Research Article

LC-MS Metabolomic Profiling Combined with HPTLC Fingerprinting of Methanolic Extract of *Baliospermum montanum* willd. Muell. Arg. Root

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ABSTRACT

Baliospermum montanum (Willd.) Muell. Arg., Danti is an important medicinal plant belonging to the family Euphorbiaceae, traditionally used in Ayurvedic practice. The present study aimed to develop a comprehensive phytochemical fingerprint and preliminary metabolomic profile of the methanolic root extract of *Baliospermum montanum (Willd.) Muell. Arg.* using High-Performance Thin Layer Chromatography (HPTLC) and High-Resolution Liquid Chromatography-Mass Spectrometry (HR-LCMS). Roots were collected, authenticated, shade-dried, powdered, and subjected to methanolic extraction by the maceration method. The HPTLC fingerprinting was carried out on silica gel 60 F254 pre-coated aluminum plates using toluene: ethyl acetate: formic acid (5:4:0.5 v/v/v) as the mobile phase. Chromatographic profiles were obtained at 254 and 366 nm. HR-LCMS analysis was performed on a reversed-phase C18 column using gradient elution and electrospray ionization in both positive and negative ionization modes. The HPTLC analysis revealed reproducible chromatographic fingerprints with several resolved phytochemical constituents with characteristic Rf values. HR-LCMS analysis revealed the presence of different metabolites like alkaloids, phenolic acids, flavonoids, fatty acids, fatty acid amides, and terpenoid derivatives. The extract also contains several metabolites that have been reported in previous metabolomic studies of medicinal plants and can be responsible for the phytochemical complexity and ethnopharmacological importance of the plant. The integrated analytical approach adopted in the present study provides a preliminary metabolome data set and chromatographic reference profile of *Baliospermum montanum (Willd.) Muell. Arg.* roots. The results may provide supportive baseline data for future quality standardization, comparative phytochemical investigation, chemometric analysis, and advanced pharmacological studies.

Keywords: *Baliospermum montanum Willd. Muell. Arg.*, Extract, HPTLC Fingerprinting, HR-LC-MS, Metabolomic profiling.

INTRODUCTION

Medicinal plants continue to be important sources of bioactive compounds and play a crucial role in the discovery and development of new medications[1]. Advances in analytical technologies have enabled detailed characterization of complex phytochemical matrices, facilitating improved understanding of plant-derived pharmacological activities[2]. Among these technologies, High-Performance Thin-Layer Chromatography (HPTLC) is widely employed for chromatographic fingerprinting[4], standardization, and authentication of herbal materials[5], while Liquid Chromatography-Mass Spectrometry (LC-MS) has become a highly effective method for detailed metabolomic analysis[6,7].

Baliospermum montanum Willd. Muell. Arg (Euphorbiaceae), commonly referred to as Danti, has been traditionally utilized for its purgative properties[13]. Although its therapeutic relevance has been mentioned in ethno-pharmacological and traditional medical sources, systematic analytical investigations focusing on its phytochemical composition remain limited[14]. In particular, the lack of integrated chromatographic and metabolomic studies has restricted a clear understanding of the chemical constituents that may contribute to its reported biological effects.

HPTLC fingerprinting provides a rapid and reproducible method for establishing the chemical identity and consistency of herbal raw materials, which is crucial for ensuring quality assurance and standardization[8]. Complementarily, LC-MS-based metabolomics enables the high-resolution detection and tentative identification of a wide spectrum of metabolites, including both low- and high-molecular-weight compounds, thereby providing deeper insight into the phytochemical complexity of medicinal plants[9].

This present study was designed to generate an analytical fingerprint and comprehensive metabolomic profile of *Baliospermum montanum* Willd. Muell. Arg roots using HPTLC and LC-MS techniques. By integrating chromatographic fingerprinting with advanced mass spectrometric analysis, this work aims to provide a scientific foundation for future pharmacological and mechanistic studies of *Baliospermum montanum* Willd. Muell. Arg.

MATERIALS AND METHODS

Plant Collection and Authentication

Roots of *Baliospermum montanum (Willd.) Muell. Arg* were collected from their natural habitat and authenticated by the Department of Blatter Herbarium, St. Xavier's College, Mumbai. The specimen was identified using standard botanical references and authenticated by comparison with

herbarium materials. Verification was further performed in accordance with the Blatter Herbarium specimen no. K. V. S.-5479 of K.V. Shenoy. A voucher specimen was prepared and kept in the departmental herbarium for future reference.

Root Extract Preparation

Methanolic maceration (1:5 ratio) was performed for 48 hours, purified *Baliospermum montanum (Willd.) Muell. Arg*. Extracts were filtered, concentrated, and stored at 4 °C.

HPTLC Study

HPTLC fingerprinting was conducted using pre-coated aluminum plates with silica gel 60 F254. Samples were applied in bands using an automated applicator, with a volume of 5 µL. The chromatographic development took place in a twin-trough chamber that was pre-saturated with the mobile phase, which consisted of toluene, ethyl acetate, and formic acid in a ratio of 5:4:0.5 (v/v/v).

After the development process, the plates were allowed to air dry and were then visualized under ultraviolet light at wavelengths of 254 nm and 366 nm. Densitometric scanning was carried out with an HPTLC scanner, and Rf values along with peak area percentages were calculated using specialized chromatography software.

LC-MS Analysis

A high-resolution LC-MS analysis of the root extract from *Baliospermum montanum Willd. Muell. Arg* was conducted using a reversed-phase C18 column with a gradient elution. This involved solvent A (water with 0.1% formic acid) and solvent B (acetonitrile with 0.1% formic acid), at a flow rate of 0.3 mL/min and an injection volume of 5 µL. Mass spectrometric detection utilized an electrospray ionization (ESI) source in positive ionization mode, with data collected over a mass-to-charge (m/z) range of 100-1500 under high-resolution full scan conditions. Auto-MS/MS experiments were employed for selected precursor ions to obtain fragmentation data. Metabolites were preliminarily identified by analyzing accurate mass measurements, isotopic patterns, MS/MS fragmentation characteristics, and by comparing them with spectral databases like METLIN and MassBank, as well as relevant literature.

Data Processing and Interpretation

LC-MS data were processed using instrument-specific data analysis software for peak detection, alignment, and annotation. Identified metabolites were classified into major phytochemical groups to facilitate interpretation of the metabolomic profile[17]. The integrated use of HPTLC fingerprinting and HR-LCMS analysis enabled comprehensive chemical characterization of *Baliospermum montanum Willd. Muell. Arg* root extract.

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RESULTS

HPTLC fingerprinting was carried out using *toluene: ethyl acetate: formic acid (5:4:0.5, v/v/v)* as the mobile phase. Chromatograms were recorded at 254 nm, shown in Figure 1, and 366 nm, shown in Figure 2, for *Baliospermum montanum (Willd.) Muell. Arg* roots. The number of peaks, their Rf values, and relative area percentages are summarized in Tables 1 & 2.

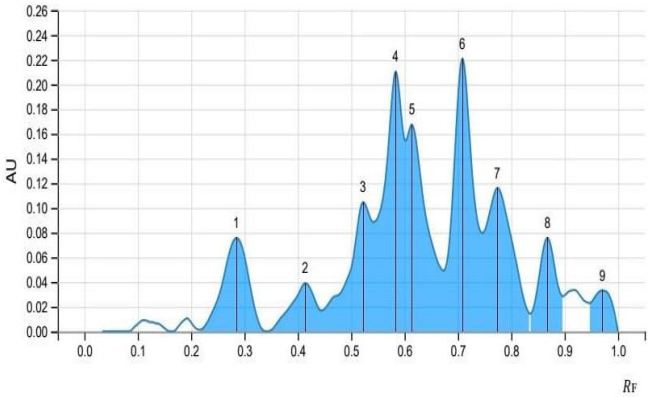

Figure 1: HPTLC destinogram of *Bloodserum montanum (Willd.) Muell. Arg* root extract recorded at 254 nm, showing resolved peaks corresponding to UV-absorbing phytoconstituents.

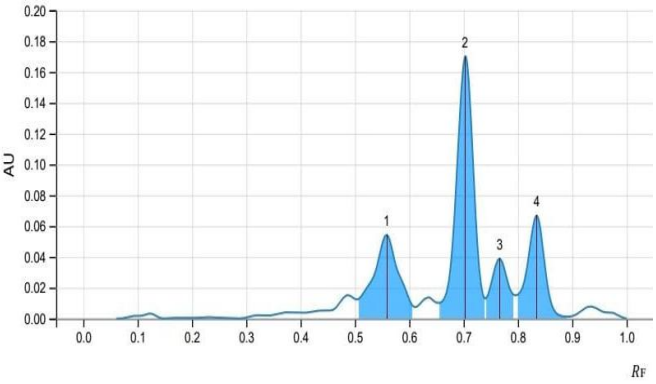

Figure 2: HPTLC densitogram of *Baliospermum montanum (Willd.) Muell. Arg* root extract recorded at 366 nm, indicating fluorescent phytochemical constituents.

Table 1: HPTLC profile of *Baliospermum montanum (Willd.) Muell. Arg* Root at 254 nm

Peak No.	Sample Rf	% Area
1	0.215	8.7
2	0.342	4.2
3	0.444	11.0
4	0.542	18.1
5	0.601	15.7
6	0.672	19.6
7	0.746	13.9
8	0.836	5.9
9	0.946	2.9

Table 2: HPTLC profile of Purified *Baliospermum montanum (Willd.) Muell. Arg* Root at 366 nm

Peak No.	Purified sample Rf	% Area
1	0.504	22.9
2	0.654	47.2
3	0.740	10.7
4	0.796	19.1

Following HPTLC fingerprinting, high-resolution LC-MS analysis was performed to achieve comprehensive metabolomic profiling of the methanolic root extract of *Baliospermum montanum (Willd.) Muell. Arg*.

The analysis enabled the detection of metabolites across a broad range of polarities and molecular weights. Tentative identification of compounds was based on accurate mass measurements, ionization mode, retention time, and comparison with established mass spectral databases and literature reports, as shown in Figures 3 and 4. The identified phytoconstituents were classified into major chemical groups such as nucleobases, alkaloids, phenolic acids, flavonoids, fatty acid amides, and terpenoid/ diterpenoid derivatives. The detailed list of metabolites detected through HR-LCMS analysis is present in Table 3.

Table 3: HR-LCMS identified phytoconstituents of the methanolic root extract of *Baliospermum montanum (Willd.) Muell. Arg*

Sr. No.	RT (min)	Ion mode	Compound identified	Molecular formula	m/z
1.	0.52	ESI+	Cytosine	C4H5N3o	111.043
2.	0.97	ESI+	Adenine	C5H5N5	135.054
3.	1.03	ESI+	Trigonelline	C7H7NO2	137.047
4.	1.24	ESI-	Uracil derivative	C4H4NO2	111.020
5.	1.86	ESI+	Nicotinic acid derivative	C6H5NO2	123.032
6.	2.11	ESI-	Citric acid	C6H8O7	191.019
7.	2.41	ESI-	Caffeic acid	C9H8O4	179.056
8.	2.88	ESI+	L-DOPA-related metabolite	C9H11NO4	195.087
9.	3.14	ESI-	Sinapic acid	C11H12O5	223.061
10.	3.42	ESI-	Ferulic acid	C10H10O4	193.050
11.	3.76	ESI-	Palmitic acid	C16H32O2	255.233
12.	4.04	ESI+	8-Hydroxyquinoline	C9H7NO	145.053
13.	4.52	ESI-	Linoleic acid	C18H32O2	279.233
14.	4.96	ESI-	Oleic acid	C18H34O2	281.248
15.	5.41	ESI+	Terpenoid-type compound	C20H28O2	301.144
16.	6.02	ESI+	Oxygenated terpene	C20H33O3	329.248
17.	6.37	ESI+	Diterpenoid ester	C24H37O5	413.266
18.	7.11	ESI+	Norharman	C11H8N2	168.069
19.	7.84	ESI-	p-Coumaric acid	C9H8O3	163.040
20.	8.23	ESI-	Quercetin	C15H10O7	302.042
21.	8.91	ESI-	Kaempferol	C15H10O6	286.047
22.	9.39	ESI-	Rutin	C27H30O16	610.153
23.	9.96	ESI+	Euphorbiaceae-type diterpene	C26H41O6	457.295
24.	10.56	ESI+	10-Hydroxycanthin-6-one	C14H8N2O2	236.058
25.	11.22	ESI-	12-Oxy-phytodienoic acid	C18H28O3	292.203
26.	12.47	ESI+	Hexadecanamide	C16H22NO	255.256
27.	13.18	ESI+	Fatty acid amide derivative	C18H35NO	285.267
28.	13.64	ESI+	Diterpene alcohol	C24H41O6	425.308
29.	14.76	ESI+	Terpenoid alcohol	C20H34O2	306.255
30.	15.92	ESI-	Stearic acid	C18H36O2	283.264
31.	17.43	ESI+	Triterpenoid-related ion	C30H48O3	456.360
32.	18.34	ESI+	Terpenoid ester	C22H34O4	362.245
33.	20.18	ESI-	Dihydroxy fatty acid	C18H34O4	313.238
34.	22.06	ESI+	Euphorbiaceae diterpene analog	C25H38O5	418.271
35.	24.81	ESI+	Non-polar terpenoid	C20H32)	288.245
36.	26.37	ESI+	Fatty acid	Amide derivative	C20H39NO

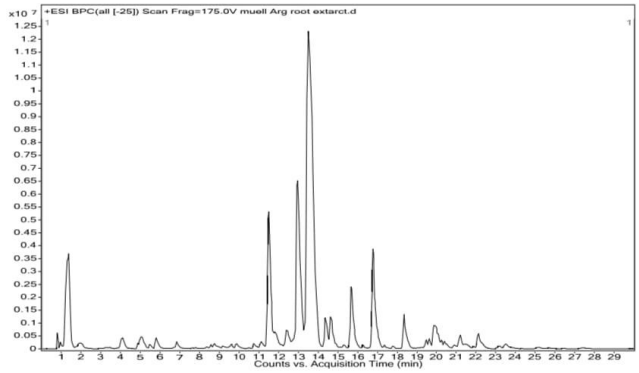

Figure 3: LC-MS total ion chromatogram of the methanolic root extract of *Baliospermum montanum (Willd.) Muell. Arg* obtained in ESI positive ion mode

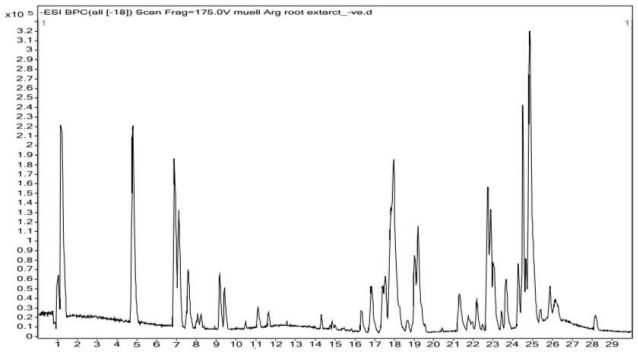

Figure 4: LC-MS total ion chromatogram of the methanolic root extract of *Baliospermum montanum (Willd.) Muell. Arg* obtained in ESI negative ion mode

DISCUSSION

This study showcases a comprehensive analytical approach that merges High-Performance Thin-Layer Chromatography (HPTLC) fingerprinting with high-resolution Liquid Chromatography-Mass Spectrometry (HR-LC-MS) to analyze the phytochemical makeup of the roots of *Baliospermum montanum (Willd.) Muell. Arg*. Such multi-platform analytical approaches are increasingly recommended for comprehensive chemical profiling and quality evaluation of medicinal plant materials[10].

HPTLC fingerprinting produced well-resolved and reproducible chromatographic profiles at both 254 nm and 366 nm, indicating the presence of diverse UV-absorbing and fluorescent phytoconstituents. The detection of multiple peaks across a broad Rf range reflects the chemical complexity of the root extract and confirms the suitability of the selected mobile phase for routine quality assessment. The produced HPTLC fingerprint can act as a reference profile for verifying authenticity, identifying adulteration, and assessing consistency between different batches of *Baliospermum montanum (Willd.) Muell. Arg* root samples.

HR-LC-MS analysis enabled detailed metabolomic characterization and tentative identification of a wide range of primary and secondary metabolites. The identified compounds included nucleobases, alkaloids, phenolic acids, flavonoids, fatty acids, fatty acid amides, and terpenoid and diterpenoid derivatives[11]. The detection of both polar and non-polar metabolites highlights the effectiveness of the chromatographic and ionization conditions employed. Additionally, the presence of multiple terpenoid and diterpenoid derivatives is consistent with the known phytochemical characteristics of members of the Euphorbiaceae family and supports the chemotaxonomic relevance of the findings[12].

Phenolic acids and flavonoids detected in the extract are commonly reported plant secondary metabolites and are frequently used as marker compounds in herbal quality assessment due to their stability and detectability. Fatty acids and their amide derivatives represent important primary metabolites that contribute to the overall chemical profile, while alkaloids and nitrogen-containing compounds add further complexity to the metabolomic spectrum. Together, these findings emphasize that *Baliospermum montanum (Willd.) Muell. Arg* roots possess a chemically diverse metabolite composition rather than being dominated by a single class of constituents.

Overall, the combined use of HPTLC and HR-LC-MS provides complementary qualitative information, enabling both rapid fingerprint-based assessment and high-resolution metabolite annotation. This integrated analytical framework strengthens the phytochemical documentation of *Baliospermum montanum (Willd.) Muell. Arg* and establishes a reliable baseline dataset for future studies related to standardization, comparative phytochemistry, and advanced pharmacological or toxicological investigations.

CONCLUSIONS

This study presents a systematic phytochemical characterization of *Baliospermum montanum (Willd.) Muell. Arg* roots using complementary chromatographic and mass spectrometric techniques. The HPTLC method produced distinct and reproducible fingerprints, enabling rapid assessment of chemical consistency. High-resolution LC-MS analysis revealed a broad spectrum of metabolites representing multiple phytochemical classes, reflecting the inherent chemical complexity of the plant material. Detection of both polar and non-polar constituents confirms the effectiveness of the applied analytical condition. The generated metabolomic dataset serves as a reliable chemical reference for this species. Overall, the integrated analytical approach enhances the documentation of *Baliospermum montanum (Willd.) Muell. Arg* and contributes valuable data for future quality evaluation and comparative phytochemical studies.

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Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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