot no.	Color	R_f	Spot no.	Color	R_f
Petroleum ether extract of prickles, toluene:ethyl acetate (9.5: 0.5)	1	Pink	0.33	1	Violet	0.20
	2	Fluorescent yellow	0.43	2	Violet	0.36
	3	Pink	0.48	3	Dark Violet	0.60
Methanol extract of prickles, toluene:ethyl acetate (9: 1)	1	Fluorescent Green	0.05	1	Navy blue	0.13
	2	Blue	0.2	2	Grey	0.16
	3	Fluorescent Green	0.55	3	Violet	0.41
	-	-	-	4	Violet	0.56
	-	-	-	5	Violet	0.66
	-	-	-	5	Green	0.71
	-	-	-	6	Greenish blue	0.82
	-	-	-	7	Blue	0.99

Thin layer chromatography analysis

The methanol and petroleum ether extracts were obtained by Soxhlet solvent extraction process. Extracts were subjected to thin-layer chromatography (TLC) to optimize the mobile phase for further HPTLC fingerprinting. Different composition of the different mobile phases was tested in order to obtain high resolution and reproducible spots. The mobile phases developed for petroleum ether and methanol extract were toluene: ethyl acetate (9.5:0.5) and toluene: ethyl acetate (9:1), respectively. The number of spots found, their color in UV 366 nm and in daylight after spraying and related R_f values obtained from the TLC study as shown in Table 6 and Figure 3.

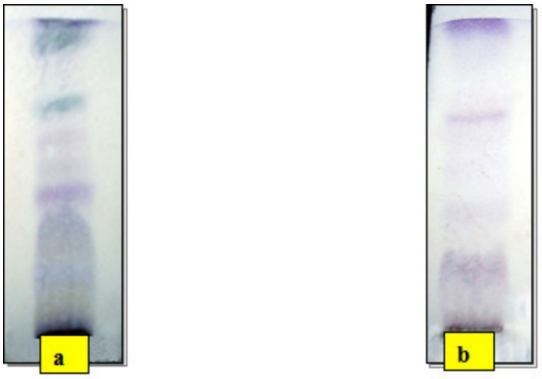

Figure 3. Thin-layer chromatography of *Bombax ceiba* prickle after spraying in visible light a) - methanol extract with toluene: ethyl acetate (9:1) and b)- petroleum ether extract with toluene: ethyl acetate (9.5:0.5).

HPTLC Fingerprinting profile

HPTLC fingerprinting was performed for both methanol and petroleum ether extracts of prickle of *Bombax ceiba* using mobile phase toluene: ethyl acetate (9:1) and toluene: ethyl acetate (9.5:0.5), respectively. Eight peaks were detected upon HPTLC of petroleum ether extract of *Bombax ceiba* prickle at 366nm using mobile phase toluene: ethyl acetate (9.5:0.5), while three peaks were detected upon HPTLC of methanol extract of *Bombax ceiba* prickle at 366 nm using mobile phase toluene: ethyl acetate (9:1). R_f values for ethyl acetate extract for peak 1 to 8 were found 0.07, 0.14, 0.25, 0.31, 0.36, 0.43, 0.5 and 0.75 respectively. R_f values for methanol extract for peaks 1 to 3 were found 0.07, 0.16 and 0.53, respectively. Peaks, their R_f values, area under curve and area % for petroleum ether and methanol extract of prickle of *Bombax ceiba* are depicted in Table 7 and Table 8. 2D chromatogram, 3D chromatogram and HPTLC fingerprint profile of petroleum ether extract of *Bombax ceiba* prickle are shown in Figure 4, 5 and 6 respectively. 2D chromatogram, 3D Chromatogram and HPTLC fingerprint profile of methanol extract of *Bombax ceiba* prickle are shown in Figures 7, 8 and 9.

Table 7. HPTLC fingerprinting of petroleum ether extract of *Bombax ceiba* prickle.

Peak	R_f	Area under curve	Area %
1	0.07	7884	13.77
2	0.14	4656	8.13
3	0.25	986.5	1.72
4	0.31	1858	3.24
5	0.36	3155	5.51
6	0.43	8001	13.97
7	0.5	1842	3.22
8	0.75	28892	50.44

Table 8. HPTLC fingerprinting of methanol extract of *Bombax ceiba* prickle.

Peak	R_f	Area under curve	Area %
1	0.07	13671	84.57
2	0.16	919.8	5.69
3	0.53	1575	9.74

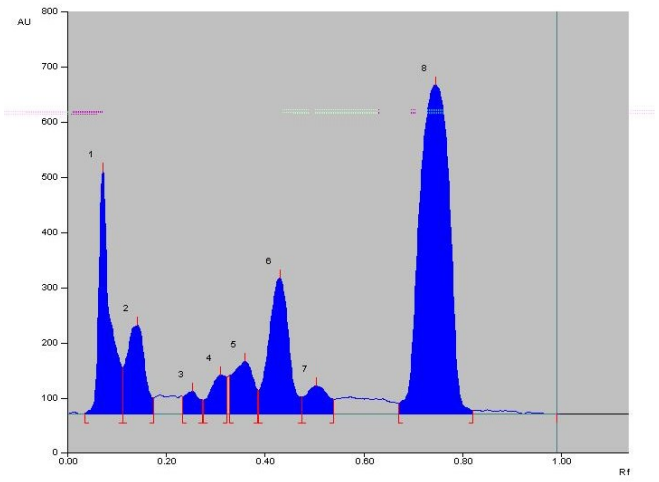

Figure 4. HPTLC 2D chromatogram of petroleum ether extract of *Bombax ceiba* prickle at 366nm (toluene:ethyl acetate 9.5:0.5).

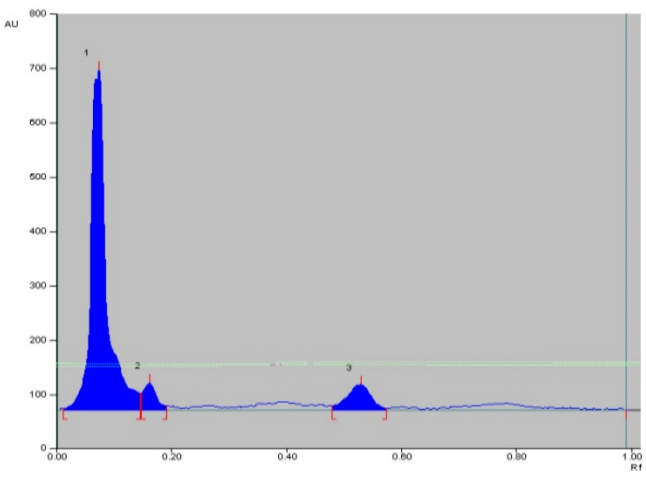

Figure 7. HPTLC 2D chromatogram of methanol extract of *Bombax ceiba* prickle at 366nm (toluene:ethyl acetate 9:1).

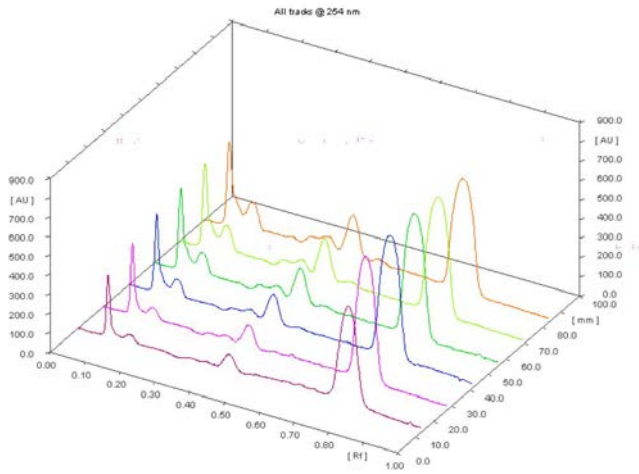

Figure 5. HPTLC 3D chromatogram of petroleum ether extract of *Bombax ceiba* prickle at 366nm (toluene:ethyl acetate 9.5:0.5).

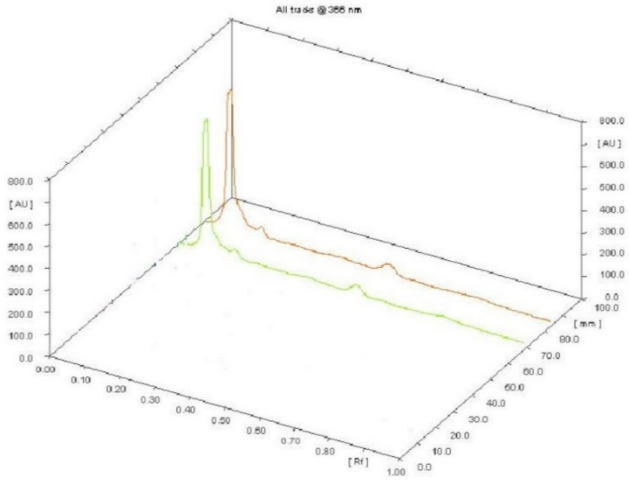

Figure 8. HPTLC 3D chromatogram of methanol extract of *Bombax ceiba* prickle at 366nm (toluene:ethyl acetate 9:1).

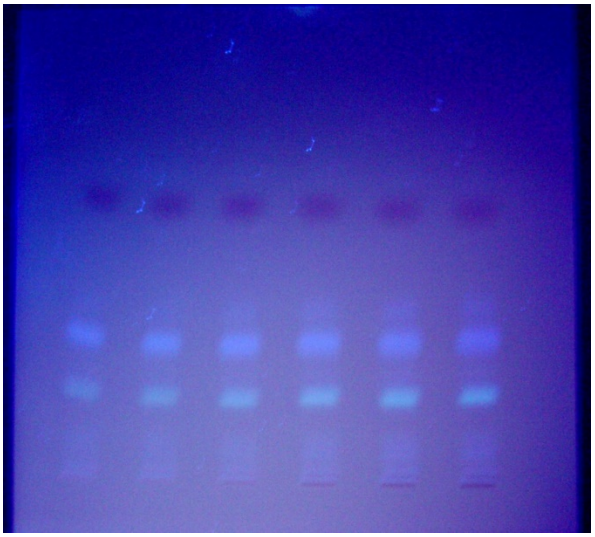

Figure 6. HPTLC profile of petroleum ether extract of *Bombax ceiba* prickle at 366nm (toluene:ethyl acetate 9.5:0.5).

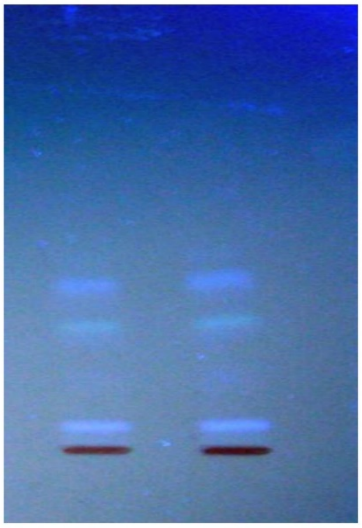

Figure 9. HPTLC profile of methanol extract of *Bombax ceiba* prickle at 366nm (toluene:ethyl acetate 9:1).

CONCLUSION

Standardization of herbal drugs can be used as an important scientific tool to ensure the quality of the crude drug, extracts and formulation prepared from crude drugs. The present study was planned to establish pharmacognostic standards for prickle of *Bombax ceiba* so as to have a reliable scientific parameter to authenticate the prickles, prickle powder, and extracts. The macroscopical and microscopical study provided important data to identify crude as well as a powder form of prickle of *Bombax ceiba*. Preliminary phytochemical screening revealed the presence of phenolics, tannins, flavonoids, saponins, carbohydrates and phytosterols/ triterpenoids in the extracts of prickle of *Bombax ceiba.* Further total phenol and total tannin content of methanol extract can be used as an important standardization parameter for the alcoholic formulation of prickle of *Bombax ceiba.* The physico-chemical analysis provided important data on loss on drying, ash values, extractive values, foaming index and swelling index of prickle of *Bombax ceiba* that can be used to authenticate it in raw as well as powder form. TLC and HPTLC fingerprinting have provided important data for both polar and non-polar extracts, which can be further useful for standardization of both types of extracts used to prepare formulation.

The present study can be specifically useful for authentication of the raw material of the prickles, identification in powder and raw form and in the detection of adulteration, which will ultimately benefit the people who use *Bombax ceiba* prickle formulations.

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