

Original Research Article

Integrated HPLC–HPTLC Characterization of Methanolic Extracts of *Hemidesmus indicus* (Root) and *Dioscorea bulbifera* (Tuber) Based on Rutin and Gallic Acid

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Dioscorea bulbifera (DB) and *Hemidesmus indicus* (HI) are widely recognized ethnomedicinal plants in Ayurveda that are traditionally used for managing metabolic disorders, oxidative stress, and diabetes. The present study aimed to establish a reliable comparative phytochemical profile of methanolic extracts of DB (tuber) and HI (root) using integrated High-Performance Thin-Layer Chromatography (HPTLC) and High-Performance Liquid Chromatography (HPLC), using rutin and gallic acid as marker flavonoids and phenolic compounds, respectively. Preliminary qualitative phytochemical screening confirmed the presence of phenols and flavonoids in both extracts; alkaloids were detected exclusively in HI, and terpenoids were absent in both plants. HPLC based quantitative analysis revealed marked differences in flavonoid and phenolic contents between the two species. The HI extract exhibited a prominent rutin peak at a retention time of 2.810 min with a relative concentration of 97.14%, whereas DB showed a corresponding peak at 2.821 min with a significantly lower relative concentration of 50.71%, indicating nearly a two-fold higher flavonoid abundance in HI compared to DB. HPTLC fingerprinting further demonstrated distinct and reproducible chromatographic bands corresponding to the marker compounds in both plant extracts, confirming their characteristic phenolic and flavonoid compositions. Overall, the integrated HPTLC–HPLC analysis provides a robust and reproducible framework for the authentication, standardization, and comparative evaluation of DB and HI, while the observed quantitative differences underscore their differential therapeutic potential, particularly regarding antidiabetic and antioxidant applications.

Introduction

Western biomedical researchers are investigating not only the usefulness of traditional herbal treatments, but also developing improved methods to identify and quantify active components [1]. Plant-based

foods rich in phenolic bioactive compounds have health benefits that can help combat diet and lifestyle-related chronic diseases [2]. Plant phytochemicals, particularly flavonoids and phenolic substances, play important roles in preventing hyperglycemia and oxidative damage. Ayurveda, Siddha, Unani, Sowa Rigpa,

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and other classical and folk medical systems are examples of India's significant traditional medicine legacy [3]. Medicinal plants have been used to treat a wide range of diseases for millennia. The search for safer and more effective drugs is driving an increase in the exploration of natural medicinal sources. Recently, there has been increased use of Indian medicinal plant resources in drug discovery and pharmaceutical business [4]. Nature provides basic resources for medicines, which have been employed as medicaments since antiquity [5]. *HI* is a semi-erect shrub that grows throughout India, from the upper Gangetic Plain to Assam, as well as in central, western, and southern India [6]. *HI* ("Indian sarsaparilla") has long been used to purify blood, protect the liver, and treat diabetes. Its main phytochemicals are flavonoids, phenolic acids, and triterpenoids [7]. *DB*, often known as yam or air potato, is a medicinal plant widely used to treat gastric cancer, rectal carcinoma, goiter, and sore throat [8]. Various bulb extracts have been shown to exhibit antihyperlipidemic, anticancer, antioxidant, anorexiant, analgesic, anti-inflammatory, plasmid curing, and antihyperglycemic effects [9]. Chromatographic techniques are critical for the standardization of medicinal plants. HPLC is the most widely used technique for pharmaceutical and phytochemical investigations of diverse bioactive chemicals [10]. HPLC allows for exact compound quantification and identification, whereas HPTLC enables rapid fingerprinting and comparative phytochemical evaluation. (HPLC) is a modified variant of thin-layer chromatography; sample components are separated on high-performance layers with detection and acquisition utilizing an improved workstation, increasing separation efficiency [11]. Rutin and gallic acid were selected as marker compounds because of their well-documented antidiabetic, antioxidant, and enzyme-inhibitory activities. The purpose of this study was to compare the HPLC and HPTLC profiles of *HI* and *DB* methanolic extracts to identify phenolic/flavonoid indicators associated with their antioxidant and antidiabetic potential.

Comparative chromatographic fingerprinting using both HPTLC and HPLC for closely related plant species is still rare, despite numerous studies reporting the phytochemical profiles of specific therapeutic plants. Using these different analytical platforms, the current study offers a methodical evaluation of the two medicinal plants, highlighting their distribution of marker compounds and phytochemical similarities. The objective of the present study was to comparatively evaluate the phytochemical fingerprints of two medicinal plants using HPTLC and HPLC, with rutin and gallic acid as marker compounds.

Methods for collection, identification, and authentication of the selected medicinal plants

The selected plants, *HI* (root) and *DB* (tuber), were collected from the regions of the Western Ghats near Pune (Maharashtra) during July 2022. The plants were identified and authenticated by the Botanical Survey of India (BSR) institute in Pune Maharashtra. The collected plants were deposited at the herbarium center of the host institute. The *HI* (root) and *DB* (tuber) and shade-dried powdered plant samples were preserved for further experiments.

Sequential extraction of the plant samples

Both plant shade-dried and powdered plant samples (10 g) were sequentially extracted in methanol, using the boiling points (79 °C), for up to 4 h using a Soxhlet apparatus. The extracted samples were then evaporated under reduced pressure at room temperature. The yield of the individual plant extracts was measured, and the dried extracts were stored at 4 °C in a refrigerator for further analysis [12].

Qualitative screening

Qualitative screening of the plant extracts was performed to detect the presence of flavonoids, gallic acid, and other major classes of secondary metabolites, such as alkaloids, phenols, flavonoids, and terpenoids. Standard qualitative phytochemical tests were employed for this purpose [13]. The presence of alkaloids was

assessed by Wagner's test [14]. Iodine (1.27 g) and potassium iodide (2 g) were dissolved in 5 mL of distilled water, and the volume was made up to 100 mL using distilled water. A few drops of this reagent were added to the plant extract filtrate, and the formation of a dark-colored precipitate indicated a positive result for alkaloids. Phenolic compounds were detected using the ferric chloride test [15]. Four to five drops of ferric chloride solution were added to 1 mL of the plant extract, and the development of a bluish-dark coloration confirmed the presence of phenols. Flavonoids were identified by the Shinoda test [16]. The extract (5 mg) was treated with a small quantity of magnesium, followed by the addition of a few drops of concentrated hydrochloric acid. The appearance of pink coloration indicated the presence of flavonoids, while color variations ranging from orange to red, red to crimson, and crimson to magenta suggested the presence of flavones, flavonoids, and flavanones, respectively. Terpenoids were qualitatively detected using the Salkowski test [17]. The extract (5 mL) was mixed with chloroform (2 mL), and concentrated sulfuric acid was carefully added along the sides of the test tube. The formation of a reddish-brown coloration at the interface confirms the presence of terpenoids.

HPTLC analysis

HPTLC was performed using a CAMAG instrument (Germany). Thin-layer chromatography (TLC) plates (silica gel >60 F254, 20 cm × 10 cm; Merck) were prewashed with methanol. The plates were activated in an oven at 100 °C for 10 min. Plant extracts (1 mg/mL) were spotted onto precoated plates using the CAMAG Linomat 5 application system.

Rutin and gallic acid (1 mg /mL) were used as standards. Flavonoids and phenols were separated using ethyl acetate: dichloromethane: glacial acetic acid (10:2.5:200 μ L) as the mobile phase. The developed spots were visualized under a CAMAG UV cabinet (254 nm) and digitized using the CAMAG photo documentation system [18,19].

HPLC analysis

The instrument was equipped with an auto sampler, an isocratic mobile phase with acetonitrile: ortho phosphoric acid 0.1% (75:25 v/v), a nucleodur C18 column (250 4.6 mm, 5 μ m) at 30 °C, and LC solution software. Plant samples and standard solutions (rutin and gallic acid) were prepared using methanol as the diluent, and detection was performed at 210 nm using a PDA detector. The calibration curve was created by plotting peak areas versus concentration using an MS Excel spreadsheet to quantify phenolic and flavonoid compounds. In analytical chemistry, HPLC is used to separate, identify, and quantify bioactive components in plant extracts [20,21].

Results

Preliminary phytochemical analysis

Screening of crude methanolic extracts *HI* of plant samples revealed the presence of some secondary metabolites such as phenols, flavonoids, and alkaloids, whereas terpenoids were absent. Crude methanolic extracts *DB* of plant samples revealed the presence of some secondary metabolites such as phenols and flavonoids, while alkaloids and terpenoids were absent (Table 1).

Table 1. Qualitative phytochemical profile of selected plants

Sr./ No.	Phytochemical test	<i>Hemidesmus indicus</i>	<i>Dioscorea bulbifera</i>
1	Alkaloids	+	-
2	Phenols	+	+
3	Flavonoids	+	+
4	Terpenoids	-	-

HPLC analysis

HPLC equipped with (210 nm) PDA detector was employed for the analysis of flavonoids and phenols in the ethanolic leaf extracts of the selected plant samples. Rutin and gallic acid standards were purchased from Chemfaces Biochemical Co. Ltd. Wuhan. HPLC) chromatograms of standard rutin and gallic acid showed a sharp single peak at a retention

time of rutin (2.818 min) with 98.80 % concentration and gallic acid (2.817 min) with 99.53 % concentration. (Table 2, Figure 1.) Whereas plant samples confirmed the presence of flavonoid (STD Rutin) and phenols (STD gallic acid), showed peaks at retention times as follows: *HI* (2.810 min) with 97.14 % concentration and *DB* (2.821 min) with 50.71% concentration (Figure 1).

Table 2. Represents the presence of a peak at the mentioned retention time

Sample	Major Peak RT (min)	Conc. (%)	Area	Match to Standards
Rutin	2.818	98.80	8,020,302	Reference
Gallic Acid	2.817	99.53	8,920,042	Reference
<i>Hemidesmus indicus</i>	2.810	97.14	5,446,940	Match
<i>Dioscorea bulbifera</i>	2.821	50.71	2,654,493	Match

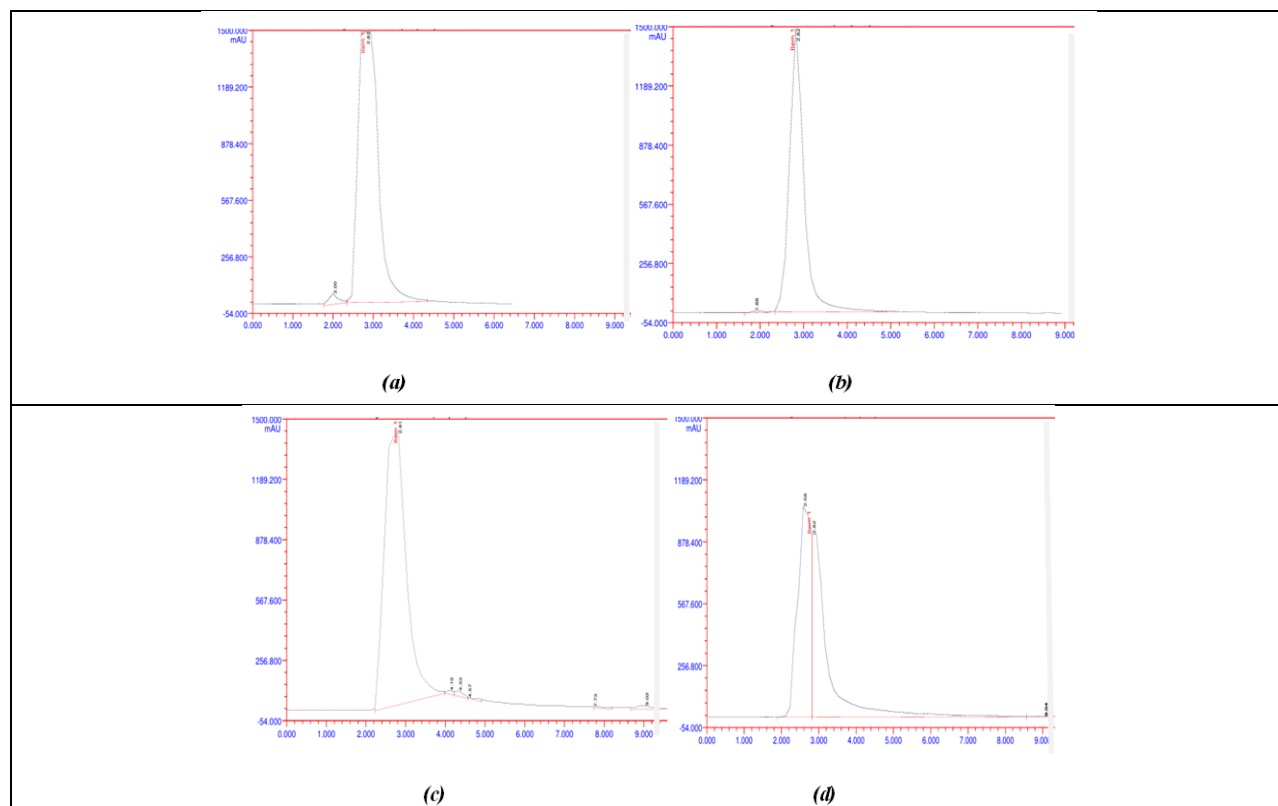


Figure 1. HPLC chromatogram of fractions at 210 nm. (a) Rutin, (b) gallic acid (c) *Hemidesmus indicus*, and (d) *Dioscorea bulbifera*

Sequential extraction and HPTLC profiling

HPTLC analysis was performed as part of the quality control of the selected plant samples. The HPTLC fingerprint of methanol-soluble samples was prepared using rutin and gallic acid as marker flavonoid and phenolic

compounds, respectively (Figure 2). The results of the HPTLC analysis showed the diversity of flavonoid and phenolic contents in both *HI* and *DB* . samples *HI* and *DB* (Figure 2), containing rutin and gallic acid (Figure 2).

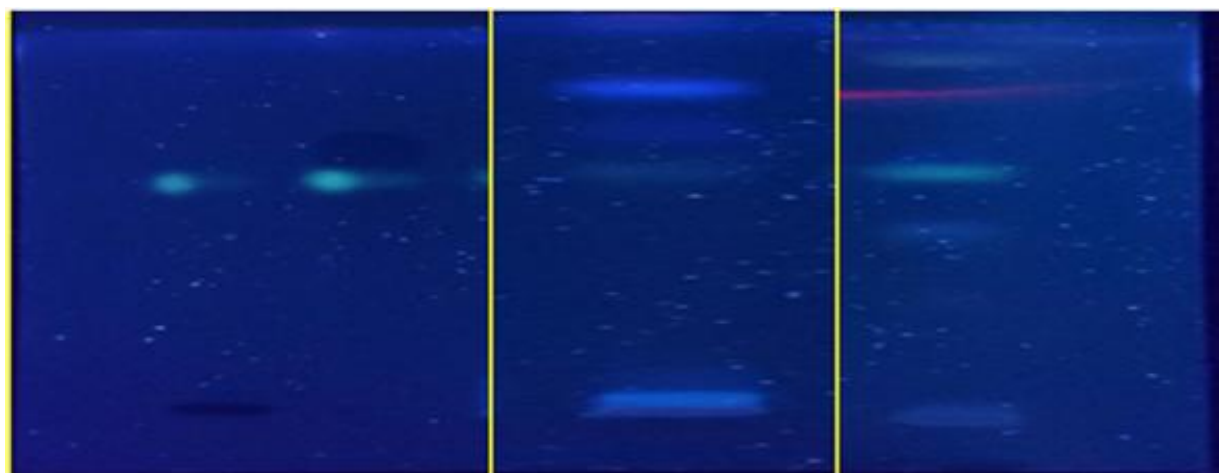


Figure 2. HPTLC profile of flavonoid and phenol fingerprints of methanol extract of selected medicinal plants using rutin and gallic acid as a marker compounds. Track no.1 and 2: (a) rutin (4 μ L), (b) gallic acid (4 μ L), (c) *Hemidesmus indicus* (4 μ L), and (d) *Dioscorea bulbifera* (4 μ L)

Discussion

Preliminary phytochemical screening confirmed the chromatographic results obtained by HPTLC and HPLC. Both *HI* and *DB* extracts contained phenols and flavonoids, but no terpenoids. Alkaloids were only found in *HI*, indicating a slight phytochemical difference between the two species. The presence of phenolic and flavonoid content in both plants is compatible with the HPTLC fingerprint profiles, which show distinctive bands for the marker chemicals rutin and gallic acid. In HPLC analysis, rutin and gallic acid standards had distinct and reproducible retention times that nearly matched the respective peaks in both plant extracts.

This similarity in retention behavior supports the presence of these bioactive markers in both plants. The similarity between HPTLC fingerprinting and HPLC profiling revealed that both species are chemically equivalent in terms of their primary phenolic and flavonoid content. Preliminary phytochemical assays, HPTLC fingerprinting, and HPLC comparison showed that both plants have a shared phenolic-flavonoid chemical framework, with rutin and gallic acid serving as dominant marker chemicals. They represent two distinct phytochemical methods for their antidiabetic and antioxidant activities.

Conclusion

The present comparative study effectively demonstrated the chemical similarities and subtle phytochemical differences between *DB* and *HI* through integrated phytochemical screening, HPTLC fingerprinting, and HPLC profiling. Both plants exhibited strong phenolic and flavonoid signatures, with the presence of rutin and gallic acid, as confirmed by the comparison of retention times with established standards, supporting their antioxidant and therapeutic relevance. The exclusive detection of alkaloids in *H. indicus* highlights a distinct phytochemical characteristic that may contribute to the variations in bioactivity between the two species. Overall, the integrated HPTLC–HPLC approach proved to be a reliable and reproducible tool for phytochemical evaluation and quality assessment of medicinal plants. However, the study is limited to selected marker compounds and *in vitro* chemical profiling, and future investigations incorporating additional bioactive markers, quantitative bioassays, and *in vivo* pharmacological validation would further strengthen the therapeutic interpretation and broaden the applicability of this analytical framework.

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None.

Ethical Issues

None.

Competing Interests

Author declared that there were no conflict of interest in this work.

Authors' Contributions

Conceptualization; data curation; formal analysis; investigation; methodology; project administration; resources; Software; supervision; validation; visualization; and writing – original draft; writing – review and editing by CNU, IMS, KAAR, KAI, and MHS.

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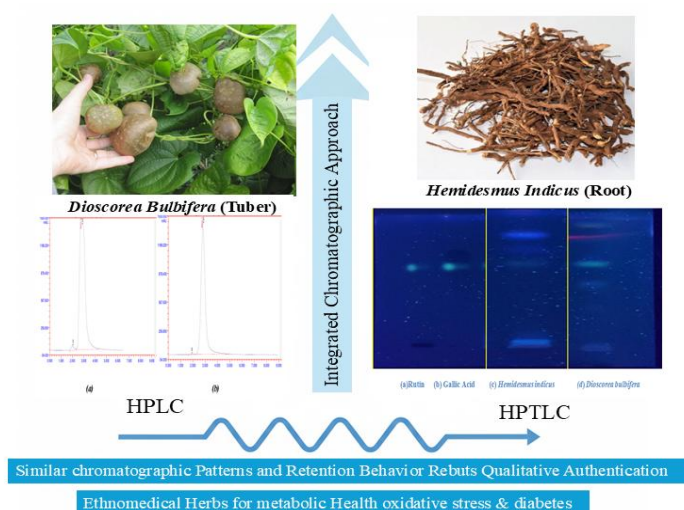
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Graphical Abstract



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