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Research Article

HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY FINGERPRINT PROFILE OF *BAUHINIA TOMENTOSA* LINN. LEAVES

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ABSTRACT

Objective: Chromatographic fingerprint is an effective method for doing the fingerprinting of a plant species. In this study, high-performance thin-layer chromatography (HPTLC) analysis of *Bauhinia tomentosa* was done in n-hexane, chloroform, and ethanol extracts.

Methods: The extract of leaves was developed using toluene:ethyl acetate:formic acid:glacial acetic acid (7:3:0.1:0.1) for n-hexane, toluene:ethyl acetate:formic acid (6:2:0.5) for chloroform, and chloroform:methanol:formic acid (8:1.5:0.2) for ethanol extract as mobile phase using standard procedures and scanned under ultraviolet at 254 nm, 366 nm, and 520 nm.

Results: The HPTLC fingerprinting results showed several peaks with different R_f values. The HPTLC fingerprinting of n-hexane extract at 266 nm showed 15 peaks. The HPTLC fingerprinting of chloroform extract at 520 nm showed 22 peaks. The HPTLC fingerprinting of the ethanol extract at 366 nm showed 13 peaks.

Conclusion: These fingerprinting results will be helpful in the identification and authentication of the species and also to identify new bioactive components in this medicinal plant.

Keywords: High-performance thin-layer chromatography, *Bauhinia tomentosa*, Ethanol extract, Chromatography, Fingerprinting, Medicinal plants.

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INTRODUCTION

Indian traditional medicine is one of the richest medicinal systems available around the world. The phytochemicals identified from traditional medicinal plants are providing an excellent opportunity for the development of new types of therapeutics [1]. This plant-based traditional medicine system continues to play an essential role in health care [2]. The revival of significant and the emergent market of herbal medicine products necessitate strong commitment by stakeholders to safeguard the end users. Furthermore, various hazardous side effects, hypersensitivity reactions, effects from adulterants, and interactions with herbal drugs have been confirmed, drawing the consideration of many regulatory agencies for the standardization of plant-based drugs [3]. The World Health Organization has developed specific guiding principles to support the associated countries to instigate nationalized policies on plant-based drugs and to study their prospective safety, efficacy, and quality, as a prerequisite for global harmonization [4-6]. Standardization of the plant material is need of the day. Several pharmacopeia containing monographs of the plant materials describe only the physicochemical characters. Hence, the modern methods describing the identification and quantification of active constituents in the plant material may be useful for proper standardization of herbs and its formulations [7,8].

Technological advancements which take place in the processes of isolation, purification, and structural elucidation of natural compounds have made it possible to generate different strategies for the analysis and standardization of plant-based medicines [4]. A variety of sophisticated methods such as spectrophotometric, chromatographic, polarography, electrophoresis, and the use of biomarkers in fingerprints are presently employed in the standardization procedures. Thin-layer chromatography (TLC) and high-performance TLC (HPTLC) fingerprint profiles are used for ensuring the identity, transparency, and potency of herbal formulations [9].

HPTLC fingerprint is mostly used for evaluating the compounds with low or moderate polarities [10]. The advantage of HPTLC is that several samples can be run simultaneously using a same quantity of mobile phase, thus lowering analysis time and cost per analysis, and it has an added advantage in that the resolution of chemically similar compounds is better than the conventional TLC and low amount of sample is required [11]. HPTLC can serve as a tool for identification, authentication, and quality of herbal drug [12].

Bauhinia tomentosa commonly known as yellow bell orchid tree belongs to Fabaceae family is one of the best, versatile, and most commonly used household remedies for many manifestations. The generic name commemorates the Bauhin brothers Jean and Gaspard, the Swiss botanists; the two lobes of the leaf exemplify the two brothers. *Tomentosa* derived from tomentose, meaning with dense, interwoven hairs. It is commonly known as "Kanchini" in Tamil and "Phalgu" in Sanskrit [13].

In this study, fingerprinting of *B. tomentosa* leaves was done by successive extraction using hexane, chloroform, and ethanol solvents with the appropriate mobile phases.

METHODS

Instrumentation

A Camag HPTLC system (Muttenz, Switzerland) equipped with a sample applicator LinomatV, twin trough plate development chamber, TLC scanner 3, win CATS software, and Hamilton (Reno, Nevada, USA) Syringe (100 µL) was used.

Material and reagents
HPLC grade ethanol, ethyl acetate, hexane, acetic acid, and formic acid were obtained from E. Merck, India).

Sample collection
The leaves of *B. tomentosa* Linn. were collected from Villivakkam, Chennai, and authenticated by Dr. S. Jayaraman, Director of Plant and Anatomy Research Centre, West Tambaram, Chennai (Authentication No. PARC/2014/2294).

Sample preparation
2 g of the sample was loaded in Millipore cellulose thimble and extracted with 100 ml of n-hexane exhaustively in a Soxhlet distillation apparatus. After that, the extract was concentrated in a water bath by distillation process and was transferred into a beaker using minimum quantity of hexane and dried over a water bath to free hexane. This extract was dissolved in hexane and made up to 10 ml in a standard flask. The process was again continued with chloroform and then with ethanol.

Chromatographic conditions
Stationary phase: Silica gel GF_{254}

Mobile phase
i. For n-hexane extract: Toluene: EA:FA: GAA (7:3:0.1:0.1)
ii. For chloroform extract: Toluene: EA:FA (6:2:0.5)
iii. For ethanol extract: Chloroform:methanol: FA (8:1.5:0.2)

Scanning wavelength: 254 nm, 366 nm, and 520 nm

Sample concentration: Extract (50 mg/ml)

Applied volume: Track 1 (10 µl), track 2 (15 µl), and track 3 (20 µl),

Development mode: Ascending mode.

Then, the plate was scanned using Camag's Scanner 4 at λ254 nm (D2 lamp, absorption mode) and λ366 nm (Hg lamp, fluorescence mode), respectively, and fingerprint profiles of the extract were detected. Subsequently, the plate was dipped in 5% sulfuric acid in alcohol followed by heating at 105°C till the development of the coloration of the spots. The plate was then photo documented in white light using Camag's TLC visualizer and scanned at λ520 nm (W light, Absorption mode).

RESULTS

The HPTLC fingerprinting of n-hexane extract of Bauhinia tomentosa was shown in Fig. 1. The chromatograms shown in Fig. 1a indicate that all sample constituents were clearly separated without any diffusion and tailing. Table 1 shows the Rf values of various bands in chromatogram (track 3). It is observed from Table 1a that, in 20 µL (track 3) of n-hexane extract of *B. tomentosa* leaves (at 254 nm), there are 15 spots with R_f values of 0.01, 0.08, 0.15, 0.17, 0.20, 0.25, 0.30, 0.40, 0355, 0.61, 0.67, 0.71, 0.77, 0.86, and 0.95. Of the 15 components in 20 µL of hexane extract, the compounds with R_f value 0.67 and 0.01 were found to be more predominant as the percentage area was more with 25.78% and 12.32%, respectively. The remaining components were found to be very less in quantity as the percent area of all the spots was <10%.

It is observed from Table 1b that, in 20 µL of n-hexane extract of *B. tomentosa* leaves, there are 11 spots (at 366 nm) with R_f values of 0.01, 0.08, 0.26, 0.45, 0.63, 0.66, 0.69, 0.76, 0.80, 0.90, and 0.98. Of the 11 components in 20 µL of hexane extract, the compounds with R_f value 0.63, 0.66, 0.76, and 0.80 were found to be more predominant as the percentage area was 47.25%, 14.96%, 11.13%, and 10.07%, respectively. The remaining components were found to be very less in quantity as the percent area of all the spots were <10%. The chromatograms shown in Fig. 1b indicate that all the constituents were clearly separated without diffusion and tailing.

Table 1: R_f values of various bands in chromatogram (track-3)

λ=254 nm		λ=366 nm		λ=520 nm (derivatized)	
Color	**R_f value (s)**	**Color**	**R_f value (s)**	**Color**	**R_f value (s)**
Green	0.06	Red	0.26	Dark	0.17
Green	0.16	Red	0.36	Dark	0.21
Green	0.20	Red	0.40	Pink	0.26
Green	0.39	Red	0.45	Dark	0.40
Green	0.60	Red	0.55	Violet	0.49
Green	0.65	Red	0.59	Green	0.66
Green	0.68	Red	0.65	Maroon	0.69
Green	0.77	Red	0.70	Maroon	0.72
				Dark	0.78

Table 1a: R_f values of various bands in chromatogram (track 3) at 254 nm

Peak	Max R_f	Max height	Area %
1	0.01	181.6	12.32
2	0.08	56.3	3.82
3	0.15	40.0	2.72
4	0.17	59.3	4.03
5	0.20	108.7	7.37
6	0.25	59.0	4.01
7	0.30	29.4	2.00
8	0.40	29.5	2.00
9	0.55	10.7	0.73
10	0.61	88.1	5.98
11	0.67	379.9	25.78
12	0.71	86.9	5.90
13	0.77	142.8	9.69
14	0.86	56.5	3.84
15	0.95	144.7	9.82

Table 1b: R_f values of various bands in chromatogram (track 3) at 366 nm

Peak	Max R_f	Max height	Area %
1	0.01	40.4	2.31
2	0.08	21.4	1.22
3	0.26	11.2	0.64
4	0.45	26.5	1.51
5	0.63	827.4	47.25
6	0.66	262.0	14.96
7	0.69	42.9	2.45
8	0.76	194.9	11.13
9	0.80	176.3	10.07
10	0.90	14.2	0.81
11	0.98	133.9	7.65

Table 1c: R_f values of various bands in chromatogram (track 3) at 520 nm (derivatized)

Peak	Max R_f	Max height	Area %
1	0.01	119.6	6.86
2	0.09	56.8	3.26
3	0.17	35.4	2.03
4	0.21	70.8	4.06
5	0.26	29.9	1.72
6	0.40	67.1	3.85
7	0.49	252.4	14.48
8	0.57	134.4	7.71
9	0.69	326.1	18.70
10	0.73	225.0	12.91
11	0.78	303.7	17.42
12	0.89	122.3	7.01

The chromatogram shown in Fig. 1c indicates that all sample constituents were clearly separated without any diffusion and tailing. It is observed from Table 1c that, in 20 µL (track 3) of n- hexane extract of *B. tomentosa* leaves, there are 12 spots with R_f values of 0.01, 0.09, 0.17, 0.21, 0.26, 0.40, 0.49, 0.57, 0.69, 0.73, 0.78, and 0.89. Of the 12 components in 20 µL of n-hexane extract, the compounds with R_f value of 0.69, 0.78, 0.49, and 0.73 were found to be more predominant as the percentage area was more with 18.70%, 17.42%, 14.48%, and 12.91%, respectively. The remaining components were found to be very less in quantity as the percent area of all the spots was <10%.

Fig. 2 shows the HPTLC fingerprinting of chloroform extract of Bauhinia tomentosa. The chromatogram shown in Fig. 2a indicates that all sample constituents were clearly separated into distinct bands without any diffusion and tailing. The Rf values of various bands in chromatogram (track 2) was depicted in Table 2. It is observed from Table 2a that, at 254 nm, 15 µL (track 2) of chloroform extract of *B. tomentosa* leaves was separated into 11 bands with R_f values of 0.00, 0.10, 0.20, 0.30, 0.35, 0.42, 0.51, 0.56, 0.65, 0.75, and 0.94, respectively. Of the 11 components in 15 µL of chloroform extract, the compounds with Rf value 0.75, 0.00, and 0.42 were found to be more predominant as the percentage area was more with 29.76%, 14.91%, and 12.34%, respectively. The remaining components were found to be very less in quantity as the percent area of all the spots was <10%.

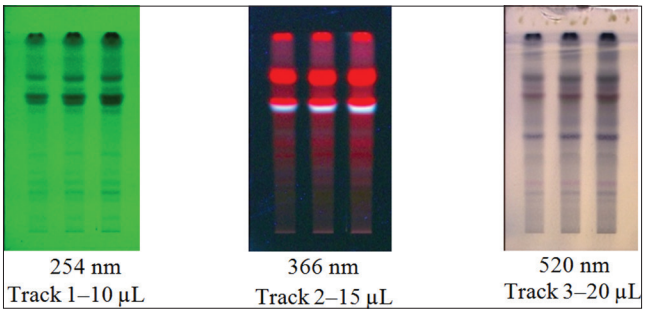

Fig. 1: High-performance thin-layer chromatography fingerprinting of hexane extract of *Bauhinia tomentosa*

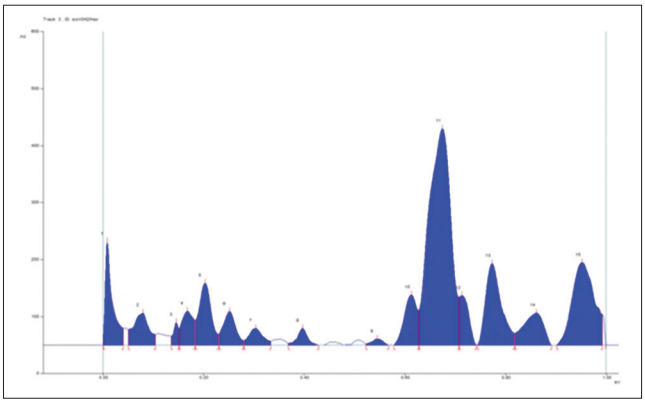

Fig. 1a: High-performance thin-layer chromatography (track 3) at 254 nm

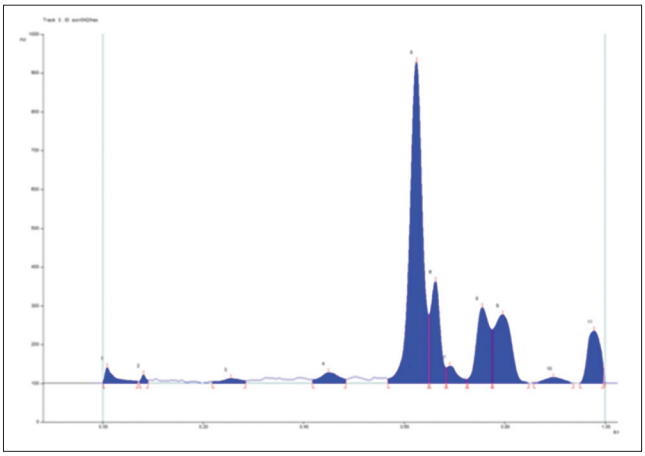

Fig. 1b: High-performance thin-layer chromatography of n-hexane extract (track 3) at 366 nm

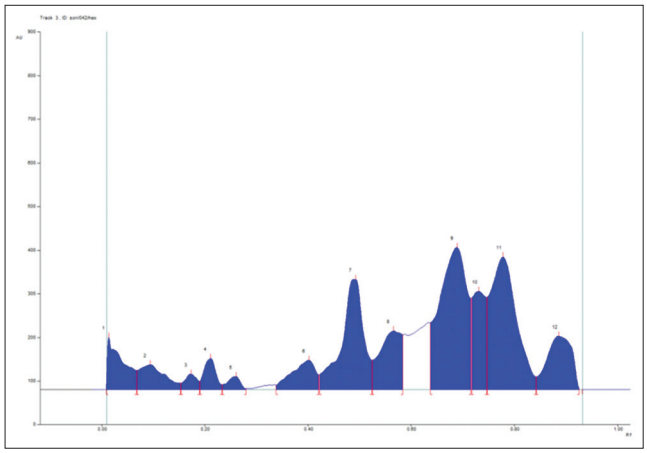

Fig. 1c: High-performance thin-layer chromatography of n-hexane extract (track 3) at 520 nm (derivatized)

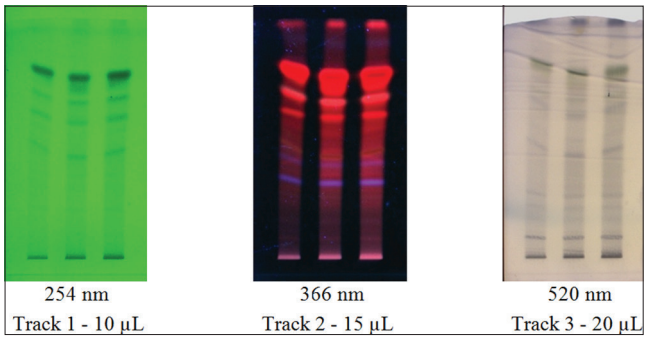

Fig. 2: High-performance thin-layer chromatography fingerprinting of chloroform extract of *Bauhinia tomentosa*

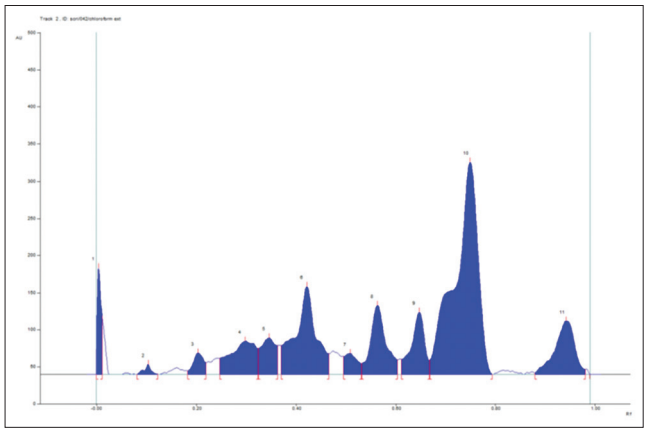

Fig. 2a: High-performance thin-layer chromatography (track 2) at 254 nm

It is observed from Table 2b that, in 15 µL (track 2 at 366 nm) of chloroform extract of *B. tomentosa* leaves, there are 21 spots with R_f values of 0.00, 0.09, 0.16, 0.23, 0.26, 0.31, 0.35, 0.39, 0.41, 0.43, 0.52, 0.59, 0.65, 0.70, 0.73, 0.77, 0.83, 0.86, 0.89, 0.90, and 0.97 values. Of the 21 components in 15 µL of chloroform extract, the compounds with R_f values 0.65 and 0.77 were found to be more predominant as the percentage area was more with 14.93% and 13.89%, respectively. The remaining components were found to be very less in quantity as the percent area of all the spots was <10%. The chromatogram shown in Fig. 2b indicates that all the constituents were clearly separated without any diffusion and tailing.

The chromatogram shown in Fig. 2c indicates that all the sample constituents were clearly separated into distinct bands without any diffusion and tailing. It is observed from Table 2c that, in 15 µL of chloroform extract of *B. tomentosa* leaves (track 2 at 520 nm), there are 22 spots with R_f values of 0.01, 0.03, 0.08, 0.16, 0.20, 0.26, 0.30, 0.39, 0.42, 0.49, 0.59, 0.61, 0.65, 0.68, 0.70, 0.72, 0.77, 0.81, 0.84, 0.89, 0.92, and 0.97. Of the 22 components in 15 µL of chloroform extract, the compounds with R_f value 0.77 was found to be more predominant as the percentage area was more with 13.84%, respectively. The remaining components were found to be very less in quantity as the percent area of all the spots was <10%.

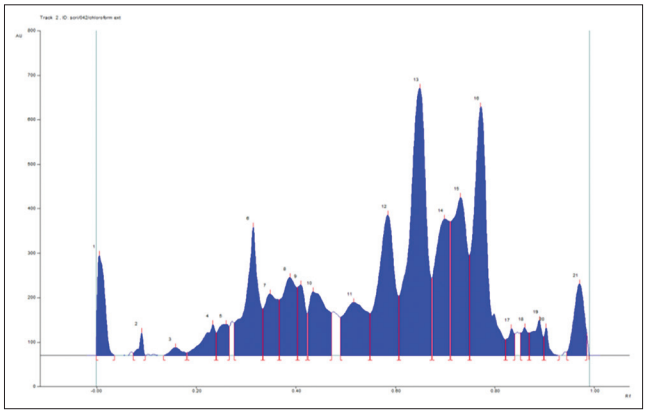

Fig. 2b: High-performance thin-layer chromatography (track 2) at 366 nm

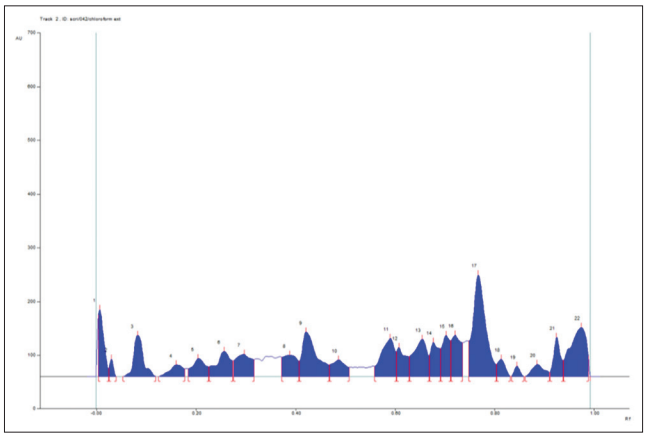

Fig. 2c: High-performance thin-layer chromatography (derivatized; track 2) at 520 nm

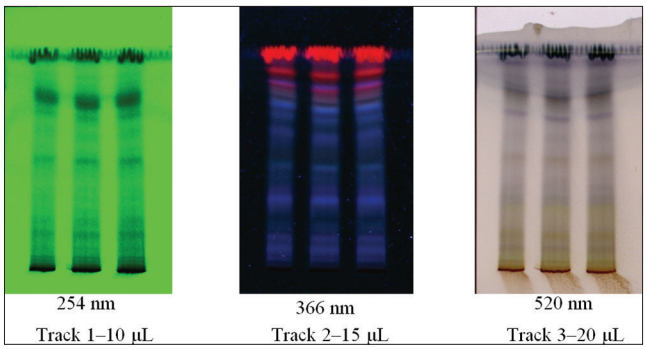

Fig. 3: High-performance thin-layer chromatography fingerprinting of ethanol extract of *B. tomentosa*

The HPTLC fingerprinting of ethanol extract of Bauhinia tomentosa was illustrated in Fig. 3. The chromatogram shown in Fig. 3a indicates that

Table 2: Rf values of various bands in chromatogram (track 2)

λ=254 nm		λ=366 nm		λ=520 nm (derivatized)	
Color	**R_f value (s)**	**Color**	**R_f value (s)**	**Color**	**R_f value (s)**
Green	0.20	Red	0.09	Dark	0.09
Green	0.35	Red	0.17	Dark	0.21
Green	0.43	Violet	0.33	Dark	0.26
Green	0.57	Red	0.40	Dark	0.43
Green	0.66	Red	0.53	Dark	0.60
Green	0.77	Red	0.66	Dark	0.67
				Green	0.78

Table 2a: R_f values of various bands in chromatogram (track 2) at 254 nm

Peak	Max R_f	Max height	Area %
1	0.00	143.3	14.91
2	0.10	13.6	1.42
3	0.20	28.8	3.00
4	0.30	44.7	4.65
5	0.35	49.1	5.11
6	0.42	118.6	12.34
7	0.51	28.3	2.95
8	0.56	93.0	9.68
9	0.65	83.7	8.71
10	0.75	285.9	29.76
11	0.94	71.9	7.48

Table 2b: R_f values of various bands in chromatogram (track 2) at 366 nm

Peak	Max R_f	Max height	Area %
1	0.00	225.6	5.60
2	0.09	52.3	1.30
3	0.16	17.8	0.44
4	0.23	69.9	1.74
5	0.26	69.9	1.74
6	0.31	289.3	7.19
7	0.35	138.5	3.44
8	0.39	175.3	4.36
9	0.41	159.1	3.95
10	0.43	143.2	3.56
11	0.52	118.9	2.95
12	0.59	316.2	7.86
13	0.65	601.1	14.93
14	0.70	306.9	7.62
15	0.73	355.9	8.84
16	0.77	559.2	13.89
17	0.83	60.9	1.51
18	0.86	62.7	1.56
19	0.89	78.7	1.96
20	0.90	62.4	1.55
21	0.97	161.5	4.01

all the sample constituents were clearly separated without any diffusion and tailing. The Rf values of various bands in track 3 of chromatogram was depicted in Table 3. It is observed from Table 3a that, in 20 µL of ethanol extract of *B. tomentosa* leaves (track 3 at 254 nm), there are 13 spots with R_f values 0.04, 0.10, 0.16, 0.22, 0.27, 0.32, 0.35, 0.49, 0.61, 0.69, 0.75, 0.86, and 0.94. Of the 13 components in 20 µL of ethanol extract, the compounds with R_f value 0.94, 0.69, and 0.75 were found to be more predominant as the percentage area was more with 28.51%, 26.37%, and 11.11%, respectively. The remaining components were found to be very less in quantity as the percent area of all the spots was <10%.

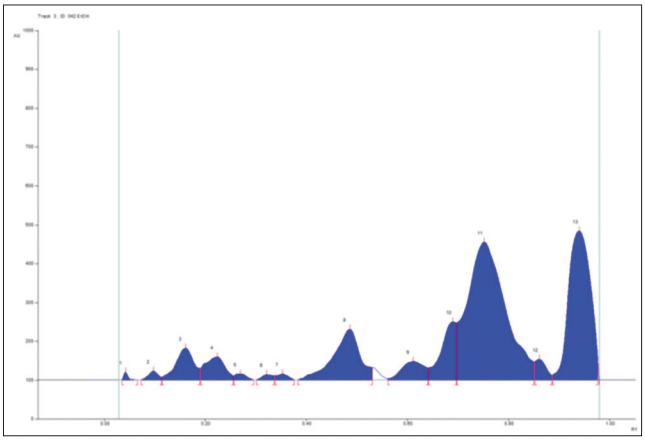

Fig. 3a: High-performance thin-layer chromatography (track 3) at 254 nm

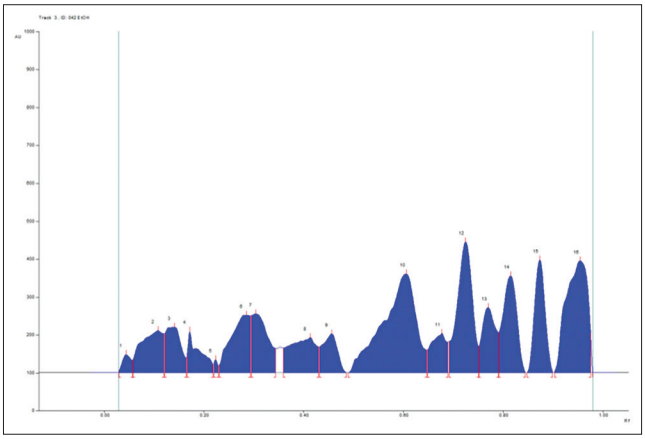

Fig. 3b: High-performance thin-layer chromatography (track 3) at 366 nm

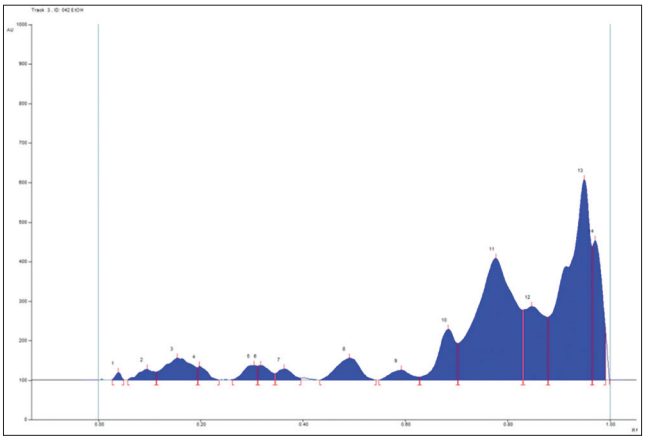

Fig. 3c: High-performance thin-layer chromatography (track 3; derivatized) at 520 nm

It is observed from Table 3b that, in 20 µL of ethanol extract of *B. tomentosa* leaves (track 3 at 366 nm), there are 16 spots with R_f values 0.04, 0.11, 0.14, 0.17, 0.22, 0.29, 0.30, 0.41, 0.46, 0.61, 0.68, 0.73, 0.77, 0.82, 0.87, and 0.96, respectively. Of the 16 components in 20 µL of

Table 2c: R_f values of various bands in chromatogram (track 2) at 520 nm

Peak	Max R_f	Max height	Area %
1	0.01	124.8	9.13
2	0.03	32.3	2.37
3	0.08	76.7	5.61
4	0.16	21.6	1.58
5	0.20	33.6	2.46
6	0.26	46.6	3.41
7	0.30	41.3	3.02
8	0.39	39.6	2.90
9	0.42	82.7	6.05
10	0.49	30.9	2.26
11	0.59	70.8	5.18
12	0.61	53.5	3.92
13	0.65	69.4	5.08
14	0.68	63.4	4.64
15	0.70	76.4	5.59
16	0.72	76.8	5.62
17	0.77	189.1	13.84
18	0.81	31.9	2.34
19	0.84	19.0	1.39
20	0.89	22.1	1.62
21	0.92	73.0	5.34
22	0.97	91.2	6.67

Table 3: R_f values of various bands in chromatogram (track)

λ=254 nm		λ=366 nm		λ=520 nm (derivatized)	
Color	R_f value (s)	Color	R_f value (s)	Color	R_f value (s)
Green	0.07	Indigo	0.14	Dark	0.05
Green	0.17	Indigo	0.32	Violet	0.10
Green	0.33	Fluorescent blue	0.46	Yellow	0.17
Green	0.50	Indigo	0.60	Yellow	0.28
Green	0.61	Indigo	0.67	Violet	0.38
Green	0.68	Indigo	0.72	Brown	0.49
Green	0.87	Red	0.80	Violet	0.60
		Red	0.88	Violet	0.68

Table 3a: R_f values of various bands in chromatogram (track 3) at 254 nm

Peak	Max R_f	Max height	Area %
1	0.04	19.8	1.47
2	0.10	22.7	1.69
3	0.16	81.4	6.05
4	0.22	59.5	4.42
5	0.27	15.2	1.13
6	0.32	13.6	1.01
7	0.35	14.9	1.11
8	0.49	130.1	9.68
9	0.61	47.4	3.53
10	0.69	149.4	11.11
11	0.75	354.6	26.37
12	0.86	5.6	3.91
13	0.94	383.4	28.51

Table 3b: R_f values of various bands in chromatogram (track 3) at 366 nm

Peak	Max R_f	Max height	Area %
1	0.04	47.4	1.79
2	0.11	110.4	4.17
3	0.14	119.7	4.53
4	0.17	109.3	4.13
5	0.22	34.2	1.29
6	0.29	151.0	5.71
7	0.30	154.5	5.84
8	0.41	91.9	3.47
9	0.46	101.9	3.85
10	0.61	259.8	9.82
11	0.68	103.4	3.91
12	0.73	344.3	13.02
13	0.77	171.5	6.48
14	0.82	254.8	9.63
15	0.87	297.0	11.23
16	0.96	294.5	11.13

Table 3c: R_f values of various bands in chromatogram (track 3; derivatized) at 520 nm

Peak	Max R_f	Max height	Area %
1	0.04	18.8	1.05
2	0.09	27.1	1.51
3	0.15	55.3	3.07
4	0.20	64.6	1.92
5	0.30	37.0	2.06
6	0.32	36.8	2.04
7	0.36	27.7	1.54
8	0.49	55.4	3.08
9	0.59	24.6	1.37
10	0.68	128.0	7.12
11	0.78	307.7	17.12
12	0.85	185.6	10.32
13	0.95	506.9	28.20
14	0.97	352.4	19.60

ethanol extract, the compounds with R_f value 0.73, 0.87, and 0.96 were found to be more predominant as the percentage area was more with 13.02%, 11.23%, and 11.13%, respectively. The remaining components were found to be very less in quantity as the percent area of all the spots was <10%. The chromatograms shown in Fig. 3b indicate that all the sample constituents were clearly separated into distinct bands without any diffusion and tailing.

The chromatograms shown in Fig. 3c indicate that all the sample constituents were clearly separated without any diffusion and tailing. It is observed from Table 3c that, in 20 µL of ethanol extract of *B. tomentosa* leaves (track 3 at 520 nm), there are 14 spots with Rf values 0.04, 0.09, 0.15, 0.20, 0.30, 0.32, 0.36, 0.49, 0.59, 0.68, 0.78, 0.85, 0.95, and 0.97, respectively. Of the 14 components in 20 µL of ethanol extract, the compounds with Rf value 0.95, 0.97, 0.78, and 0.85 were found to be more predominant as the percentage area was more with 28.20%, 19.60%, 17.12%, and 10.32%, respectively. The remaining components were found to be very less in quantity as the percent area of all the spots was <10%.

DISCUSSION

HPTLC fingerprinting is a valuable tool for the analysis of phytochemicals because of sensitivity and cost-effectively. The fingerprinting of a plant will help in the identification and quality control of a particular species. It can also give information that will be useful for the isolation, purification, characterization, and identification of marker compounds of the species. In the present study, the developed chromatograms will be specific with the selected solvent systems for the hexane, chloroform, and ethanol extracts, respectively. The presence of many spots in every chromatogram indicates the presence of different phytochemicals in varying concentrations in the plant. Devaki *et al*. have reported the presence of phenols, flavonoids, tannin, and cardiac glycosides in *B. tomentosa* using HPTLC technique [14]. Pachouri and Yadav have carried out HPTLC analysis on a *Bauhinia* species to indicate the presence of various spots at different Rf values [15]. The above studies correlate with the results of present study. The R_f values obtained will be helpful in the standardization of the drug. Thus, the results of the present study will provide information for the standardization of the medicinal plant, *B. tomentosa*.

CONCLUSION

It can be concluded that the results obtained from the HPTLC fingerprint analysis will be helpful in identification and standardization of *B. tomentosa* and can be used as a reference for the identification and quality control of the drug. As per literature survey, minimal work has been carried out in this variety. The results of the present study can be taken as a reference and the efficacy of the products can be done in the future which will validate the use of this plant for treating various ailments in the folklore system of medicine.

AUTHORS CONTRIBUTION

Dr. K. Vijayalakshmi designed the research work. R. Balabhaskar executed the current study and prepared the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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