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ORIGINAL RESEARCH ARTICLE

HPTLC Fingerprinting Profile of Root of *Punarnava* (*Boerhaavia diffusa* Linn.)

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ABSTRACT

Introduction: The root of *Punarnava* (*Boerhaavia diffusa* Linn.) possesses high therapeutic value and is used in many conditions such as *Gulma* (abdominal tumor), *Pandu* (anemia), *Yakrit roga* (liver disorders), *Pleeha roga* (splenic disorders), and *Hridroga* (cardiac disorders). But, before internal administration, it is essential to confirm the drug's authenticity and quality. Obtaining authentic chromatographic fingerprints that accurately reflect the pharmacologically active and chemically distinctive components of the drugs is essential. High-performance thin-layer chromatography (HPTLC) fingerprinting helps to know the authenticity and identity of the drug. It is highly useful to avoid the unnecessary usage of adulterated drugs. Detection of the marker compound is essential to know the mechanism of action a drug in particular diseases and systems of the body.

Materials and Methods: In the present study, methanolic extract of root powder of *B. diffusa* Linn. was selected. Solvent system used was toluene, ethyl acetate, and formic acid (5:4:0.5). After development, the plate was examined under ultraviolet light 254 nm, 366 nm, and after derivatization in white light.

Results and Discussion: While analyzing the HPTLC fingerprinting profile of *Punarnava* (*B. diffusa* Linn.), at 254 nm, it showed six peaks and at 366 nm, it showed ten peaks. At 254 nm, highest peak was obtained at Rf 0.03 with a total area of 4390.5 (AU). At 366 nm, highest peak obtained at Rf 0.02 with a total area of 7211.2 (AU).

Conclusion: For identification of medicinal plants, the HPTLC fingerprinting profile is a crucial component of the standardization of herbal drugs. At 254 nm, it shows 6 peaks and at 366 nm, it shows 10 peaks.

1. INTRODUCTION

The botanical name for the trailing herb, *Punarnava*, is *Boerhaavia diffusa* Linn. It is a member of the Nyctaginaceae family and is native to all of India. *Punarnava* root is indicated for diseases affecting different body systems, such as *Gulma* (abdominal tumor), *Pandu* (anemia), *Yakrit roga* (liver disorders), *Pleeha roga* (splenic disorders), *Hridroga* (cardiac disorders), *Gara* (poison), *Kasa* (cough), and *Arsas* (hemorrhoids).[1-3] Numerous studies support the antioxidant, immunomodulatory, hepatoprotective, anthelminthic, and other properties of the *B. diffusa* Linn root. To confirm the authenticity and quality of the drug, high-performance thin-layer chromatography (HPTLC) fingerprinting profile should be done. It is essential to obtain genuine chromatographic fingerprints that authentically represent the chemically particular and pharmacologically active ingredients in the drugs. The drug's identification and authenticity can be determined with the aid of HPTLC fingerprinting. Preventing the unwarranted use of altered drugs is very important. Finding the marker chemical is important to understanding how a drug works in specific conditions and bodily systems. Thin-layer chromatography of the methanolic extract of root of *B. diffusa* Linn. was mentioned in the Ayurvedic Pharmacopoeia of India.[4] HPTLC fingerprinting profile of root powder of *B. diffusa* Linn. was previously done in ethanolic extract by Khalid and Freed and was recorded under 366 nm, after derivatization 366 nm and UV light.[5] In the present study, methanolic extract of root powder of *Punarnava* (*B. diffusa* Linn.) was used for the HPTLC fingerprinting profile and examined under 254 nm, 366 nm, and after derivatization in white light.

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2. MATERIALS AND METHODS

2.1. Materials

Choorna of root of *B. diffusa* Linn., methanol, toluene, ethyl acetate, formic acid are shown in Figure 1.

2.2. Procedure

Test solutions were made with 2 g of *choorna* (powder) of dried root of *B. diffusa* Linn. Extract 0.5 g sample in 10 mL methanol, filter, and carry out the thin-layer chromatography. In the Ayurvedic Pharmacopoeia of India, thin-layer chromatography of the root of *B. diffusa* Linn. was mentioned and used mobile phase as toluene: ethyl acetate: formic acid. Hence, the same mobile phase was selected in the present study also. Apply 5 µL of the extract on HPTLC plate and develop the plate to a distance of 8 cm using toluene: ethyl acetate: formic acid (5:4:0.5). After development, allow the plate to dry in air and examine under ultraviolet light 254 nm and 366 nm.

Develop the plate using the solvent system in twin trough chamber previously saturated with the solvent system for 30 min and wash the syringe twice with methanol. Dry the plate and place it in the scanner. Open a file and enter all parameters of scanning, integration, and spectrum. Scan the plate in UV 254 nm and 366nm. Scan all the tracks and then scan the UV spectrum of each scanning. Take the fingerprint of each track. UV spectra spots can be compared in the spectrum display.

3. RESULTS

3.1. Area and Peaks of Methanol Extract at 254 nm

Total 6 peaks were obtained for methanol extract of dried root powder of *B. diffusa* Linn. at 254 nm. These 6 peaks were defined with max Rf value of −0.03 with area 4390.5AU, max Rf value of 0.22 with area 201.9 AU, max Rf value of 0.57 with area 934.6AU, max Rf value of 0.71 with area 1130.2AU, max Rf value of 0.81 with area 2062.6 AU, max Rf value of 0.86 with area 608.9AU, respectively, which are tabulated in [Table 1, Diagram 1, Figure 2]

3.2. Area and Peaks of Methanol Extract at 366 nm

Total 10 peaks were obtained for methanol extract of dried root powder of *B. diffusa* Linn. The peaks were obtained with max Rf value of −0.03 with area 7211.2, max Rf value of 0.04 with area 745.4AU, max Rf value of 0.27 with 225.3 AU, max Rf value of 0.30 with area 207.6 AU, max Rf value of 0.57 with 945.2 AU, max Rf value of 0.66 with area 642.8 AU, max Rf value 0.72 with area 457.9 AU, max Rf value of 0.79 with area 1123.7AU, max Rf value 0.83 with area 230.4 AU, max Rf value of 1.00 with area 403.1, respectively, which are tabulated in [Table2, Diagram 2, Figure 3]

4. DISCUSSION

Thin-layer chromatography of the methanolic extract of root of *B. diffusa* Linn. was mentioned in the Ayurvedic Pharmacopoeia of India. In the present study, HPTLC fingerprinting profile of the root powder of *B. diffusa* Linn. was done. A single run of HPTLC can separate and identify multiple components of a sample, making it a quick and effective technique. The high resolution of HPTLC, which enables the separation of chemically similar compounds, is one of its main benefits. Additionally, HPTLC has a higher sensitivity because it has a detection limit of up to nanograms and can identify compounds in traces. HPTLC chromatogram of the methanol extracts of root powder of *B. diffusa* Linn. at 254 nm and 366 nm was recorded. The Rf values of the separated compounds of each extract were noted at 254 nm and 366 nm. Each peak indicates the presence of a specific chemical constituent. At 254 nm, methanol extract of the root powder showed the presence of 6 peaks, with a total area of 9328.71 (AU). Highest peak was obtained at Rf 0.03 with a total area of 4390.5 (AU). At 366 nm, total 10 peaks were obtained with a total area of 12192.67(AU). Highest peak obtained at Rf 0.02 with a total area of 7211.2 (AU). In a previous research, work ethanolic extract of root of *B. diffusa* Linn. also showed 10 peaks at 366 nm.

5. CONCLUSION

Punarnava (*B. diffusa* Linn.) has a unique pharmacological activity that is attributed to its multitude of active components. For the correct identification of medicinal plants, the HPTLC fingerprinting profile is a crucial component of the standardization of herbal drugs. At 254 nm, it shows 6 peaks and at 366 nm, it shows 10 peaks. This method is very helpful to identify the adulterations in herbal drug market. In further researches, the present HPTLC fingerprinting profile can be utilized as a diagnostic tool to identify and assess the quality and purity of the *B. diffusa* Linn.

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7. AUTHORS' CONTRIBUTIONS

All the authors contributed equally in design and execution of the article.

8. FUNDING

Nil.

9. ETHICAL APPROVALS

This study does not require ethical clearance as it is a laboratory study.

10. CONFLICTS OF INTEREST

Nil.

11. DATA AVAILABILITY

This is an original manuscript and all data are available for only review purposes from principal investigators.

12. PUBLISHERS NOTE

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Figure 1: *Boerhaavia diffusa* Linn. root powder

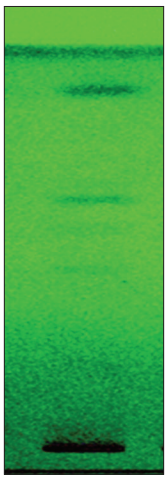

Figure 2: TLC views of methanol extract of dried root powder of *Boerhaavia diffusa* Linn. at 254 nm

Figure 3: TLC views of methanol extract of dried root powder of *Boerhaavia diffusa* Linn. at 366 nm

Figure 4: TLC views of methanol extract of dried root powder of *Boerhaavia diffusa* Linn. after derivatization in white light

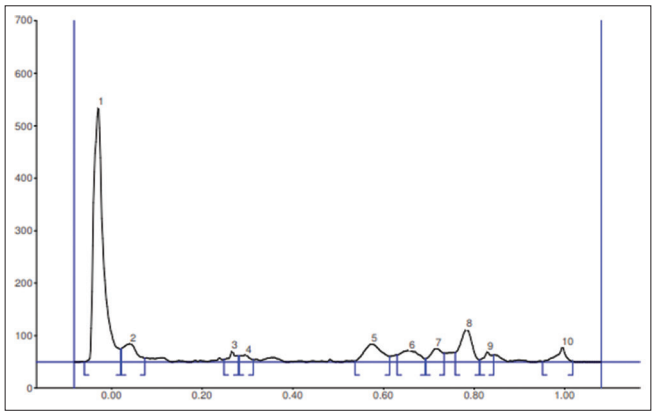

Diagram 1: Overview graph of methanol extract of dried root powder of *Boerhaavia diffusa* Linn. at 254 nm

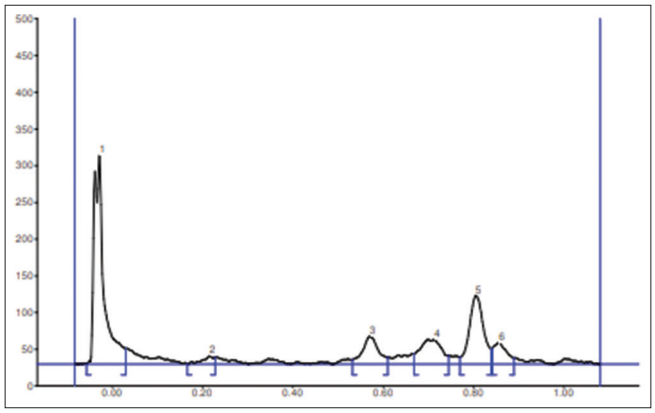

Diagram 2: Overview graph of methanol extract of dried root powder of *Boerhaavia diffusa* Linn. at 366 nm

Table 1: Area and peaks of methanol extract of dried root powder at 254 nm

Peak	Start Rf	Max Rf	End Rf	Area (AU)	Area %
1	−0.06	−0.03	0.03	4390.5	47.06
2	0.17	0.22	0.23	201.9	21.16
3	0.53	0.57	0.61	934.6	10.02
4	0.67	0.71	0.75	1130.2	12.12
5	0.77	0.81	0.84	2062.6	22.11
6	0.84	0.86	0.89	608.9	6.53

Table 2: Area and peaks of methanol extract of dried root powder at 366 nm

Peak	Start Rf	Max Rf	End Rf	Area	Area %
1	−0.06	−0.03	0.02	7211.2	59.14
2	0.02	0.04	0.07	745.4	6.11
3	0.25	0.27	0.28	225.3	1.85
4	0.28	0.30	0.31	207.6	1.70
5	0.54	0.57	0.62	945.2	7.75
6	0.63	0.66	0.69	642.8	5.27
7	0.69	0.72	0.74	457.9	3.76
8	0.76	0.79	0.81	1123.7	9.22
9	0.81	0.83	0.85	230.4	1.89
10	0.95	1.00	1.02	403.1	3.31