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HPTLC fingerprint profile of *Bauhinia variegata* Linn. leaves

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ABSTRACT

Objective: To develop the finger print of medicinally and economically important leaves of *Bauhinia variegata* Linn. **Methods:** Ethanol extract of the leaves were developed in the mobile phase of n-Hexane: Ethyl acetate: Formic acid: Acetic acid (70:30:1.0:1.0) using standard procedures and scanned under UV at 254 nm, 366nm and under visible light. **Results:** The HPTLC fingerprinting of the ethanol extract has shown several peaks with different Rf values. 2.5 μL of ethanol extract showed 11 spots while 5 μL and 10 μL has shown 13 spots. 15 μL concentration gave 14 spots in the above said solvent system. **Conclusions:** This finger print would be helpful in the identification and authentication of this species.

1. Introduction

Modern medicine has evolved from folk medicine and traditional system only after thorough chemical and pharmaceutical screening; plants remain a major source of medicinal compounds. Synthetic drugs causes side effects as a result, people are more favorable to use natural compounds obtained from plants [1]. It has been estimated that 56% of the lead compounds for medicines in the British National Formulary are natural products [2]. Phytochemical analysis of plants which were used in folklore has yielded a number of compounds with various pharmacological activities. Standardization of the plant material is need of the day. Several pharmacopoeia containing monographs of the plant materials describe only the physico-chemical 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 [3,4].

Currently HPTLC is often used as an alternative to HPLC for the quantification of plant products because of its simplicity, accuracy, cost-effectiveness and rapidity [5]. HPTLC fingerprint has better resolution and estimation of active constituents is done with reasonable accuracy in a shorter time [6]. Chromatographic fingerprint is a rational option to meet the need for more effective and powerful quality assessment to ITM (IndianTraditional Medicine) and TCHM (Chinese traditional herbalmedicine). The optimized chromatographic finger print is not only an alternative analytical tool for authentication, but also an approach to express the various patterns of chemical ingredients distributed in the herbal drugs. HPTLC finger print analysis has become the most potent tool for quality control of herbal medicines because of its simplicity and reliability. It can serve as a tool for identification, authentication and quality control of herbal drug [7].

Bauhinia variegata linn. (Mandharai) is a medium sized, deciduous tree, found throughout India, ascending to an attitude of 1,300 m in the Himalayas. It is commonly known as Kanchnar(Sanskrit), Mountain Ebony (English), Mandharai (Tamil) and Raktakanchan (Hindi)[8]. The various parts of the plant viz., flower buds, flowers, stem, stem bark, leaves, seeds and roots are practiced in various indigenous systems of medicine and popular among the various ethnic groups in India for the cure of variety of aliments[9,10]. The leaves of other bauhinia species are reported to have antiophidian[11],antidiabetic[12], antimalarial[13], antimicrobial[14] and antioxidant potential[15,16].Previously reported phytochemical constituents from the leaves of

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B.variegata are lupeol, β-sitosterol, tannins, kaempferol-3-glucoside[17], amides, carbohydrates, reducing sugars, crude protein, vitamin C, fibers[18], calcium, phosphorus[19], rutin, quercetin, quercitrin, apigenin, apigenin -7-O-glucoside[20],dotetracont-15-en-9-ol and heptatriacontan-12,13-diol[21]. Inspite of its abundant uses, the chromatographic finger print profile of *Bauhinia variegata* leaves have not been reported.

The main objective of this study was to evaluate and optimize the HPTLC fingerprint method in standardization of *Bauhinia variegata*.The present study may serve as a basis for their use in medicinal preparations.

2. Material and methods

2.1. Instrumentation

A Camag HPTLC system (Muttenz,Switzerland) equipped with a sample applicator LinomatV, twin trough plate development chamber, TLC Scanner3, winCATS software and Hamilton (Reno, Nevada, USA)Syringe (100 μL).

2.2. Material and reagents

HPLC grade ethanol, ethyl acetate, Hexane, acetic acid and formic acid were obtained from *E.Merck*, India).

2.3. Sample Collection

B.variegata leaves were collected from Chennai in the month of December and it was authenticated by Dr.P.Jayaraman, Director, National Institute of Herbal Science (authentication reference no. PARC/2010/670 dated 22/12/2010).

2.4. Sample preparation

The leaves were washed with water and then shade dried. The petioles were separated from the lamina portion and powdered coarsely. Crude extract was obtained after maceration with 95% ethanol at room temperature for 72 hrs, and repeated till exhaustion of the material. Thereafter, the ethanol crude extract was distilled, evaporated and dried under reduced pressure to yield ethanol extract of *B.variegata* leaves,EBV(yield 8%). A stock solution was prepared at a concentration of 25mg/ml and it was for the analysis.

2.5. Chromatographic conditions

Chromatograph was performed on 10x10 cm aluminum packed TLC plate coated with 0.2 mm layer of silica gel 60F254 ((E. Merck Ltd, Darmstadt, Germany) stored in a dessicator, application was done by Hamilton microsyringe(Switzerland), mounted on a Linomat V applicator. Spotting was done on the TLC plate, ascending development of the plate, migration distance 80 mm (distance to the lower edge was 10 mm) was performed at 25±20°C with n-Hexane:ethyl acetate: formic acid: acetic acid (70:30:1.0:1.0 v/v)as a mobile phase in a camag chamber previously saturated for 30 min. Various concentrations of the sample(2.5 μL, 5 μL, 10 μL and 15 μL) were applied in four tracks as 8 mm bands at a spraying rate of 15s μL^{-1}. After development the plate was dried at 60°C in an oven for 5 minutes. Densitometric scanning was then performed with a Camag TLC Scanner 3 equipped with win CATS Software Version 1.3.0 at λ max = 254 nm and 366nm using Deuterium light source, the slit dimensions were 6.00 X 0.45 mm and at λ max = 620 nm using Tungsten light source.The chromatograms were recorded.

3. Results

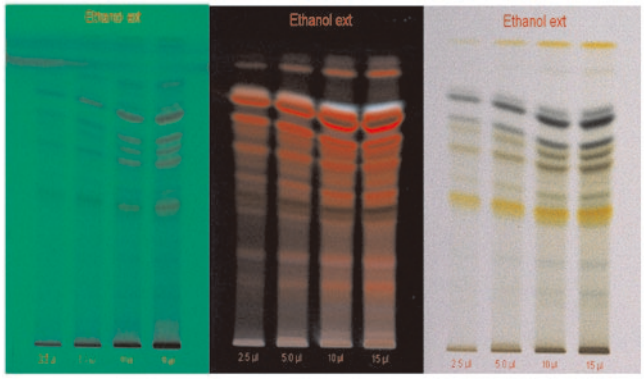
The Chromatograms shown in Fig. 1 indicate that all sample constituents were clearly separated without any tailing and diffuseness.It is evident from Table 1 that in 2.5 μL of ethanol extract of *Bauhinia variegata* leaves, there are 11 spots at the following Rf 0.24, 0.36, 0.55, 0.6, 0.63, 0.69, 0.75, 0.79, 0.89, 0.96, 0.99 as shown in Fig. 2, indicating the occurrence of atleast 11 different components in 2.5 μL of ethanol extract. Out of 11 components, the component with Rf values 0.51, 0.67, 0.73, 0.76 and 0.83 were found to be more predominant as the percentage area was more with 23.04%, 13.91%, 10.15%, 13.84% and 27.65% respectively. The remaining components were found to be very less in quantity as the percent area for all the spots were less than 4.0%.

It is revealed from Table 2 that in 5 μL of ethanol extract of *Bauhinia variegata* leaves there are 13 spots as shown in Fig. 3 indicating the occurrence of at least 13 different components in ethanol extract. Out of 13 components, the component with Rf values 0.49, 0.64, 0.7, 0.73 and 0.8 were found to be more predominant as the percentage area was more with 21.21%, 15.03%, 10.86%, 14.85% and 27.3% respectively. The remaining components were found to be very less in quantity as the percent area for all the spots were less than 5.5%.

Table 3 shows that in 10 μL of ethanol extract of *Bauhinia variegata* leaves there are 13 spots as shown in Fig. 4 indicating the occurrence of at least 13 different components in ethanol extract. Out of 13 components, the component with Rf values 0.46, 0.6, 0.65, 0.68 and 0.76 were found to be more predominant as the percentage area was more with 19.99%, 14.24%, 10.03%, 14.87% and 27.41% respectively. The remaining components were found to be very less in quantity as the percent area for all the spots were less than 6.1%.

Table 4 indicate that in 15 μL of ethanol extract, there are 14 spots at the following Rf 0.18,0.23,0.26 ,0.35,0.5,0.54,0.63,0.66,0.71,0.77,0.84,0.9,0.96,0.99 as

shown in Fig.5 indicating the occurrence of at least 14 different components in ethanol extract. Out of 14 components, the component with Rf values 0.46, 0.6, 0.64, 0.68, 0.75 and 0.78 were found to be more predominant as the percentage area is more with 20.14%, 14.47%, 10.05%, 12.82% , 19% and 8.79% respectively. The remaining components were found to be very less in quantity as the percent area for all the spots were less than 6.6%.



A. 254 nm B. 366 nm C. Visible light

Fig 1. HPTLC chromatogram of ethanol extract of *B.variegata* leaves(EBV)

Track 1: 2.5 μL of EBV , Track 2: 5.0 μL of EBV, Track 3: 10 μL of EBV, Track 4: 15 μL of EBV

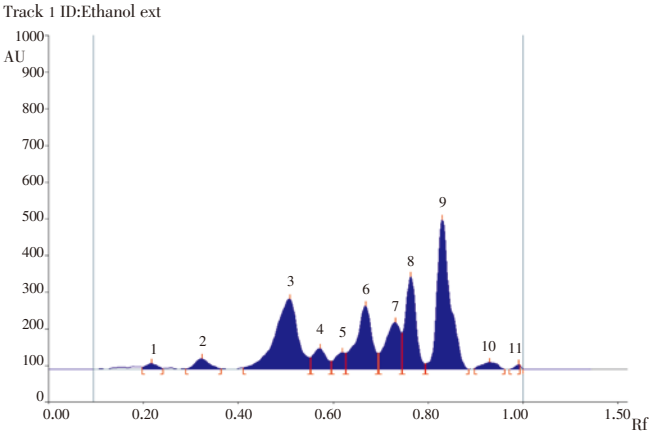


Fig 2. Fingerprint of *B.variegata* (Track 1) leaves.

Table 1

Peak list and Rf value of the chromatogram of 2.5 μL of Ethanol Extract of *Bauhinia variegata* Linn.leaves.

Track	Peak	Max Rf	Max Height	Area %
1	1	0.22	16.2	0.96
1	2	0.32	29.6	2.19
1	3	0.51	191.3	23.04
1	4	0.57	56.6	3.9
1	5	0.62	46.1	2.46
1	6	0.67	172.5	13.91
1	7	0.73	128.9	10.15
1	8	0.76	252.6	13.84
1	9	0.83	407.7	27.65
1	10	0.93	19.1	1.58
1	11	0.99	13	0.33

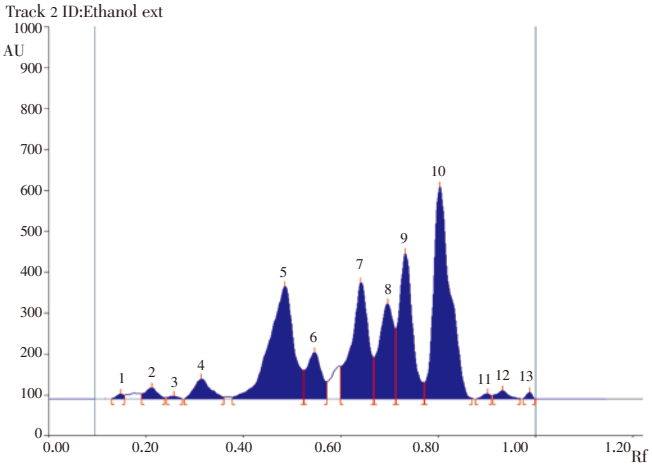


Fig 3 Fingerprint of *B.variegata* (Track 2) leaves.

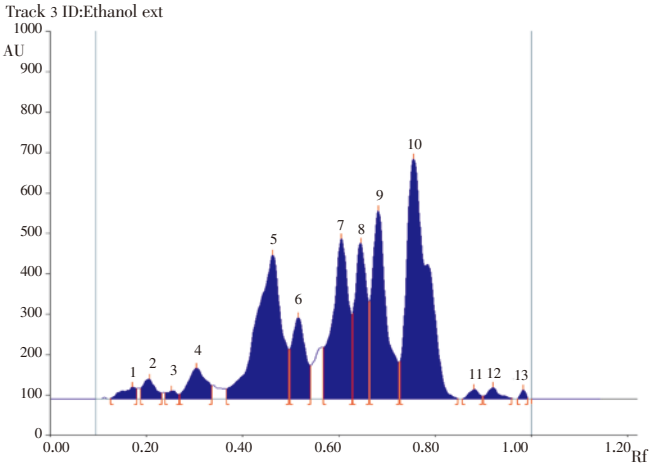


Fig 4. Fingerprint of *B.variegata* (Track 3) leaves.

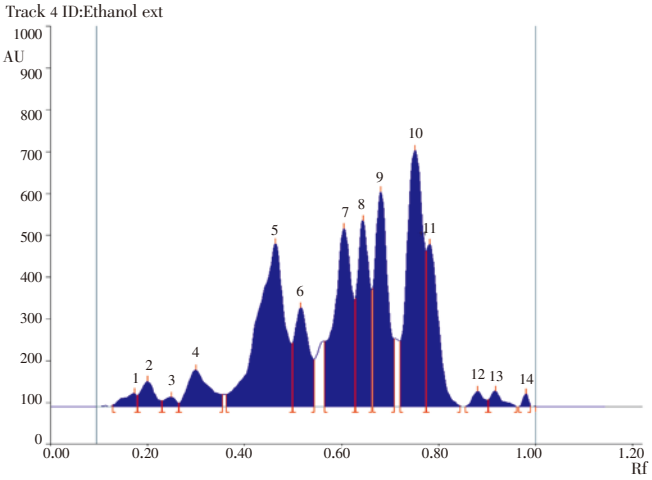


Fig 5 Fingerprint of *B.variegata* (Track 4) leaves.

4. Discussion

Thus the developed chromatogram will be specific with selected solvent system n-Hexane: ethyl acetate: formic acid: acetic acid (70:30:1.0:1.0 v/v), Rf value and serve the

better tool for standardization of the drug.

Characteristic TLC/HPTLC fingerprinting of particular plant species will not only help in the identification and quality control of a particular species but also provide basic information useful for the isolation, purification, characterization and identification of marker chemical compounds of the species. Thus the present study will provide sufficient information about therapeutic efficacy of the drug and also in the identification, standardization and quality control of medicinal plant.

Table 2

Peak list and Rf value of the chromatogram of5 μL of Ethanol Extract of *Bauhinia variegata* Linn.leaves.

Track	Peak	Max Rf	Max Height	Area %
2	1	0.15	11.8	0.29
2	2	0.21	27.2	1.08
2	3	0.26	6.9	0.21
2	4	0.31	49.2	2.55
2	5	0.49	275.4	21.21
2	6	0.55	114.3	5.21
2	7	0.64	284.8	15.03
2	8	0.7	232.8	10.86
2	9	0.73	357	14.85
2	10	0.8	519.3	27.3
2	11	0.9	12.9	0.34
2	12	0.93	20.1	0.76
2	13	0.99	16.2	0.29

Table 3

Peak list and Rf value of the chromatogram of 10 μL of Ethanol Extract of *Bauhinia variegata* Linn.leaves.

Track	Peak	Max Rf	Max Height	Area %
3	1	0.17	29.3	0.95
3	2	0.21	49.9	1.41
3	3	0.25	20.7	0.48
3	4	0.3	76.7	3.03
3	5	0.46	357.6	19.99
3	6	0.52	201.8	6.09
3	7	0.6	397.2	14.24
3	8	0.65	386.7	10.03
3	9	0.68	466.5	14.87
3	10	0.76	595.6	27.41
3	11	0.88	23.7	0.53
3	12	0.92	28.8	0.72
3	13	0.98	23.3	0.26

Table 4

Peak list and Rf value of the chromatogram of 15 μL of Ethanol Extract of *Bauhinia variegata* Linn.leaves.

Track	Peak	Max Rf	Max Height	Area %
4	1	0.17	32.2	0.85
4	2	0.2	61.1	1.54
4	3	0.25	22.7	0.5
4	4	0.3	87.6	3.6
4	5	0.46	390.2	20.14
4	6	0.52	237.1	6.55
4	7	0.6	426.4	14.47
4	8	0.64	445.1	10.05
4	9	0.68	514.3	12.82
4	10	0.75	613.4	19
4	11	0.78	389.5	8.59
4	12	0.88	37.3	0.77
4	13	0.92	37.6	0.8
4	14	0.98	31	0.31

5. Conclusion

In conclusion, the results obtained from qualitative evaluation of HPTLC fingerprint images will be helpful in the identification and quality control of the drug and ensure therapeutic efficacy. HPTLC analysis of *Bauhinia variegata* Linn.leaves can provide standard fingerprints and can be used as a reference for the identification and quality control of the drug.

Conflict of interest statement

We declare that we have no conflict of interest.

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