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Research Article

HPTLC FINGERPRINT PROFILE OF AUTHENTIC AND MARKET SAMPLE OF *HEMIDESMUS INDICUS* (Linn.) R. Br.

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Abstract

Objective: To establish the fingerprint profile for *Hemidesmus indicus* (Linn.) R. Br. roots using high performace thin layer chromatography (HPTLC) technique for quality control. **Methods:** CAMAG HPTLC system equipped with TLC autosampler 4 applicator, TLC scanner 3 and win CATS 1.4.4. software was used. Chloroform and ethyl alcohol extracts of the roots were developed in suitable mobile phase using standard procedures and scanned under UV at 254 nm, 366 nm and under visible light. **Results:** The HPTLC fingerprinting of the chloroform and ethyl alcohol extracts showed several peaks with different R_f values. **Conclusion:** HPTLC fingerprint profile of chloroform extract of authentic sample of *Hemidesmus indicus* may be useful in the identification and authentication of the plant.

Keywords: Absorption spectra, *Hemidesmus indicus*, HPTLC fingerprinting, Sveta Sariva.

1. Introduction

Sveta Sariva (Tamil: Ven Nannari) is botanically equated as *Hemidesmus indicus* (Linn.) R. Br. (Fam. Asclepiadaceae). *Hemidesmus indicus* (Linn.) R. Br. commonly known as sarsaparilla or anantmool is a prostrate or semi-erect shrub found throughout India from upper Gangetic plains east-wards to Assam, throughout Central, Western and Southern India upto an elevation of 600 m[1,2]. The roots of *H.indicus* were repoted to possess 2-hydroxy-4-methoxy benzaldehyde, hemidesmine, hemidine, hemidesine, rutin[3]. The roots are used as antipyretic, antidiarrhoeal, diuretic, diaphorectic, leucorrhoea and also for urinary diseases, leprosy, leucoderma. Methanolic extract of *H. indicus* roots showed remarkable anti-cancer potentials against MCF and breast cancer cell lines. Root decoction tested on

hepatoma HepG$_2$ cell lines showed good results[4]. It is also used in combination with other drugs for snake bite[4,5]. It is widely used in folk medicine and as ingredient in Ayurvedic and Unani preparations which are prescribed for disease of biliousness, blood diseases, diarrhoea, skin diseases, respiratory diseases, fever, bronchitis, eye diseases, burning sensation, rheumatism and gastric disorders. Root decoction helps in alleviating skin diseases, syphilis, elephanthiasis, loss of appetite, blood purification and for kidney and urinary disorders. Several biological activities like hepatoprotective, antioxidant, antithrombotic, anti-ulcerogenic, anti-inflammatory, immunomodulatory, antidiabetic have been reported from various root extracts[6,7]. The ethanol and methanol extracts showed maximum inhibition against *Escherichia coli*, *Klebseilla pnemoniae*, *Salmonella typhi*, *Staphylococcus aureus* and *Vibrio cholera*. The aqueous extract of *H. indicus* does not show any activity against the above selected pathogenic bacteria[8]. In the crude drug market there are several other similar looking materials derived from various botanicals are sold in the name of Sveta Sariva. In order to develop an important and easy tool to identify the genuine Sveta Sariva roots, this study was aimed. Thus, the present study deals with the TLC/HPTLC fingerprint profile of chloroform and ethyl alcohol extracts of auhentic and market sample of *Hemidesmus indicus.*

2. Material and Methods

2.1. Plant Material

The shade dried roots of *Hemidesmus indicus* was collected from Chengalpet, Tamil Nadu, India, identified and authenticated by the botanist of this institute. Herbarium (specimen No. 00633) has been deposited in the department of Pharmacognosy of this Institute. The market sample sold in the name of Sveta Sariva was procured from local drug market, Chennai.

2.2. Instrumentation

A CAMAG HPTLC system (Muttenz, Switzerland) equipped with a sample applicator TLC autosampler 4, twin trough plate development chamber, TLC Scanner 3, win CATS software version 1.4.4. and Hamilton (Reno, Nevada, USA) Syringe (25 µL).

2.3. Material and reagents

AR grade toluene, chloroform, ethyl acetate and ethyl alcohol were obtained from E.Merck, India.

2.4. HPTLC profile

HPTLC studies were carried out following the method of Wagner[9], Sethi[10] and Stahl [11].

2.4.1. Sample preparation: The roots were powdered coarsely. Two grams each of authentic and market powdered samples were accurately weighed and exhaustively extracted successively by chloroform and ethyl alcohol (each 100 ml) respectively using Soxhlet apparatus. The extracts were filtered and reduced under reduced pressure and made up to 10 ml in standard flasks separately.

2.4.2. Developing solvent system

The mobile phase used for developing the plant extracts of chloroform and ethyl alcohol extracts were toluene: ethyl acetate (5:1.5).

2.4.3. Sample application

The samples were spotted in the form of bands with a Hamilton syringe (25 µl) on aluminium TLC plate precoated with silica gel $60F_{254}$ (E. Merck) 0.2 mm thickness with the help of TLC autosampler 4 attached to CAMAG HPTLC system, which was programmed through win CATS software version 1.4.4. Various concentrations of the samples (5 µL and 10 µL) of chloroform and ethyl alcohol extracts of authentic and market samples were applied as 6 mm bands at a spraying rate of 10 seconds μL^{-1}. 5 µL of chloroform extract of market and authentic samples were applied respectively in track 1 and 2 and 5 µL of ethyl alcohol extract of market and authentic samples of *H. indicus* were applied respectively in track 3 and 4.

2.4.4. Development of chromatogram

Development of the plate up to a migration distance 80 mm was performed at 27 ± 2°C with the mobile phase for each extracts in a CAMAG chamber previously saturated for 30 min. After development the plate was dried at 60°C in an oven for 5 min. Densitometric scanning was then performed with a CAMAG TLC scanner 3 equipped with win CATS software version 1.4.4. at λmax 254 and 366 nm using deuterium light source, the slit dimensions were 5.00 X 0.45 mm. The chromatograms were recorded.

2.4.5. Detection of spots

The developed plate was then dipped in vanillin-sulphuric acid reagent as a derivatizing agent and dried at 105°C in hot air oven till the colour of the band appears. The plate was kept in photo-documentation chamber and captured the images. The R_f values and finger print data were recorded by win CATS software.

3. Results: The TLC profile of chloroform and ethyl alcohol extracts of market and authentic samples of *H.indicus* under UV 254 nm, UV 366 nm and visible light after derivatization with vanillin-sulphuric acid reagent are shown in figure 1. Under UV 254 nm, in market sample of *H. indicus* root, there were 10 spots at the following R_f 0.04, 0.05, 0.11, 0.20, 0.26, 0.43, 0.52, 0.69, 0.83 and 0.91, indicating the presence of 10 different components in 5 µl of chloroform extract. Out of 10 spots, the spots with R_f values 0.43 and 0.83 were found to be prominent as the percentage area was more with 14.79 and 28.52 respectively. The remaining components were found to be very less in quantity as the percent area for all the spots were less than 7. The HPTLC finger print profile of market sample of chloroform extract of *H. indicus* is shown in figure 2. Authentic sample of *H. indicus* root, there were 10 spots at the following R_f 0.04, 0.12, 0.21, 0.28, 0.43, 0.48, 0.55, 0.70, 0.84 and 0.92, indicating the presence of 10 different components in 5 µl of chloroform extract. Out of 10 spots, the spots with R_f values 0.43 and 0.84 were found to be prominent as the percentage area was more with 0.43 and 0.84 respectively. The remaining components were found to be very less in quantity as the percent area for all the spots were less than 3. The HPTLC finger print profile of authentic sample of chloroform extract of *H. indicus* is shown in figure 3. The common spot present in chloroform extract of both market and authentic sample of *H. indicus* were found to have R_f 0.43 indicating the similarity. The other spots are specific to the individual species. In market sample of *H. indicus* root, there were 6 spots at the following R_f 0.04, 0.12, 0.21, 0.44, 0.84 and 0.94, indicating the presence of 6 different components in 5 µl of ethyl alcohol extract. Out of 6 spots, the spots with R_f values 0.04 and 0.84 were found to be prominent as the percentage area was more with 60.62 and 18.80 respectively. The remaining components were found to be very less in quantity as the percent area for all the spots were less than 8. The HPTLC finger print profile of market sample of ethyl alcohol extract of *H. indicus* is shown in figure 4. In authentic sample of *H. indicus* root, there were 4 spots at the following R_f 0.04, 0.43, 0.84 and 0.95 as shown in figure 5, indicating the presence of 4 different components in 5 µl of ethyl alcohol

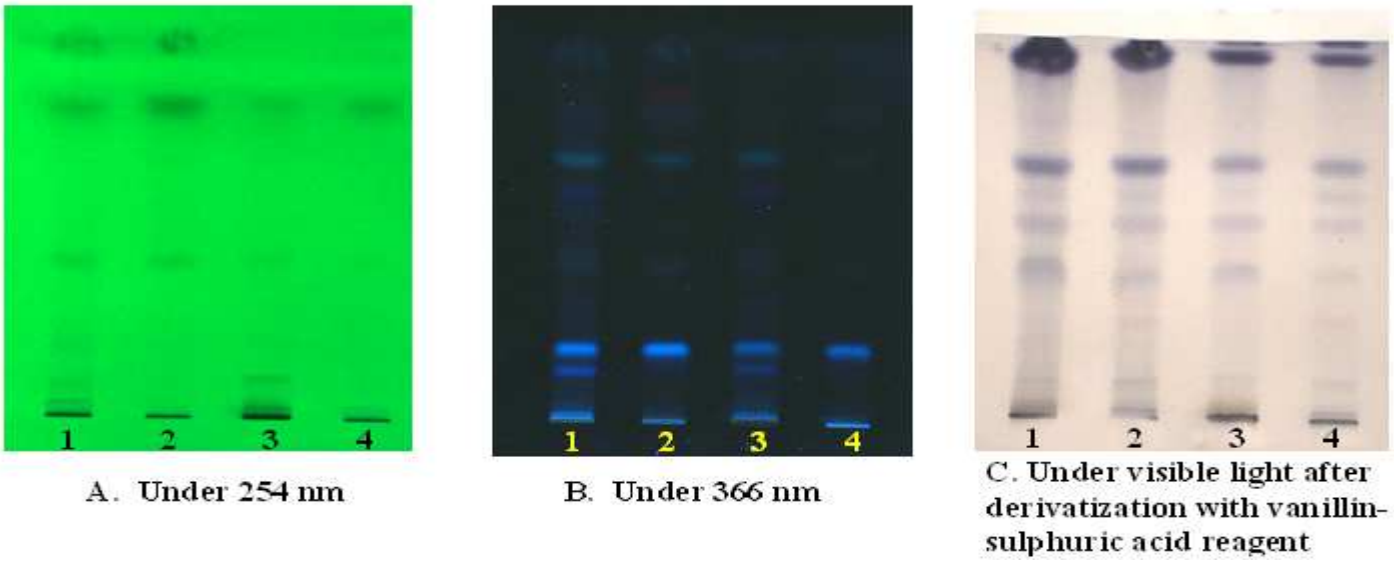
extract. Out of 4 spots, the spots with R_f values 0.84 and 0.95 were found to be prominent as the percentage area was more with 40.12 and 36.88 respectively. The remaining component was found to be very less in quantity as the percent area for all the spots were less than 5. The HPTLC finger print profile of authentic sample of ethyl alcohol extract of *H. indicus* is shown in figure 5. The common spot present in ethyl alcohol extract of both market and authentic sample of *H. indicus* were found to have R_f 0.84 indicating the similarity. The other spots are specific to the individual species.

Under UV 366nm, market sample of *H. indicus* showed 5 spots at R_f 0.19, 0.22, 0.43, 0.62 and 0.68, indicating the presence of 5 different components in 5 µl of chloroform extract. The HPTLC finger print profile of market sample of chloroform extract of *H. indicus* is shown in figure 6. Authentic sample of *H. indicus* showed 3 spots at R_f 0.22, 0.43 and 0.68, indicating the presence of 3 different components in 5 µl of chloroform extract. The HPTLC finger print profile of authentic sample of chloroform extract of *H. indicus* is shown in figure 7. Spots with R_f 0.22 and 0.68 were found to be common in chloroform extract of market and authentic sample of *H.indicus.* The common spots present in chloroform extract of both market and authentic sample of *H. indicus* were found to have R_f 0.22 and 0.68 indicating the similarity. The other spots are specific to the individual species. Market sample of *H. indicus* showed 3 spots at R_f 0.19, 0.22 and 0.68, indicating the presence of 3 different components in 5 µl of ethyl alcohol extract. The HPTLC finger print profile of market sample of ethyl alcohol extract of *H. indicus* is shown in figure 8. Authentic sample of *H. indicus* showed 2 spots at R_f 0.22 and 0.68, indicating the presence 2 different components in 5 µl of ethyl alcohol extract. The HPTLC finger print profile of authentic sample of ethyl alcohol extract of *H. indicus* is shown in figure 9. Spots with R_f 0.22 and 0.68 were found to be common in ethyl alcohol extract of market and authentic sample of *H. indicus.* The common spots present in ethyl alcohol extract of both market and authentic sample of *H. indicus* were found to have R_f 0.22 and 0.68 indicating the similarity. The other spots are specific to the individual species.

After derivatization with vanillin-sulphuric acid, market sample of *H. indicus* showed 6 spots at R_f 0.19, 0.42, 0.53, 0.58, 0.66 and 0.92, indicating the presence of 6 different components in 5 µl of chloroform extract. Authentic sample of *H. indicus* showed 7 spots at R_f 0.19, 0.36, 0.42, 0.53, 0.58, 0.66 and 0.92, indicating the presence 7 different components in 5 µl of chloroform extract. Spots with R_f 0.19, 0.42, 0.53, 0.58, 0.66 and 0.92 were found to be common in chloroform extract of market and authentic sample of *H. indicus.* The common spots present in chloroform extract of

both market and authentic sample of *H. indicus* were found to have R_f 0.19, 0.42, 0.53, 0.58, 0.66 and 0.92 indicating the similarity. The other spots are specific to the individual species. Market sample of *H. indicus* showed 6 spots at R_f 0.19, 0.42, 0.53, 0.58, 0.66 and 0.92, indicating the presence of 6 different components in 5 µl of ethyl alcohol extract. Authentic sample of *H. indicus* showed 7 spots at R_f 0.19, 0.36, 0.42, 0.53, 0.58, 0.66 and 0.92, indicating the presence 7 different components in 5 µl of ethyl alcohol extract. Spots with R_f 0.19, 0.42, 0.53, 0.58, 0.66 and 0.92 were found to be common in ethyl alcohol extract of market and authentic sample of *H. indicus.* The common spots present in ethyl alcohol extract of both market and authentic sample of *H. indicus* were found to have R_f 0.19, 0.42, 0.53, 0.58, 0.66 and 0.92 indicating the similarity. The other spots are specific to the individual species. The densitometric scan of 5 µl of chloroform and ethyl alcohol extracts of market and authentic sample of *H. indicus* under UV 254 nm were shown in figure 10. The densitometric scan of 5 µl of chloroform and ethyl alcohol extracts of market and authentic sample of *H. indicus* under UV 366 nm were shown in figure 11.

Figure-1: TLC profile of chloroform and ethyl alcohol extracts of authentic and market sample of *H. indicus.*



A. Under 254 nm

B. Under 366 nm

C. Under visible light after derivatization with vanillin-sulphuric acid reagent

Track 1: 5 µl of chloroform extract of market sample of *H. indicus.*

Track 2: 5 µl of chloroform extract of authentic sample of *H. indicus.*

Track 3: 5 µl of ethyl alcohol extract of market sample of *H. indicus.*

Track 4: 5 µl of ethyl alcohol extract of authentic sample of *H. indicus.*

HPTLC finger print profile of *H. indicus* root at UV 254 nm.

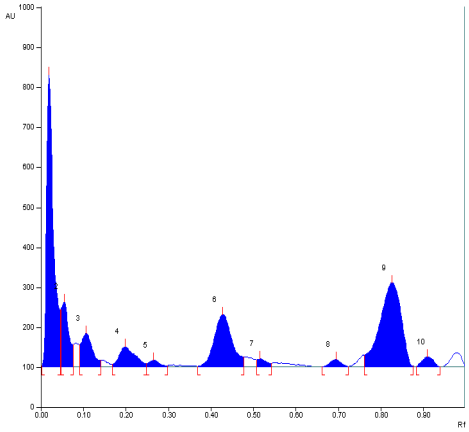


Figure-2: Chloroform extract of market sample

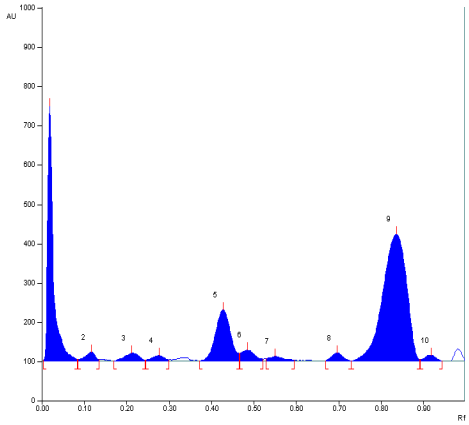


Figure-3: Chloroform extract of authentic sample

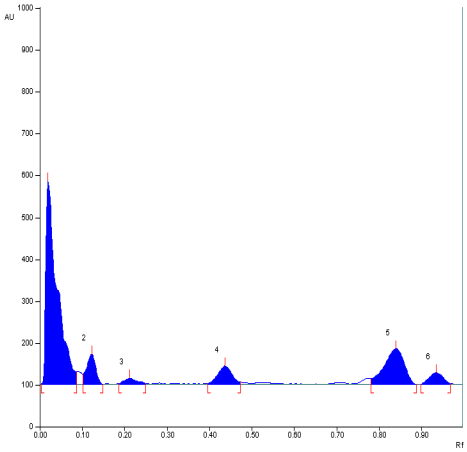


Figure-4: Ethyl alcohol extract of market sample

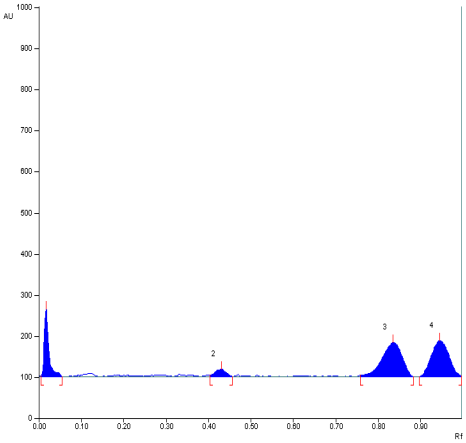


Figure-5: Ethyl alcohol extract of authentic sample

HPTLC finger print profile of *H. indicus* root at UV 366 nm

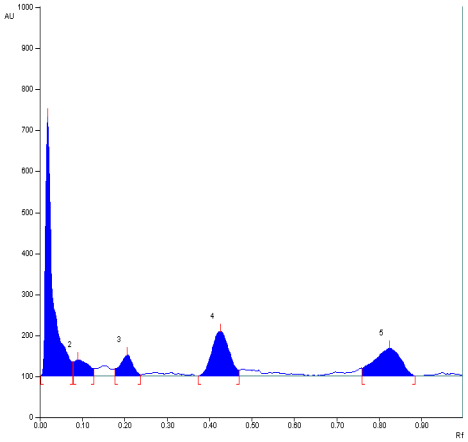


Figure-6:. Chloroform extract of market sample

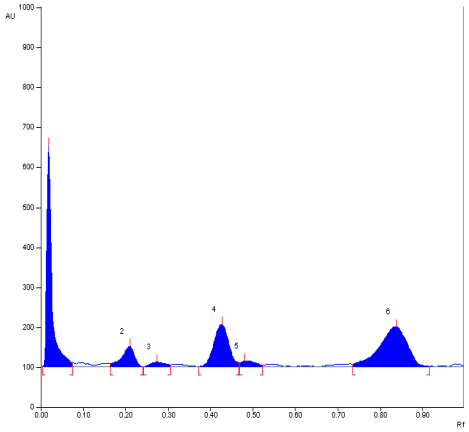


Figure-7: Chloroform extract of authentic sample

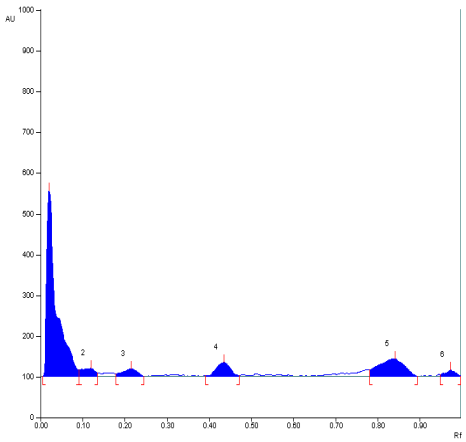


Figure-8: Ethyl alcohol extract of market sample

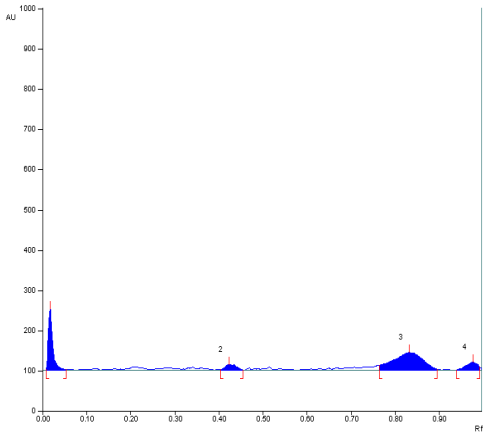


Figure-9: Ethyl alcohol extract of authentic sample

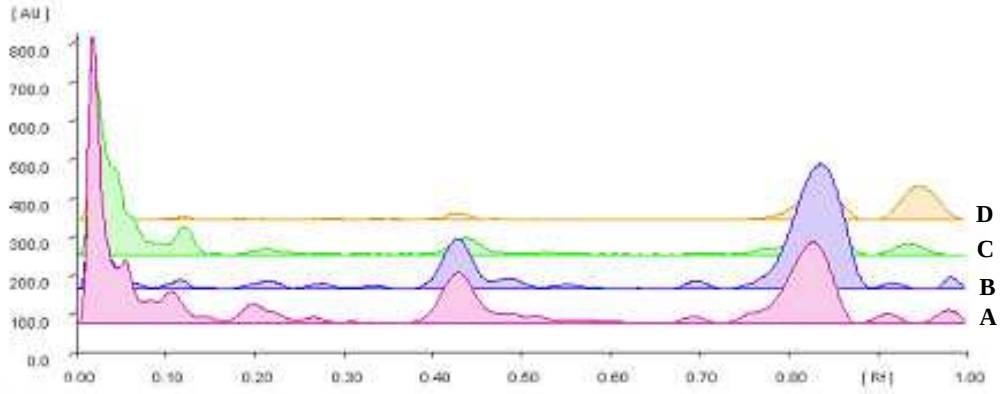


Figure 10. Denistometric scan of chloroform and ethyl alcohol extract of market and authentic sample of *H. indicus* at UV 254 nm.

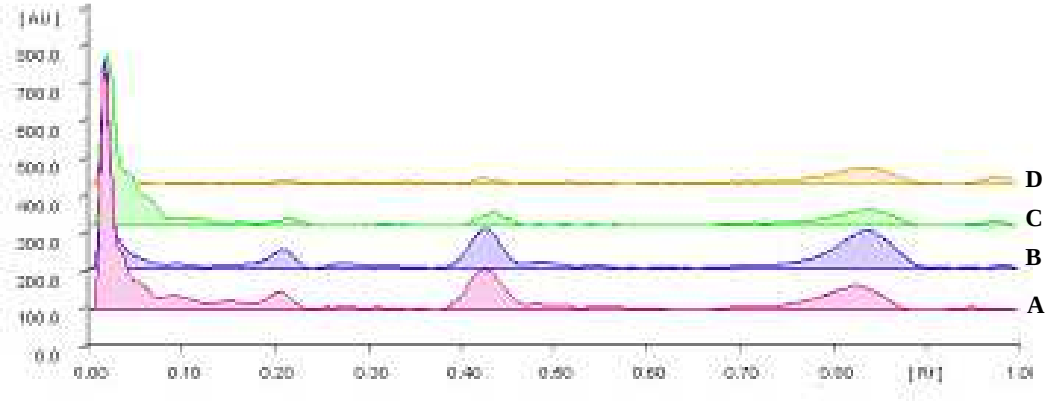


Figure 11. Densitometric scan of chloroform and ethyl alcohol extract of market and authentic sample of *H. indicus* at UV 366 nm

A. 5 µl of chloroform extract of market sample of *H. indicus.*
B. 5 µl of chloroform extract of authentic sample of *H. indicus.*
C. 5 µl of ethyl alcohol extract of market sample of *H. indicus.*
D. 5 µl of ethyl alcohol extract of authentic sample of *H. indicus.*

3. Discussion

The TLC and HPTLC finger print profile of chloroform and ethyl alcohol extracts of market and authentic sample of *Hemidesmus indicus* were recorded. The discussion in this study is with 5 µl of each extracts of market and authentic sample of *H. indicus*. HPTLC fingerprinting is proved to be a reliable, accurate and precised method for herbal identification and authentication. Thus the developed chromatogram and R_f value will be specific with selected solvent system, and serve as the better tool for standardization of the roots of *H. indicus*. The HPTLC fingerprinting is helpful in differentiating the species from the adulterant. 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 for the isolation, purification, characterization and identification of chemical marker compounds of the species. Thus the present study will provide sufficient information about the identification, standardization and quality control of *H. indicus*, from the profile of chloroform extract of the roots.

4. 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 market and authentic sample of *H. indicus* can provide standard fingerprints with selected solvent system and can be used as a reference for the proper identification/authentication and quality control of the drug. The HPTLC fingerprint pattern developed with chloroform extract will be helpful in differentiating the species. This feature can be used to differentiate the authentic root from that of market drug. In TLC and HPTLC profile of chloroform and ethyl alcohol extracts in the mobile phase of toluene: ethyl acetate (5:1.5), the spots were common in both the samples. Thus the market sample procured from local drug market is same as that of *H.indicus*. Densitometric scan at UV 254 and 366 nm of market and authentic sample of chloroform and ethyl alcohol extracts also clearly indicates that the sample *H.indicus* available in market is authentic.

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