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HPTLC Fingerprinting of *Tecomella undulata* Leaves as a Quality Control Parameter in Herbal Formulations

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Abstract : *Tecomella undulata* leaves have antibacterial, analgesic, anti-HIV and hepato protective activity. Many of its formulations are mentioned in Ayurvedic texts and available in the market as well. The present work focuses on developing a simple HPTLC fingerprint of *Tecomella undulata* leaves. Methanolic extract of the leaves was prepared by maceration. This extract was used to develop a suitable mobile phase for fingerprinting. After mobile phase development involving several pilot TLC, the mobile phase showing distinct spots in TLC was found to be Chloroform: Methanol: Ethyl acetate (4:1:5). It was further subjected to HPTLC fingerprinting where R_f and Area Under Curve were calculated. HPTLC fingerprinting showed12 peaks at 254nm and 11 peaks at 366nm. This work provides a simple technique for standardization and detection of adulteration of *Tecomella undulata* leaf formulations, since no such work has been done on its leaves.
Keywords: Bignoniaceae, HPTLC, Rohitak, Standardization, *Tecomella undulata,* Quality control.

Introduction

Tecomella undulata (Family – Bignoniaceae) is found in arid and semi-arid regions of India and is also known as Rohitak (Hindi)[1,2]. According to traditional medicinal texts as well as modern research, they possess antimicrobial, anti-HIV, anti-inflammatory, analgesic, and hepatoprotectiveactivity[1-8].Considering such potent applications of *T. undulata* leaves, the present work focuses on developing an HPTLC fingerprint of *T. undulate* leaves, since no such work has been done.

Figure 1. ***Tecomella undulata***

Experimental

Collection and processing

T. undulate leaves were collected from medicinal plants garden of School of Pharmacy, RK University, in July 2015. Its herbarium was prepared and authenticated by Dr. KunjalSoni, botanist of School of Science, RK University. The leaves were dried in hot air oven at a temperature less than 50°Cand then powdered in electric grinder. The powder was passed through sieve #50.

Extraction

50g dry powder of *T. undulata leaves* was macerated with 100ml methanol for 24h at room temperature. Methanolic extract was filtered and evaporated on water bath at 50°C to obtain the dried extract.

Mobile phase development

Pilot TLC were developed for methanol extract using various mobile phases prepared using solvents like toluene, chloroform, methanol, ethyl acetate, etc., in varying ratios. After observing the pilot results, further TLC were developed by adding ammonia & ethyl acetate for removal of tailing.

HPTLC

HPTLC fingerprinting of methanolic extract was performed in Dept. of Pharmaceutical Sciences, Saurashtra University, using the mobile phase Chloroform: Methanol: Ethyl acetate (4:1:5), as it gave most appropriate TLC fingerprint, under the following conditions…

Stationary phase:Silica gel 60 F 254 (E. Merck KGaA)
Sample application: CAMAG Linomat 5
Detection: CAMAG TLC Scanner 3
Lamp: D2 & W
Measurement type: Remission
Measurement mode: Absorption
Optical filter: Second order
Data filtering: Savitsky-Golay 7

Four tracks of same extract at different concentrations were run for the HPTLC fingerprinting and scanned under visible light, UV 254nm and UV 366nm.

Results and Discussion

Twelve peaks were detected at 254nm (Table 1, Fig. 2, 3) and eleven peaks were detected at 366nm (Table 2, Fig. 4, 5) upon HPTLC of methanolic extract of *T. undulata leaves* using mobile phase Chloroform: Methanol: Ethyl Acetate (4:1:5).

Table 1. R_f & Area Under Curve of HPTLC of methanol extract at 254nm

Peak	Rf	Area Under Curve	Area %
1	0.04	110.5	0.17
2	0.05	217.1	0.34
3	0.11	3785.8	5.86
4	0.23	3608.0	5.59
5	0.38	3750.8	5.81
6	0.41	2489.9	3.86
7	0.46	6301.6	9.76
8	0.52	8658.9	13.41
9	0.59	14019.5	21.72
10	0.65	2657.0	4.12
11	0.72	12010.8	18.61
12	0.85	6945.1	10.76

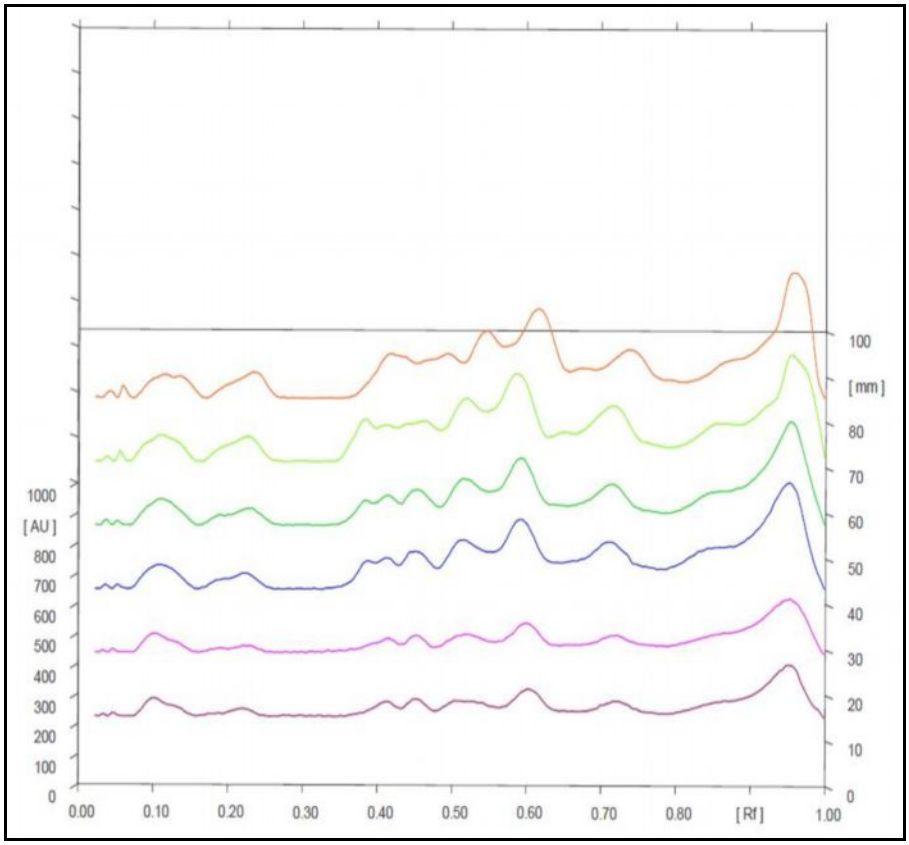
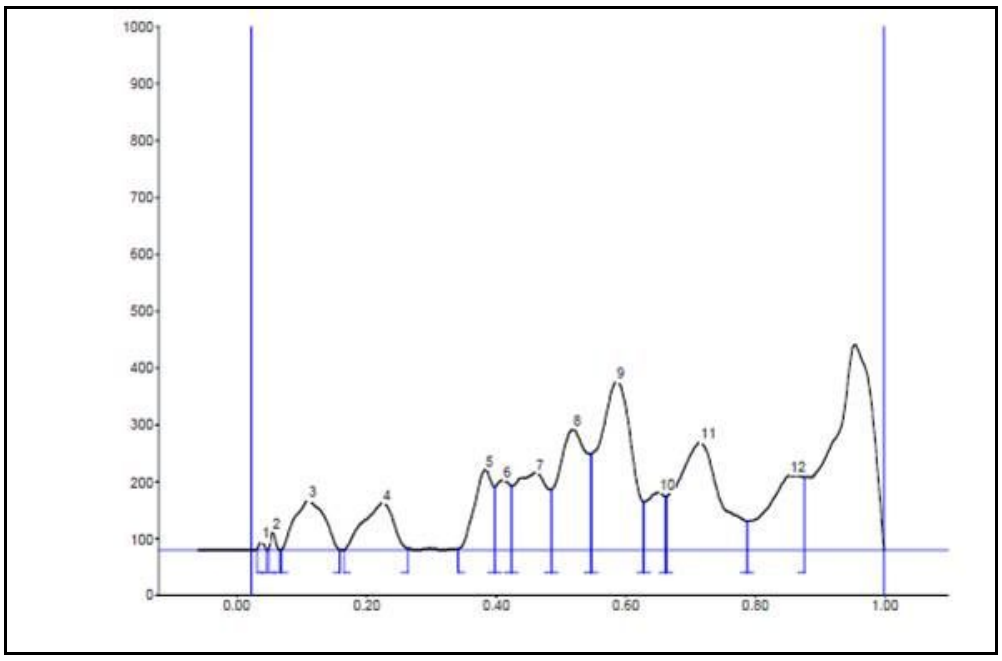


Figure 2: HPTLC 2D densitometric superimposable chromatogram of methanol extract at 254nm (chloroform: methanol: ethyl acetate-4:1:5)



-0.10 0.00 0.10 0.20 0.30 0.40 0.50 0.60 0.70 [Rf] 0.90

Figure 3: HPTLC chromatogram of methanol extract at 254nm(chloroform: methanol: ethyl acetate-4:1:5)

Table 2. R_f & Area Under Curve of HPTLC of methanol extract at 366nm

Peak	Rf	Area Under Curve	Area %
1	0.04	367	0.45
2	0.05	114.4	0.14
3	0.08	245.0	0.29
4	0.12	1708.0	2.06
5	0.23	17247.6	20.75
6	0.41	12536.6	15.08
7	0.44	13897.5	16.72
8	0.52	13633.2	16.40
9	0.58	8671.2	10.43
10	0.65	233.5	2.80
11	0.85	12354.7	14.87

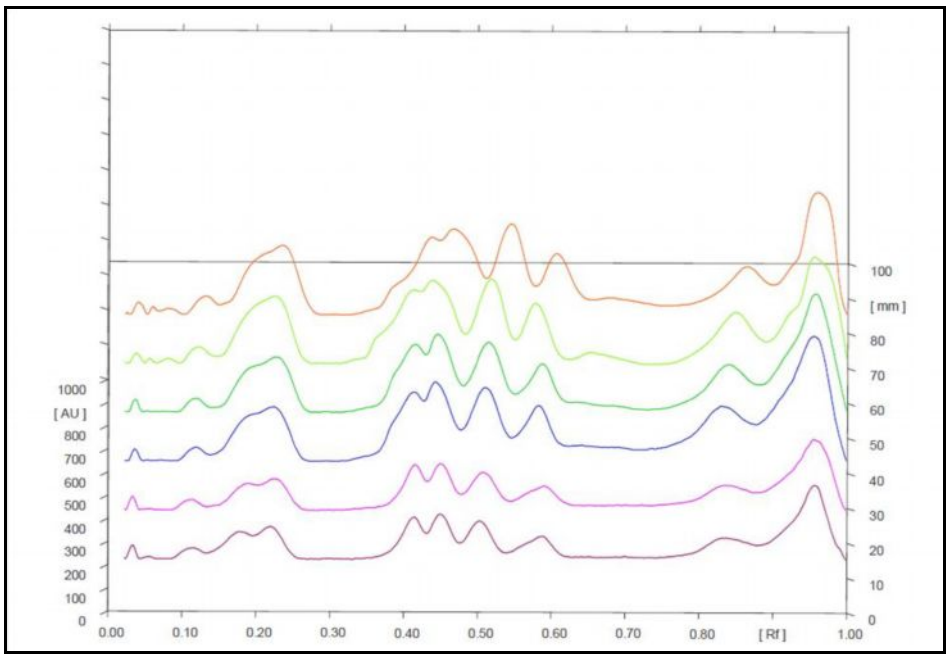


Figure 4: HPTLC 2D Densitometric superimposable chromatogram of methanol extract at 366nm (chloroform: methanol: ethyl acetate-4:1:5)

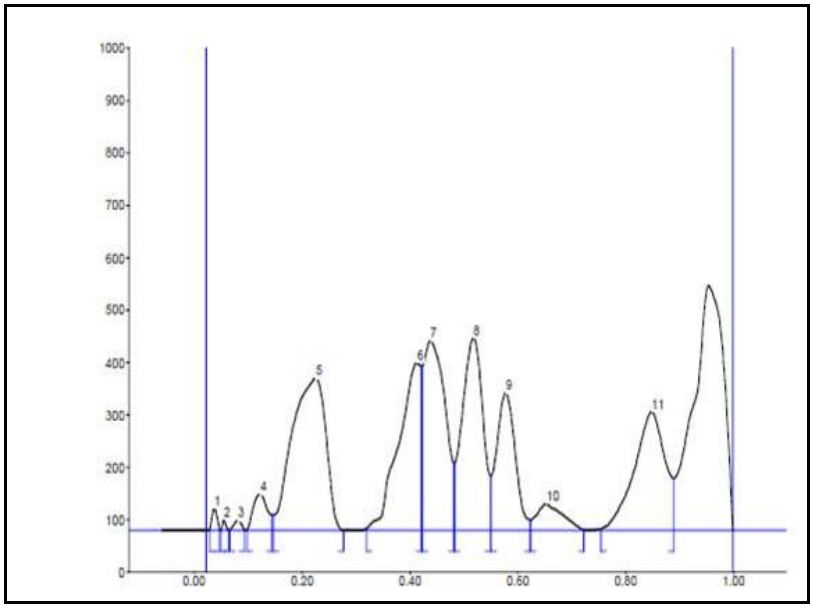


Figure 5: HPTLC chromatogram of methanol extract at 366nm (chloroform: methanol: ethyl acetate 4:1:5)

The present work can be helpful to herbal industry as an important standardization parameter of *T. undulate* leaves, and especially its alcoholic formulations and extracts, since *T. undulate* leaves are a part of several Ayurvedic and marketed herbal products, as they are indicated in many diseases[5]. This work can be specifically useful for authentication of raw material of the leaves and in detection of adulteration, which will ultimately benefit the society which consumes *T. Undulate* leaf formulations. Also, further phytochemical research such as isolation of marker compounds can be done on the basis of HPTLC fingerprint.

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