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HPTLC Fingerprinting of *Hemidesmus indicus* roots as a Quality Control Parameter in Herbal Formulations

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Abstract : *Hemidesmus indicus* roots have Anti-inflammatory, Anti-microbial, Anti-acne, Antioxidant, Hepatoprotective and Anti-arthritic activity. Many of its formulations are available in the market. The present work focuses on developing a simple HPTLC fingerprint of *Hemidesmus indicus* roots. Methanolic extract of the roots 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 (13:1:2). It was further subjected to HPTLC fingerprinting where R_f and Area Under Curve were calculated. HPTLC fingerprinting showed9 peaks at 254nm and 4 peaks at 366nm. This work provides a simple technique for standardization and detection of adulteration of *Hemidesmus indicus* root formulations, many of which are available in the market, consumed by people for treatment of various disease conditions, and also investigated upon continuously considering its wide domain of pharmacological actions.
Keywords : Anantmool, *Hemidesmus indicus*, HPTLC, Quality control, Sarivaa.

Introduction

Hemidesmus indicus(Family – Asclepiadaceae) roots are found commonly across India and are also known as Anantmool (Hindi), Sarivaa (Sanskrit) and Indian Sarsaparilla (English)[1,2].According to traditional medicinal texts as well as modern research, they possess anti-venom, anti-inflammatory, anti-pyretic, anti-microbial, anti-acne, antioxidant, hepatoprotective, anti-leprotic, anti-diarrheal, anti-cancer, anti-ulcer, anti-hyperlipidemic, anti-diabetic, chemoprotective, radioprotective, renoprotective, and anti-arthritic activity[1-32]. Many of its formulations are available in the market, including Dashmoolarishta, Manjisthadi Taila and Trifaladi Taila. The present work focuses on developing an HPTLC fingerprint of *H. indicus* roots.

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Figure 1. *Hemidesmus indicus* roots

Experimental[29-43]

Crude drug material

*H. indicus*roots were purchased from Sanjivani Aushadhalaya, Bhavnagar, in July 2015 and compared with standard literature for authentication[1-2].

Extraction

50g dry powder of *H. indicus* roots 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. After observing the pilot results, further TLC were developed by adding ammonia & ethyl acetate for removal of tailing. The mobile phases used were Chloroform : Methanol : Ethyl acetate 5:5:1 to 8:2:1, Chloroform : Methanol : Ammonia 5:5:1 to 8:2:1, Chloroform : Methanol : Ethyl acetate 4:6:2 to2:6:4, Chloroform : Methanol: Ammonia 4:6:2 to2:6:4, Chloroform : Methanol : Ethyl acetate 7:3:2, Chloroform: Methanol : Ethyl Acetate 8:2:2 and Chloroform : Methanol : Ethyl Acetate 13:1:2

HPTLC

HPTLC fingerprinting of methanolic extract was performed in Dept. of Pharmaceutical Sciences, Saurashtra University, using the mobile phase Chloroform: Methanol: Ethyl acetate (13:1:2), 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

Nine peaks were detected at 254nm (Table 1, Fig. 2, 3) and four peaks were detected at 366nm (Table 2, Fig. 4, 5) upon HPTLC of methanolic extract of *H. indicus*roots using mobile phase Chloroform: Methanol: Ethyl Acetate (13:1:2).

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

Peak	Rf	Area Under Curve	Area %
1	0.03	22380.8	58.02
2	0.09	3053.2	7.92
3	0.13	1290.1	9.46
4	0.21	1290.1	3.34
5	0.29	575.6	1.49
6	0.38	979.8	2.54
7	0.68	1188.5	3.08
8	0.75	4766.1	12.36
9	0.86	689.2	1.79

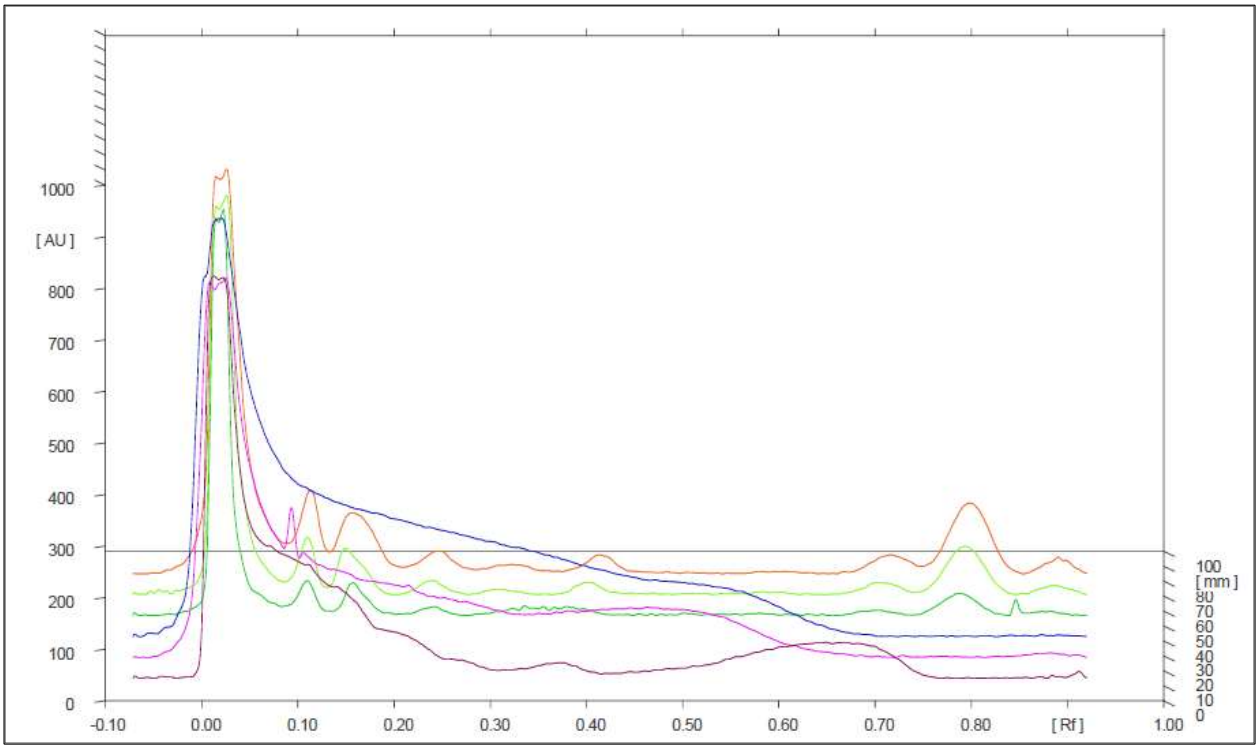
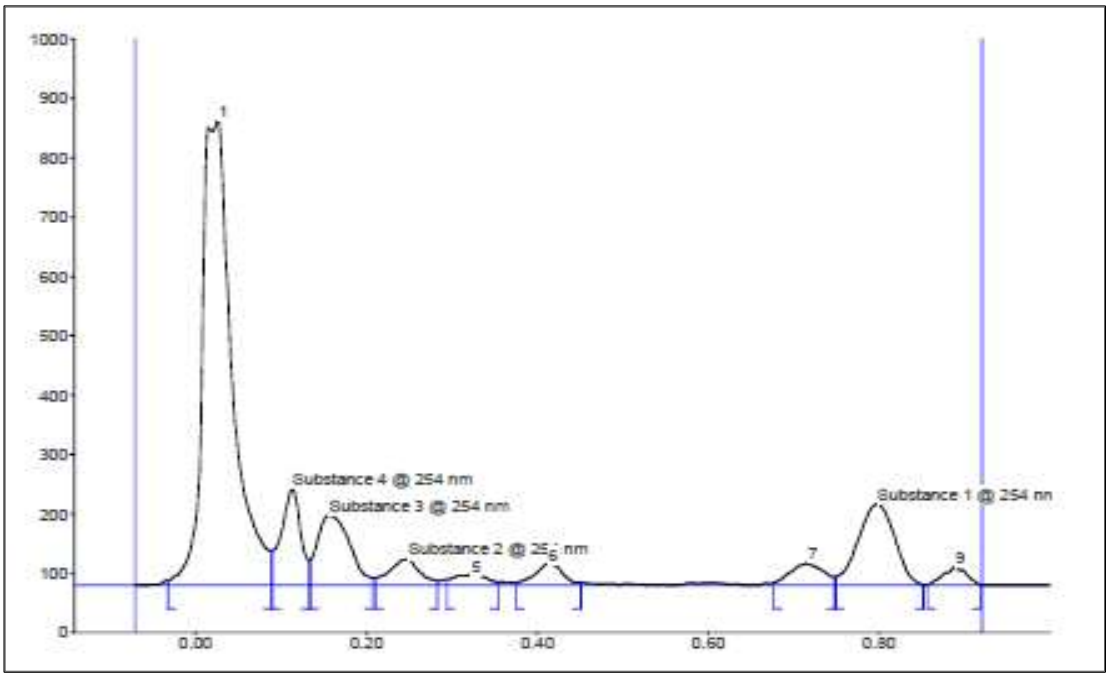


Figure 2: HPTLC 2Ddensitometric superimposable chromatogram of methanol extract at 254nm (chloroform: methanol: ethyl acetate-13:1:2)



-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-13:1:2)

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

Peak	Rf	Area Under Curve	Area %
1	0.03	14221.9	87.85
2	0.11	701.3	4.33
3	0.13	470.2	2.90
4	0.79	796.0	4.92

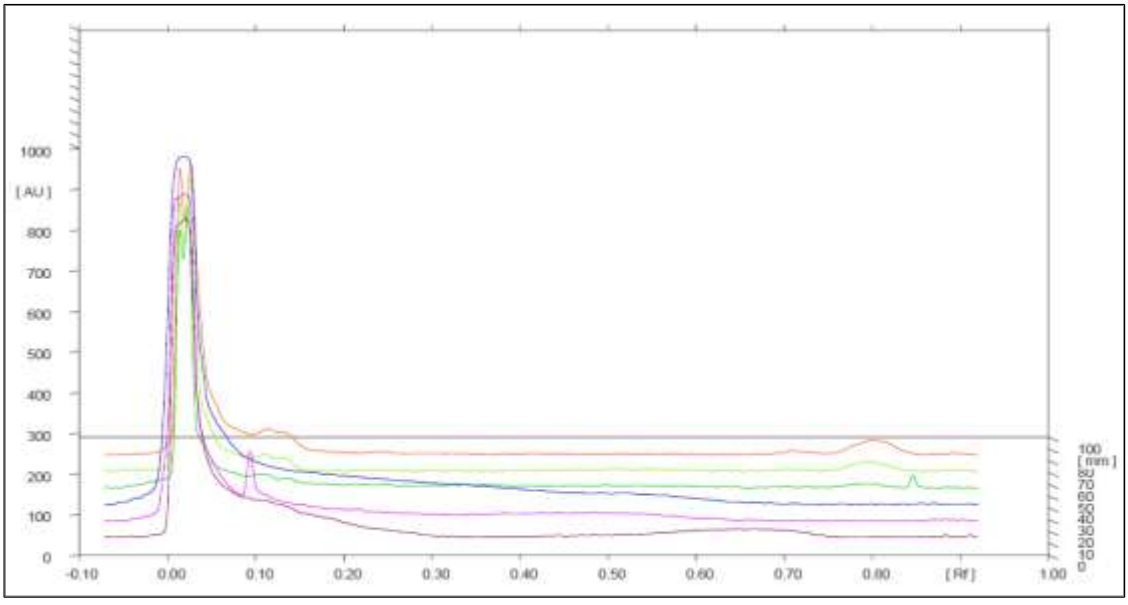


Figure 4: HPTLC 2D Densitometric superimposable chromatogram of methanol extract at 366nm (chloroform: methanol: ethyl acetate-13:1:2)

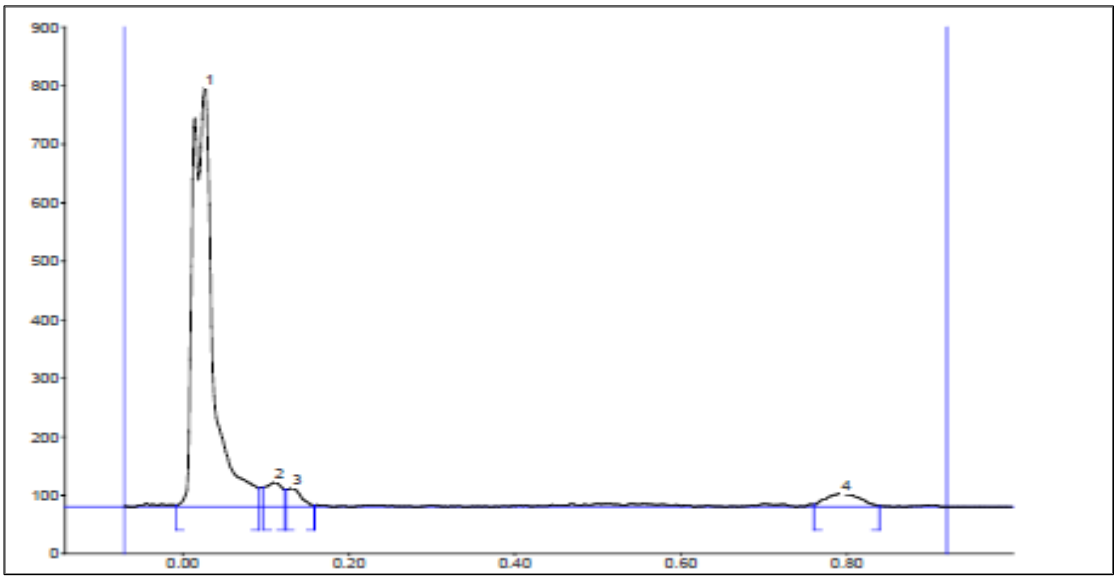


Figure 5: HPTLC chromatogram of methanol extract at 366nm (chloroform: methanol: ethyl- acetate 13:1:2)

The present work can be helpful to herbal industry as an important standardization parameter of *H. indicus* roots, and especially its alcoholic formulations and extracts, since *H. indicus* roots are a part of several Ayurvedic and marketed herbal products, as they are indicated in a broad spectrum of diseases[6]. This work can be specifically useful for authentication of raw material of the roots and in detection of adulteration, which will ultimately benefit the people who consume *H. indicus* root formulations.

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