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Phytochemical Screening And Hptlc Fingerprinting Analysis Of Ethanolic Extract Of Leucas Biflora Leaves

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ABSTRACT

The present study aimed to establish the phytochemical profile and High-Performance Thin-Layer Chromatography (HPTLC) fingerprint of the ethanolic extract of *Leucas biflora* leaves. Preliminary phytochemical screening and HPTLC analysis were performed using a CAMAG HPTLC system equipped with an Automatic TLC Sampler (ATS-4), twin-trough chamber, visualizer, scanner, and Vision CATS Server software (version 3.4). Phytochemical investigation of the ethanolic extract revealed the presence of carbohydrates, tannins, saponins, glycosides, phenolic compounds, and flavonoids, whereas alkaloids and phytosterols were absent. HPTLC fingerprinting demonstrated distinct chromatographic peaks corresponding to different classes of phytoconstituents. A total of eleven characteristic peaks with Rf values ranging from 0.03 to 0.93 were observed under optimized chromatographic conditions. The developed HPTLC fingerprint profile may serve as a reliable tool for the authentication, standardization, and quality control of *Leucas biflora*. Furthermore, the study highlights the phytochemical richness of the plant and provides baseline data for future pharmacological and phytochemical investigations.

Keywords: HPTLC fingerprinting, *Leucas biflora*, phytochemical screening, ethanolic extract, medicinal plants.

INTRODUCTION

Medicinal plants and plant-derived products constitute an integral component of both traditional and modern healthcare systems. Since ancient times, plants have been extensively utilized for the prevention and treatment of various diseases due to their therapeutic potential. Numerous medicinal plants used in Ayurvedic and other traditional systems of medicine have been scientifically investigated, while many still remain unexplored for their phytochemical and pharmacological properties.[1]

Standardization of herbal drugs is essential to ensure their identity, purity, safety, and efficacy. High-Performance Thin-Layer Chromatography (HPTLC) is a versatile, robust, and reliable analytical technique widely employed for the qualitative and quantitative analysis of phytoconstituents in medicinal plants. HPTLC fingerprinting enables the identification and authentication of herbal materials through characteristic chromatographic patterns and marker compounds. Owing to its simplicity, reproducibility, and cost-effectiveness, HPTLC has become an important tool for herbal standardization and quality control.[2]

Plants belonging to the genus *Leucas* (family: Lamiaceae) are traditionally used for the treatment of several ailments and possess considerable medicinal potential. One such species, *Leucas biflora*, is native to the northeastern region of India, particularly Tripura. It is a perennial herb characterized by a quadrangular stem, nodal roots, and ovate velvety leaves. Ethnomedicinally, the leaves are used in the management of conjunctivitis, epistaxis, and leucorrhea. [3–6]

Despite its traditional usage, limited scientific data are available regarding the phytochemical characterization and chromatographic profiling of *Leucas biflora*. Therefore, the present study was undertaken to perform preliminary phytochemical screening and establish an HPTLC fingerprint profile of the ethanolic extract of *Leucas biflora* leaves.

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

MATERIAL & METHODS:

Collection and Authentication of Plant Material

Fresh leaves of *Leucas biflora* were collected from the campus of Smt. Kishoritai Bhoyar College of Pharmacy, Kamptee, Maharashtra, India. The plant material was authenticated by Botany, department, RTM Nagpur University, Nagpur. A voucher specimen (No. 10301) was deposited for future reference.

Preparation of Ethanolic Extract

Approximately 500 g of shade-dried and coarsely powdered leaves of *Leucas biflora* were extracted with ethanol using a Soxhlet apparatus. The extract was concentrated under reduced pressure using a rotary vacuum evaporator and stored in an airtight container until further use.[7]

Preliminary Phytochemical Screening

The ethanolic extract was subjected to phytochemical screening for the detection of plant metabolites such as proteins, carbohydrates, glycosides, saponins, phenolic compounds, alkaloids, flavonoids, tannins, and steroids using standard qualitative methods. [8,9]

HPTLC ANALYSIS

Instrumentation

HPTLC analysis was carried out using a CAMAG HPTLC system equipped with an Automatic TLC Sampler (ATS-4), twin-trough development chamber, CAMAG visualizer, and TLC Scanner 4 integrated with Vision CATS Server software version 3.4.

Chromatographic Conditions

Chromatographic separation was performed on precoated silica gel 60 F254 plates (Merck, Germany). Sample application was carried out as 8 mm bands. Developed plates were visualized under UV light at 254 nm and 366 nm. Densitometric scanning was performed in absorbance mode using a slit dimension of 6 × 0.45 mm and a scanning speed of 20 mm/s. [10–12]

TABLE 1. HPTLC conditions for different phytoconstituents

Class Of Phytoconstituents	Solvent System(v/v/v/v)	Derivatizing Agent	Confirmatory Observation
Glycoside	Ethyl Acetate: Methanol: Water (100:13.5:10)	Anisaldehyde H_2SO_4	Blue, green and yellow fluorescence under 366nm
Saponin	Chloroform: Acetic Acid: Methanol: Water (6.4:3.2:1.2:0.8)	Anisaldehyde H_2SO_4	Blue, green, red fluorescence under 366nm
Flavonoid	Ethyl Acetate: Formic Acid: Glacial Acetic Acid: Water (10:0.5:0.5:1.3)	10% Methanolic H_2SO_4	Fluorescence under 366nm
Tannins	Toluene: Ethyl Acetate: Formic Acid (6:4:0.3)	Alcoholic $FeCl_3$	Dark blue colour 366nm (blue or green)
Steroids	Toluene: Methanol (9:1)	Anisaldehyde H_2SO_4	Blue-violet colour under white light

RESULTS

Preliminary Phytochemical Screening

The ethanolic extract of *Leucas biflora* leaves showed the existence of carbohydrates, glycosides, saponins, flavonoids, tannins, steroids and phenolic compounds and absence of proteins and alkaloids.

TABLE 2. Preliminary phytochemical screening of ethanolic extract of *Leucas biflora* leaves

Sr. No.	Phytochemical Test	Observation
1	Carbohydrates	Present
2	Glycosides	Present
3	Saponins	Present
4	Alkaloids	Absent
5	Phytosterols/Steroids	Present
6	Flavonoids	Present
7	Tannins and Phenolic Compounds	Present

(+ indicates presence; – indicates absence)

HPTLC analysis details

GLYCOSIDES

The chromatogram revealed six distinct peaks with Rf values ranging from 0.004 to 0.834, indicating the existence of multiple glycosidic compounds. The peak at Rf 0.834 represented the major constituent with 73% peak area, suggesting the predominance of a relatively non-polar glycoside.

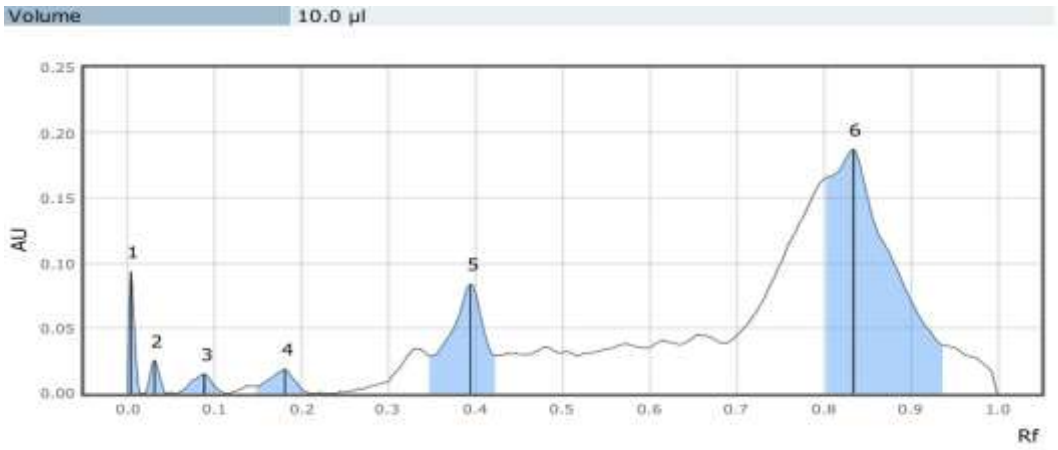


FIGURE 1. HPTLC Fingerprinting Chromatogram of Ethanolic extract of *Leucas biflora* leaves.

Peak #	Start		Max			End		Area		Manual peak	Substance Name
	Rf	H	Rf	H	%	Rf	H	A	%		
1	0.000	0.0000	0.004	0.0935	22.06	0.015	0.0000	0.00062	2.85	No	
2	0.020	0.0000	0.030	0.0254	6.00	0.044	0.0000	0.00030	1.39	No	
3	0.054	0.0000	0.088	0.0151	3.56	0.113	0.0000	0.00041	1.88	No	
4	0.148	0.0054	0.180	0.0186	4.39	0.213	0.0000	0.00062	2.85	No	
5	0.346	0.0288	0.394	0.0838	19.78	0.424	0.0291	0.00395	18.03	No	
6	0.800	0.1644	0.834	0.1874	44.21	0.938	0.0368	0.01598	73.00	No	

TABLE 3. Peak analysis data of HPTLC Chromatogram for Ethanolic extract of *Leucas biflora* leaves.

SAPONINS

HPTLC analysis indicated the existence of saponins with characteristic peaks observed at Rf values of 0.521 and 0.945, corresponding to 40.71% and 25.39% peak areas, respectively. Lower Rf values indicated the presence of more polar saponin derivatives.

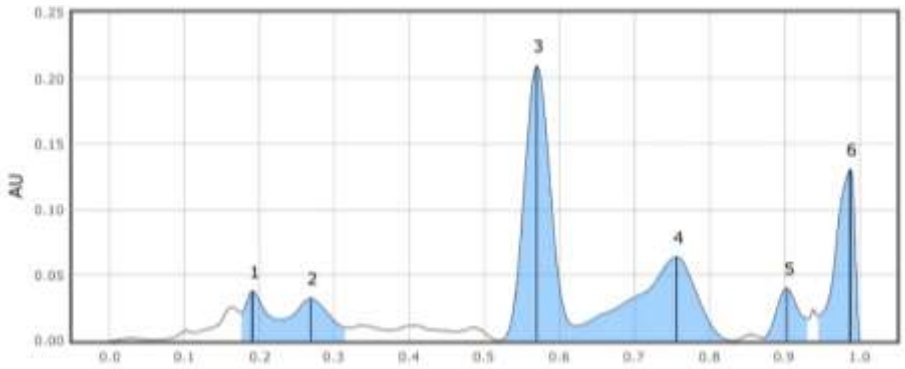


FIGURE 2. HPTLC Fingerprinting Chromatogram of Ethanolic extract of *Leucas biflora* leaves.

Peak #	Start		Max			End		Area		Manual peak	Substance Name
	Rf	H	Rf	H	%	Rf	H	A	%		
1	0.176	0.0212	0.190	0.0379	7.37	0.224	0.0162	0.00127	5.50	No	
2	0.224	0.0162	0.268	0.0323	6.30	0.316	0.0100	0.00196	8.46	No	
3	0.521	0.0000	0.570	0.2091	40.71	0.621	0.0115	0.00847	36.61	No	
4	0.621	0.0115	0.756	0.0639	12.44	0.829	0.0000	0.00627	27.12	No	
5	0.871	0.0021	0.904	0.0400	7.78	0.930	0.0167	0.00137	5.92	No	
6	0.945	0.0187	0.988	0.1304	25.39	1.000	0.0003	0.00379	16.39	No	

TABLE 4. Peak Analysis Data of HPTLC Chromatogram for Ethanolic extract of *Leucas biflora* leaves.

FLAVONOIDS

Seven peaks corresponding to flavonoid constituents were observed. The dominant peak at Rf 0.950 accounted for 74.18% of the total peak area, indicating the presence of a major flavonoid compound in the extract.

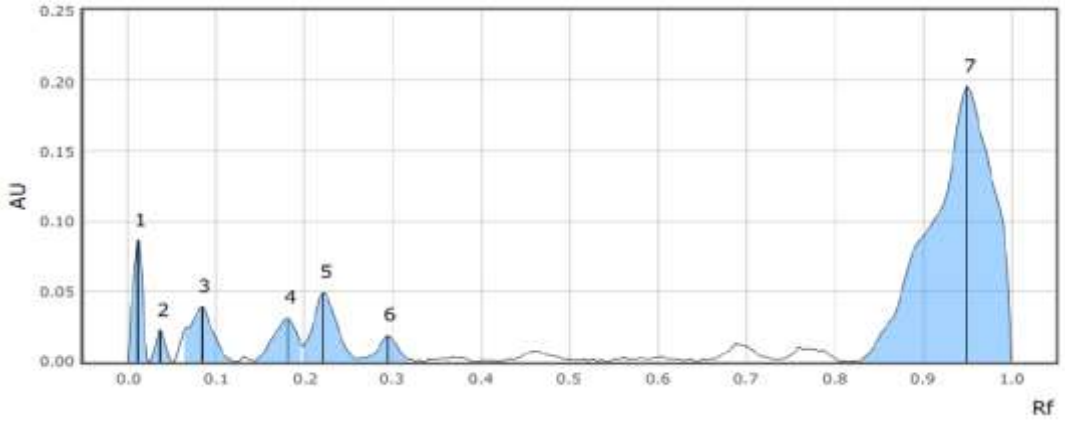


FIGURE 3. HPTLC Fingerprinting Chromatogram of Ethanolic extract of *Leucas biflora* leaves.

Peak #	Start		Max			End		Area		Manual peak	Substance Name
	Rf	H	Rf	H	%	Rf	H	A	%		
1	0.000	0.0000	0.011	0.0862	19.44	0.023	0.0000	0.00107	5.00	No	
2	0.024	0.0000	0.037	0.0228	5.13	0.050	0.0000	0.00027	1.28	No	
3	0.061	0.0190	0.084	0.0394	8.89	0.122	0.0001	0.00123	5.72	No	
4	0.143	0.0008	0.180	0.0313	7.05	0.198	0.0112	0.00096	4.46	No	
5	0.199	0.0112	0.221	0.0493	11.11	0.259	0.0023	0.00151	7.05	No	
6	0.260	0.0023	0.294	0.0184	4.15	0.318	0.0018	0.00049	2.30	No	
7	0.827	0.0004	0.950	0.1961	44.22	1.000	0.0005	0.01592	74.18	No	

TABLE 5. Peak analysis data of HPTLC Chromatogram for Ethanolic extract of *Leucas biflora* leaves.

TANNINS

Tannin analysis demonstrated distinct peaks at Rf values of 0.251 and 0.327 with significant peak areas of 39.82% and 29.50%, respectively. Visualization with ferric chloride reagent confirmed the presence of polyphenolic compounds

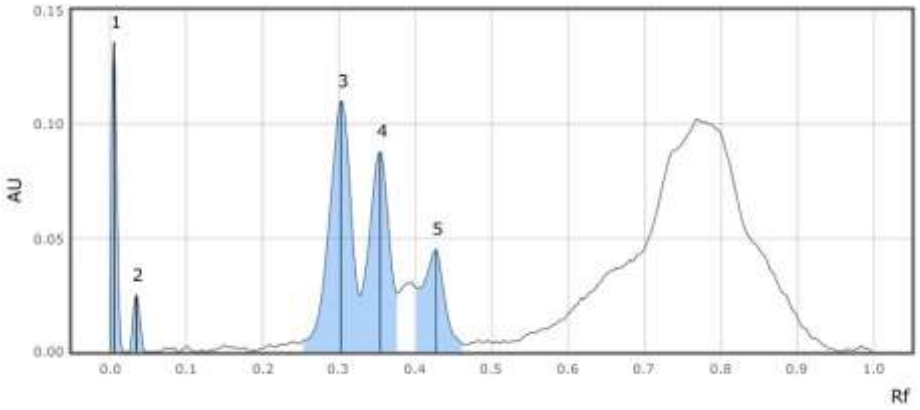


FIGURE 4. HPTLC Fingerprinting Chromatogram of Ethanolic extract of *Leucas biflora* leaves.

Peak #	Start		Max			End		Area		Manual peak	Substance Name
	Rf	H	Rf	H	%	Rf	H	A	%		
1	0.000	0.0000	0.005	0.1359	33.67	0.017	0.0000	0.00105	11.38	No	
2	0.026	0.0000	0.034	0.0250	6.18	0.045	0.0000	0.00025	2.74	No	
3	0.251	0.0042	0.302	0.1101	27.27	0.327	0.0247	0.00368	39.82	No	
4	0.327	0.0247	0.354	0.0878	21.75	0.377	0.0256	0.00273	29.50	No	
5	0.401	0.0279	0.427	0.0449	11.13	0.467	0.0029	0.00153	16.55	No	

TABLE 6. Peak analysis data of HPTLC Chromatogram for Ethanolic extract of *Leucas biflora* leaves.

STEROIDS

Three well-resolved peaks with Rf values of 0.091, 0.639, and 0.727 were detected. The major steroidal constituent was observed at Rf 0.727 with 68.96% peak area.

FIGURE 5. HPTLC Fingerprinting Chromatogram of Ethanolic extract of *Leucas biflora* leaves.

Peak #	Start		Max			End		Area		Manual peak	Substance Name
	Rf	H	Rf	H	%	Rf	H	A	%		
1	0.091	0.0001	0.122	0.0334	9.37	0.138	0.0083	0.00070	2.69	No	
2	0.639	0.0827	0.688	0.1010	28.33	0.718	0.0879	0.00736	28.35	No	
3	0.727	0.0874	0.820	0.2220	62.30	0.839	0.2120	0.01789	68.96	No	

TABLE 7. Peak Analysis Data of HPTLC Chromatogram for Ethanolic extract of *Leucas biflora* leaves.

DISCUSSION

Preliminary phytochemical screening of ethanolic extract of *Leucas biflora* leaves confirmed the presence of several biologically active secondary metabolites, including glycosides, steroids flavonoids, tannins, saponins, and phenolic compounds. These constituents are known to contribute significantly to the therapeutic properties of medicinal plants belonging to the family Lamiaceae. HPTLC fingerprinting provided detailed chromatographic separation of the phytoconstituents and demonstrated the chemical complexity of the extract. Distinct peaks with characteristic Rf values were obtained for different classes of compounds,

thereby confirming the reliability and reproducibility of the analytical method.

The glycoside profile showed a dominant non-polar compound, while the flavonoid analysis revealed the predominance of a major flavonoid constituent that may contribute to antioxidant and anti-inflammatory activities. Similarly, tannin and saponin profiles demonstrated the occurrence of multiple constituents with varying polarity.

The generated HPTLC fingerprint profile can therefore serve as a valuable reference for the authentication, standardization, and quality assessment of *Leucas biflora*. Additionally, the fingerprinting data may assist in detecting adulteration and evaluating chemotype variation among plant populations.

CONCLUSION

This study successfully established a comprehensive HPTLC fingerprint profile of the ethanolic extract of *Leucas biflora* leaves along with preliminary phytochemical characterization. The findings confirmed the presence of several bioactive phytoconstituents, including glycosides, flavonoids, tannins, saponins, and phenolic compounds.

The developed HPTLC method proved to be simple, reliable, reproducible, and effective for the identification and standardization of *Leucas biflora*. The characteristic chromatographic peaks and Rf values obtained in this study may serve as reference markers for future quality control and authentication of herbal formulations containing this plant.

Furthermore, the study provides baseline phytochemical data that may support future investigations involving isolation, structural characterization, and pharmacological evaluation of bioactive compounds from *Leucas biflora*.

FUTURE SCOPE

This study provides a primary phytochemical and chromatographic profile of *Leucas biflora* leaves; however, further investigations are warranted to fully explore its therapeutic potential. Isolation and structural characterization of individual bioactive constituents using advanced analytical techniques such as HPLC, LC-MS/MS, and NMR spectroscopy may help identify the compounds responsible for the plant's pharmacological activities. In addition, quantitative estimation of major phytoconstituents and validation of the developed HPTLC method according to ICH guidelines would strengthen its application in herbal standardization and quality control. Future pharmacological studies involving antioxidant, anti-inflammatory, antimicrobial, and cytotoxic activities may further substantiate the traditional medicinal uses of *L. biflora*. The established HPTLC fingerprint profile may also serve as a reference for chemotaxonomic studies, detection of adulteration, and development of standardized herbal formulations.

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DECLARATION OF COMPETING INTEREST

The authors declare that they do not have any known competing financial interests or personal relationships that could have influenced the work stated in this paper.

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