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Comparative HPTLC Fingerprint Profiling of *Boerhavia diffusa* L. and *Boerhavia erecta* L.: A Tool for Phytochemical Characterization and Quality Assessment

[1]Aafree Hashmi and [2]Ritesh Vaidya

[1,2]Department of Science, Gokul Global University,
Sidhpur - 384151 (Inia)

Abstract

Medicinal plants are important sources of biologically active secondary metabolites and play a significant role in traditional and modern healthcare systems. The genus *Boerhavia* (Nyctaginaceae) comprises several medicinally valuable species, among which *Boerhavia diffusa* L. and *Boerhavia erecta* L. are widely recognized for their ethnopharmacological importance. Accurate authentication and quality assessment of herbal materials are essential for ensuring their efficacy, safety, and consistency. High-performance thin-layer chromatography (HPTLC) has emerged as a rapid, reliable, and cost-effective analytical technique for developing characteristic phytochemical fingerprints of medicinal plants. The present study aimed to compare the HPTLC fingerprint profiles of methanolic extracts prepared from the leaf, stem, and root of *B. diffusa* L. and *B. erecta* L. Methanolic extracts were prepared by Soxhlet extraction and chromatographically analysed on silica gel 60 F, … „ plates. The developed chromatograms were evaluated based on Rf values, number of peaks, and peak area percentages to establish comparative phytochemical fingerprints. Distinct chromatographic patterns at 366nm were observed among different plant parts of both species, indicating variations in their phytochemical composition.

Key words : *Boerhavia diffusa* L., *Boerhavia erecta* L., HPTLC, phytochemical fingerprinting.

Medicinal plants have served as an indispensable source of therapeutic agents for thousands of years and remain fundamental to healthcare systems across the world. According to the World Health Organization[15] (WHO), approximately 80% of the global population depends directly or indirectly on herbal medicines for primary healthcare, particularly in developing countries where traditional medicine continues to complement

[1]Research Scholar, [2]Asociate Professor

modern healthcare practices (World Health Organization[16] [WHO]). The growing interest in herbal medicines has increased the need for scientific validation, standardization, and quality assessment of medicinal plant materials to ensure their safety, efficacy, and authenticity[7].

Plants synthesize a diverse array of secondary metabolites including alkaloids, flavonoids, phenolic compounds, tannins, terpenoids, glycosides, steroids, and saponins. Although these metabolites are not directly involved in plant growth and development, they perform important ecological functions such as defense against pathogens, insects, and environmental stress. More importantly, many of these compounds possess significant pharmacological activities including antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, anticancer, antidiabetic, and immunomodulatory properties[4,5]. The qualitative and quantitative variation of these metabolites among species and even among different plant parts makes phytochemical characterization an essential component of medicinal plant research.

The genus *Boerhavia* belongs to the family Nyctaginaceae and comprises nearly 40 species distributed throughout tropical and subtropical regions of the world. Among these, *Boerhavia diffusa* L., commonly known as Punarnava, is one of the most extensively studied medicinal plants owing to its wide range of pharmacological activities. It has been traditionally employed in Ayurveda for the treatment of liver disorders, urinary diseases, inflammation, edema, jaundice, diabetes, and gastrointestinal ailments.

In contrast, *Boerhavia erecta* L. has received comparatively less scientific attention despite its widespread distribution and traditional medicinal use. Various ethnobotanical reports describe its application in the management of fever, skin diseases, inflammation, digestive disorders, and microbial infections. Preliminary phytochemical investigations have revealed the presence of flavonoids, phenolic compounds, alkaloids, steroids, and terpenoids, suggesting that the species may possess pharmacological properties comparable to those of *B. diffusa*. However, comprehensive phytochemical characterization and comparative analytical studies involving *B. erecta* remain limited, highlighting the need for systematic investigations using advanced chromatographic techniques[3].

One of the major challenges associated with herbal medicines is ensuring their authenticity and consistency. Environmental conditions, geographical location, harvesting season, processing methods, and storage conditions may significantly influence the phytochemical composition of medicinal plants. Consequently, reliable analytical techniques are required to establish characteristic chemical fingerprints that can be used for quality control and authentication[7].

High-performance thin-layer chromatography (HPTLC) has become one of the most widely accepted chromatographic techniques for the qualitative evaluation of herbal drugs. Compared with conventional thin-layer chromatography, HPTLC provides improved resolution, higher sensitivity, better reproducibility, and rapid analysis of multiple samples simultaneously. The technique enables visualization of characteristic chromatographic

fingerprints that facilitate the identification of phytochemical constituents based on their retention factor (Rf), peak area, and densitometric characteristics[10]. Furthermore, HPTLC requires relatively small quantities of solvents and samples, making it an economical and environmentally friendly analytical approach suitable for routine quality assessment of herbal materials[13].

The selection of an appropriate stationary phase and mobile phase is crucial for obtaining well-resolved chromatographic fingerprints. Silica gel 60 F_{254} plates are commonly employed because they effectively separate compounds possessing different polarities, while optimization of the mobile phase ensures efficient migration and resolution of phytochemical constituents[14]. Comparative HPTLC fingerprinting of different plant parts offers additional insight into the distribution of bioactive metabolites within a species. Since leaves, stems, and roots often accumulate secondary metabolites in different proportions, comparative chromatographic analysis provides valuable information regarding the chemical diversity of medicinal plants.

Although numerous pharmacological studies have been conducted on *Boerhavia diffusa* L., comparative chromatographic investigations involving *Boerhavia* erecta L. remain scarce. Furthermore, limited information is available regarding the comparative phytochemical fingerprints of different vegetative parts of these two medicinal species. Therefore, the present investigation was undertaken to establish comparative HPTLC fingerprint profiles of methanolic extracts obtained from the leaves, stems, and roots of *Boerhavia diffusa* L. and *Boerhavia erecta* L.

Plant Material :

Fresh and healthy specimens of *Boerhavia erecta* L. and *Boerhavia diffusa* L. were collected from different locations in Banaskantha district, Gujarat, India. The leaves, stems, and roots were separated, washed thoroughly with distilled water to remove adhering impurities, shade-dried at room temperature, and powdered separately using a mechanical grinder. The powdered samples were stored in airtight containers until extraction[5] (WHO, 2011). Methanolic extracts of each plant part were prepared separately by Soxhlet extraction. Approximately 25 g of powdered material was extracted with methanol for 24 h. The extracts were filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator at 40°C. The concentrated extracts were dried and preserved at 4°C until chromatographic analysis[7].

HPTLC Fingerprint Analysis :

High-performance thin-layer chromatography (HPTLC) was employed to compare the phytochemical fingerprints of methanolic extracts obtained from different plant parts of *Boerhavia erecta* L. and *Boerhavia diffusa* L. Silica gel 60 F_{254} pre-coated aluminium plates (20 × 10 cm, Merck, Germany) were selected as the stationary phase because of their excellent resolution, reproducibility, and suitability for separating phytochemicals with different polarities[10].

Several solvent combinations were evaluated to obtain satisfactory separation of phytochemical constituents. Among the tested

systems, Ethyl Acetate: Formic acid: Glacial Acetic acid: H_2O (8:0.5:0.5:1, v/v/v/v) produced sharp, distinct, and well-resolved chromatographic bands and was therefore selected as the mobile phase.

The extracts were dissolved in methanol, and an equal volume of each sample was applied onto silica gel plates using a micros-yringe. Chromatographic development was carried out in a glass chamber pre-saturated with the optimized mobile phase. After development, the chromatographic plates were air-dried and observed under ultraviolet light.

Outcome :

Analysis demonstrated clear differences in chromatographic complexity among the investigated plant parts at 366nm. The leaf extract of *B. diffusa*. (B1) produced the highest number of peaks (8 peaks), whereas the root extract of *B. diffusa* (B3) exhibited only two major peaks. Similarly, *B. erecta* leaf extract (A1) showed seven peaks, while its root extract (A3) displayed only three peaks. A prominent chromatographic band was consistently observed in both species, suggesting the occurrence of phytochemical constituents with similar chromatographic behaviour. However, the percentage peak areas differed considerably among the samples, indicating quantitative variation in the abundance of these constituents. The chroma-tographic fingerprints also revealed species-specific differences.

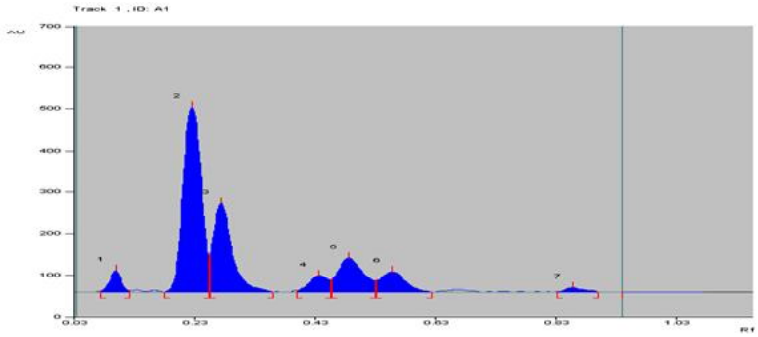

Figure 1. Densitogram at 366 nm (Sample A1)

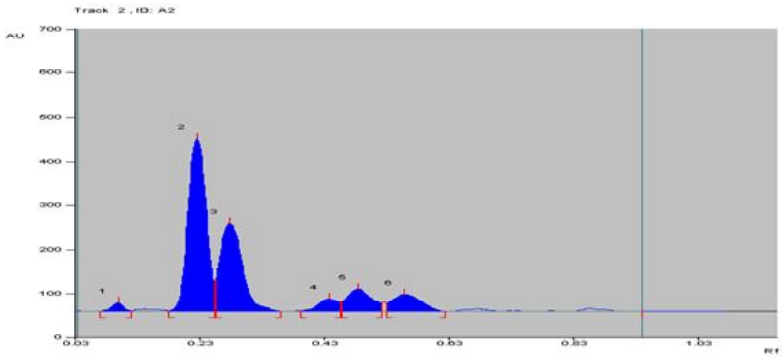

Figure 2. Densitogram at 366 nm (Sample A2)

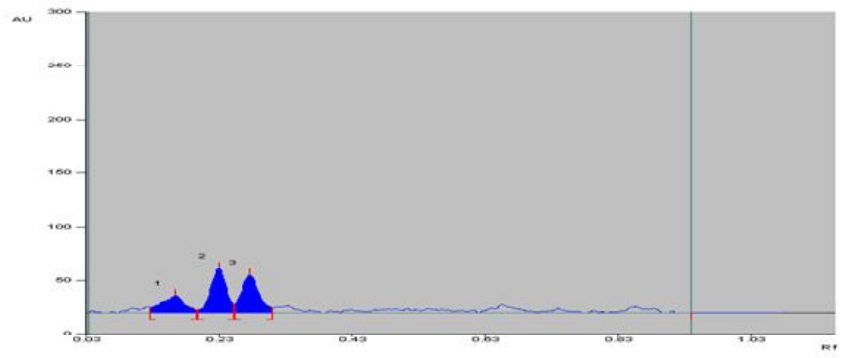

Figure 3. Densitogram at 366 nm (Sample A3)

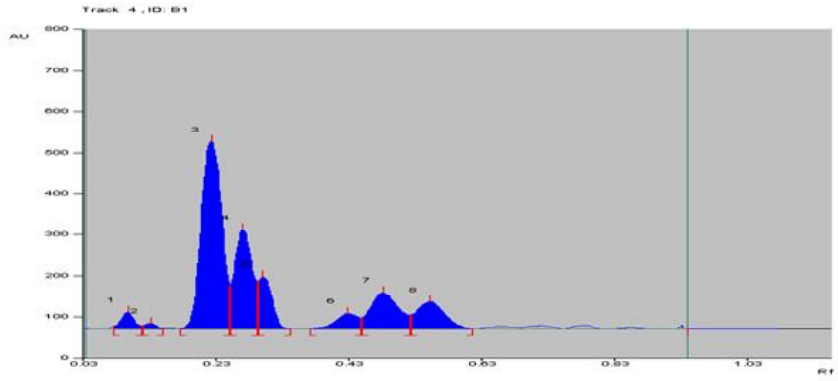

Figure 4. Densitogram at 366 nm (Sample B1)

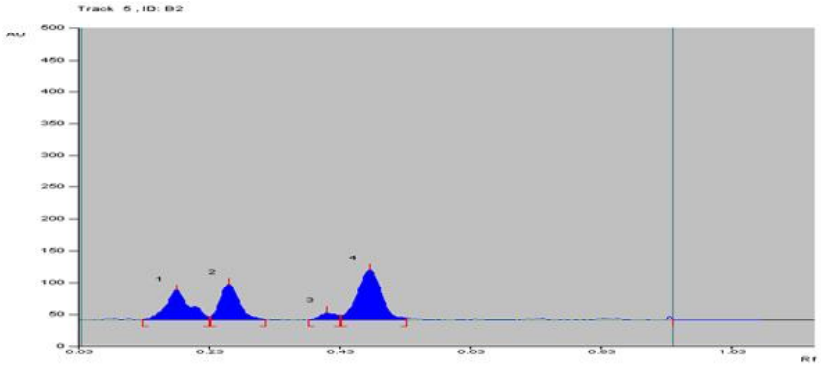

Figure 5. Densitogram at 366 nm (Sample B2)

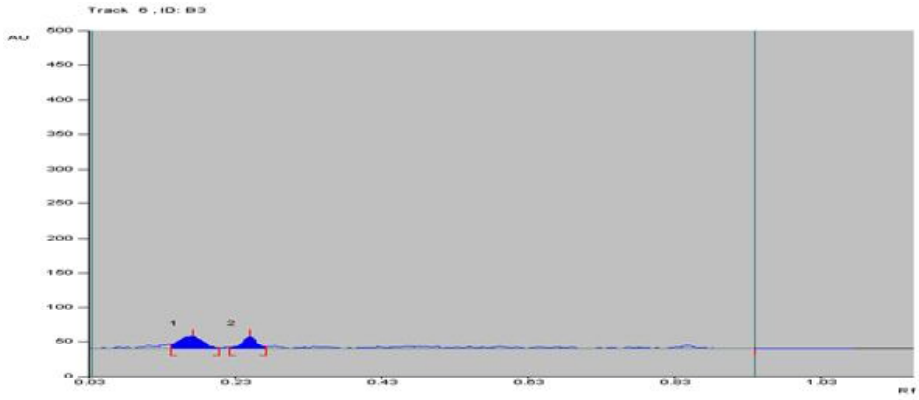

Figure 6. Densitogram at 366 nm (Sample B3)

Table-1. Comparative HPTLC Densitometric Data of all Samples

Sample	Number of Peaks	Major Rf Values	Highest Peak Area (%)
A1	7	0.10,0.23,0.28,0.44,0.49,0.56,0.86	47.74
A2	6	0.10,0.23,0.28,0.44,0.49, 0.56	48.49
A3	3	0.17, 0.23, 0.28	39.37
B1	8	0.10,0.13,0.23,0.27,0.30,0.43,0.48,0.55	44.34
B2	4	0.18, 0.26,0.41,0.48	43.15
B3	2	0.17, 0.25	62.73

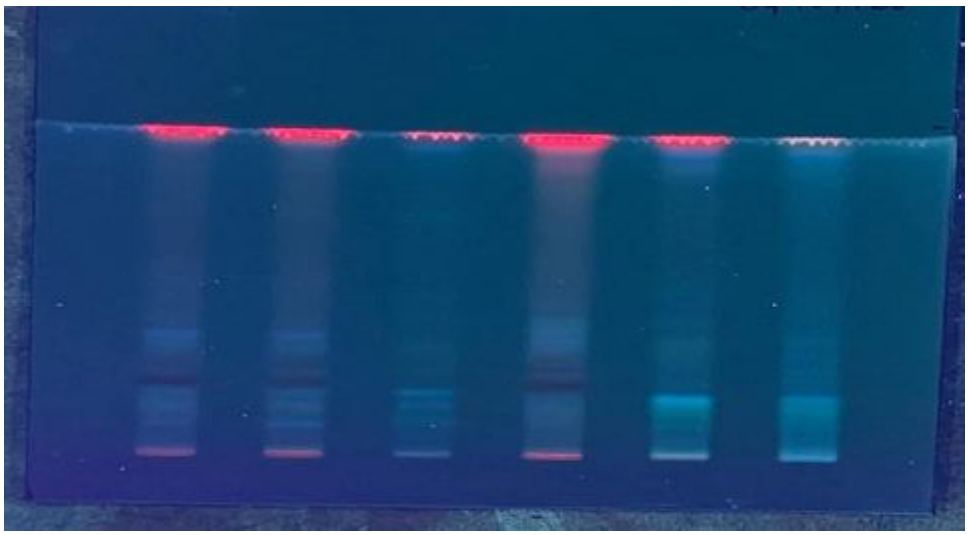

Figure 7. Chromatographic Fingerprint under Short-Wave UV (366 nm)

The present study established comparative HPTLC fingerprint profiles of methanolic extracts obtained from the leaves, stems, and roots of *Boerhavia erecta* and *Boerhavia diffusa*. Distinct chromatographic patterns were observed among the different plant parts, demonstrating variation in the number of peaks, Rf values, and peak area percentages. The leaf extracts of both species exhibited more complex chromatographic profiles than the corresponding root extracts, indicating differences in the distribution of phytochemical constituents. A common major peak was observed in most samples, suggesting the presence of phytochemicals with similar chromatographic behaviour, whereas variations in peak area percentages reflected species-species and plant part-specific differences in phytochemical abundance.

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