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Advances in Bioresearch

ORIGINAL ARTICLE

HPTLC-Based Comparative Analytical Standardization and Phytochemical Fingerprinting of *Centella asiatica* and *Nardostachys Jatamansi*

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ABSTRACT
Mandukparni (Centella asiatica) and Jatamansi (Nardostachys Jatamansi) are classical Ayurvedic Medhya Rasayana herbs with neurological, anxiolytic, and adaptogenic properties. Standardization and chemical fingerprinting are critical for ensuring quality, reproducibility, and safety. To develop and validate high-performance thin-layer chromatography (HPTLC) profiles of extracts of Mandukparni (Centella asiatica) and Jatamansi (Nardostachys Jatamansi), compare their chemical fingerprints, and integrate a comprehensive phytochemical and pharmacological review of both herbs. Methanolic (or relevant solvent) extracts of both herbs were prepared. HPTLC was performed on silica gel 60 F254 plates, using mobile phase systems optimized for separation (e.g., toluene: ethyl acetate: formic acid in varying proportions). Detection was done at 254 nm and 366 nm. Rf values, peak areas, and relative intensities were recorded. Validation (linearity, repeatability) was carried out. Literature was reviewed from PubMed, Scopus, Medline, and other sources to gather phytochemical, pharmacological, and toxicological data of the two herbs. Distinct fingerprint profiles were established. For Mandukparni (Centella asiatica), major peaks were observed at Rf ~0.34–0.35, ~0.44–0.53, and ~0.63–0.64. For Jatamansi, major peaks at ~0.38–0.40, ~0.55–0.60, ~0.70–0.78, and ~0.93–0.97 were seen. Fluorescent bands under 366 nm provided additional differentiation. The linearity of peak area vs concentration was confirmed. The analysis reveals that Centella contains triterpenoid saponins (asiaticoside, madecassoside, asiatic acid) with neuroprotective, anxiolytic, wound-healing, and cognition-enhancing effects. Jatamansi (Nardostachys Jatamansi) is rich in sesquiterpenoids (jatamansone, valeranone, nardosinone) and has demonstrated sedative, anticonvulsant, antioxidant, and neuroprotective activities. The HPTLC fingerprints provide reliable quality control markers to distinguish and standardize Mandukparni (Centella asiatica) and Jatamansi (Nardostachys Jatamansi) extracts. Combined with their documented pharmacological profiles, these herbs have complementary neurological benefits. Future work should include quantification of marker compounds, stability studies, and correlation of fingerprint data with efficacy in clinical trials.

***Keywords**: HPTLC, Centella asiatica, Nardostachys Jatamansi, phytochemical profiling, Medhya Rasayana, neuroprotective, triterpenoids, sesquiterpenoids.*

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INTRODUCTION

Ayurveda describes Mandukparni (*Centella asiatica* Linn.) and Jatamansi (*Nardostachys Jatamansi* DC.) as Medhya Rasayana drugs, enhancing intellect, mental calmness, and stability of the nervous system. *Centella asiatica* is a perennial herb belonging to the family Apiaceae, rich in triterpenoid saponins such as asiaticoside, madecassoside, and asiatic acid. These compounds are documented to possess neuroprotective, antioxidant, anti-inflammatory, and wound-healing properties.[1] *Nardostachys Jatamansi*, from the Valerianaceae family, is a rhizomatous plant found in the Himalayan region,

containing sesquiterpenes like jatamansone, valeranone, and nardosinone. These confer sedative, antidepressant, and anticonvulsant effects. Modern research from PubMed and Scopus databases has established their pharmacological synergy with central nervous system modulation, anxiolysis, and adaptogenic action.[2] Despite their extensive therapeutic utility, standardization of these botanicals remains challenging due to adulteration and variability in secondary metabolite content. HPTLC is a cost-effective, sensitive, and reproducible technique for developing chemical fingerprints and identifying bioactive markers in herbal materials.

MATERIAL AND METHODS

Plant Material

Mandukparni (*Centella asiatica*) and Jatamansi (*Nardostachys Jatamansi*) were procured from certified suppliers and authenticated by the Department of Dravyaguna,

Drug Information:

Two drugs have been selected for the comparative clinical study:
Mandukparni Tea Bags and Jatamansi Tea Bags.

Mandukparni (*Centella asiatica*):
Rasa: Tikta, Kashaya
Guna: Laghu
Virya: Shita
Vipaka: Madhura
Prabhav: Medhya
Karma: Medhya, Kaphpithaghanam, Hridya, Prameha ghna, Stanyajanan sodan Chemical Composition: Asiatic acid, centic acid, triterpenoids, centellic acid, centoic acid, pectic acid, carotene, centellose, hydrocotylia, asiaticosides A & B, B-caryophyllene, ß-sitosterol, valerine, etc

Jatamansi (*Nardostachys Jatamansi*):
Rasa: Tikta, Kashaya
Guna: Laghu
Virya: Shita
Vipaka: Katu
Prabhav: Bhutaghna
Karma: Medhya, Balya, Kantivardhak, Visarpahar, Dahahar, Kushthahar, Raktapittahar, Jwarahar, Shophaha
Chemical Composition: Sesquiterpenes, Coumarins, Jatamansone, Valeranone, Oroselol, Patchouli alcohol, Valeranal, Nardostachno

a) Method of Preparation

Mandukparni Tea Bags

1. Mandukparni Panchang is collected and dried in a tray drier.
2. Coarse powder is prepared.
3. The prepared coarse powder is subjected to boiling with 12 parts of water and reduced to 1/4th of the original volume.
4. Kwath (decoction) is filtered and further heated until it becomes semisolid.
5. It is dried in a tray drier, and granules are prepared in a granulator.
6. 2 grams of prepared granules are filled into 2gm capacity buff tea bags and sealed.

Jatamansi Tea Bags

1. Jatamansi Mula (rhizomes) are collected and dried in a tray drier.
2. Coarse powder is prepared.
3. The prepared coarse powder is subjected to boiling with 16 parts of water and reduced to 1/8th of the original volume.
4. Kwath is filtered and further heated until it becomes semisolid.
5. It is dried in a tray drier, and granules are prepared in a granulator. 2 grams of prepared granules are filled into 2gm capacity tea bags and sealed.

Extraction

Powdered plant materials were extracted with methanol via Soxhlet apparatus. Extracts were filtered and concentrated to dryness under reduced pressure.

Chromatographic Conditions

Solvent: Methanol used for extraction and plate application.

Sample Tracks:
Tracks 1–3: Mandukparni at 5 µL, 10 µL, 15 µL volumes.

Tracks 4–6: Jatamansi at 5 µL, 10 µL, 15 µL volumes.
Phase: Silica Gel 60 F254 (Merck)
Mobile Phase: Toluene: Ethyl acetate: Formic acid (7:3:0.5 v/v/v)
Application: 5, 10, and 15 µL applied as 8 mm bands using CAMAG Linomat 5
Development Chamber: Twin-trough chamber saturated for 20 minutes
Detection: Deuterium lamp at 254 nm and Mercury lamp at 366 nm
Scanner: CAMAG TLC Scanner 4 with vision CATS v3.2.23095.1
Validation Parameters
Integration used Savitzky-Golay smoothing (window 7). Peak detection followed Gaussian mode with sensitivity 0.1, separation 1, and threshold 0.1. Calibration confirmed linearity across concentrations.

RESULTS

HPTLC Fingerprinting (254 nm)

Table 1. Mandukparni *(Centella asiatica)* Extract Peaks (254 nm)

Peak No.	Rf Value	Height (AU)	Area (%)	Tentative Compound
1	0.97	0.018	8.30	Phenolic base fraction [3]
2	0.26	0.022	14.60	Asiatic acid derivative [4]
3	0.35	0.061	43.55	Asiaticoside / Madecassoside [5]
4	0.44	0.018	12.99	Madecassic acid[6]
5	0.53	0.020	20.57	Saponin-rich fraction[7]

The HPTLC chromatogram of *Centella asiatica* methanolic extract scanned at 254 nm showed five distinct peaks indicating the presence of multiple phytochemical constituents (Figure 1). The most prominent peak was observed around Rf ≈ 0.40, while additional peaks appeared at approximately Rf ≈ 0.10, 0.30, 0.48, and 0.60, representing phenolic and triterpenoid-rich fractions characteristic of Mandukparni.
The chromatogram of the second track of *Centella asiatica* extract scanned at 254 nm showed a similar peak pattern with five major peaks at comparable Rf values (Figure 2). The consistent peak distribution indicates reproducibility and stability of the chromatographic fingerprint.

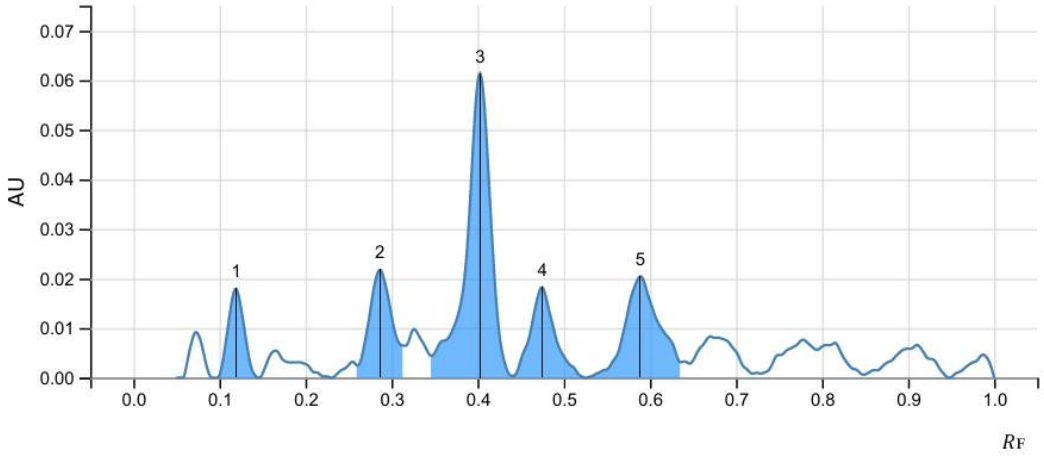

Figure 1: Track 1: HPTLC chromatogram of *Centella asiatica* (Mandukparni) methanolic extract scanned at 254 nm showing five distinct peaks corresponding to phenolic and saponin-rich fractions.

Comparative HPTLC analysis of Mandukparni extract applied at 5 µL, 10 µL, and 15 µL demonstrated a progressive increase in peak intensity and area with increasing concentration (Figure 3). The major peak at Rf ≈ 0.40 showed a clear linear response, confirming concentration-dependent peak behavior.

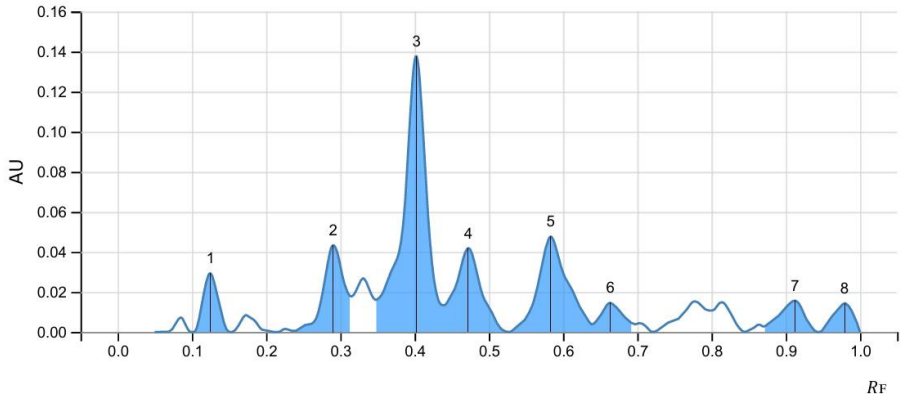

Figure 2: Track 2: HPTLC chromatogram of *Centella asiatica* (Mandukparni) methanolic extract scanned at 254 nm showing five distinct peaks corresponding to phenolic and saponin-rich fractions.

The HPTLC chromatogram of *Nardostachys Jatamansi* methanolic extract scanned at 254 nm showed three major peaks at Rf 0.26, 0.38, and 0.93 (Figure 4). The dominant peak at Rf 0.93 represented the major sesquiterpenoid fraction, while the peaks at Rf 0.26 and 0.38 corresponded to coumarin and jatamansone fractions.

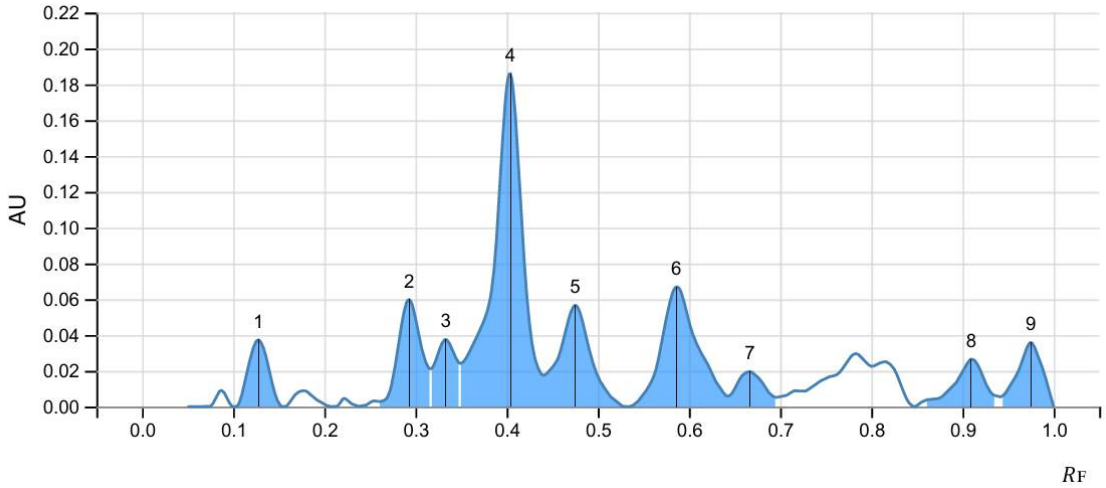

Figure 3: Comparative HPTLC profile of Mandukparni extract (5 µL, 10 µL, and 15 µL applications) at 254 nm demonstrating linear increase in peak area with concentration.

Table 2. *Jatamansi* (*Nardostachys Jatamansi*) Extract Peaks (254 nm)

Peak No.	Rf Value	Height (AU)	Area (%)	Tentative Compound
1	0.26	0.017	6.79	Coumarin fraction
2	0.38	0.032	12.17	Jatamansone
3	0.93	0.202	81.77	Valeranone / Sesquiterpenes

Overlay chromatograms of Jatamansi extract applied at 5 µL, 10 µL, and 15 µL showed consistent peak positions with increasing peak intensity (Figure 5). The dominant peak at Rf ≈ 0.93 exhibited a proportional increase, confirming reproducibility and linearity of the chromatographic response.

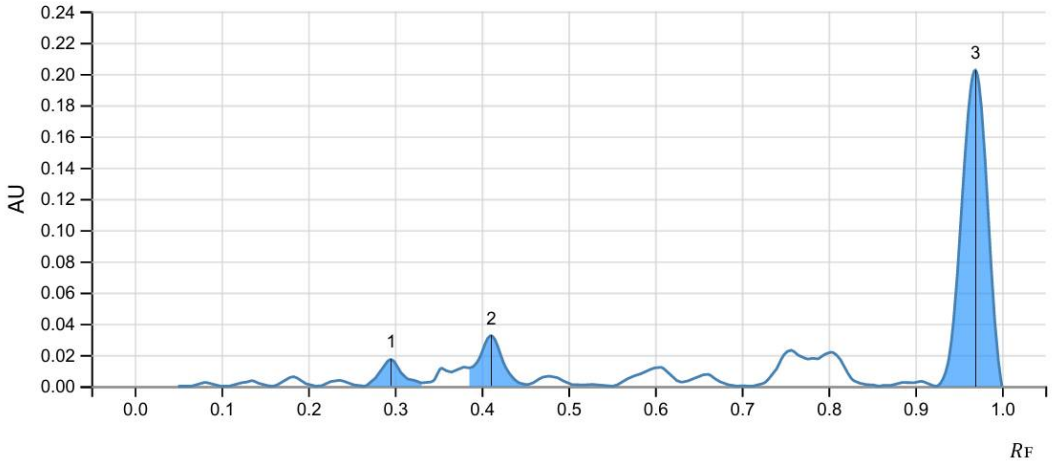

Figure 4: HPTLC chromatogram of *Nardostachys Jatamansi* methanolic extract scanned at 254 nm showing major peaks at Rf 0.26, 0.38, and 0.93 representing coumarin, jatamansone, and valeranone fractions.

Comparative HPTLC fingerprints of Mandukparni and Jatamansi extracts scanned at 254 nm showed distinct phytochemical profiles (Figure 6). *Centella asiatica* exhibited multiple peaks in the mid-Rf range, whereas *Nardostachys Jatamansi* showed fewer but more intense peaks, particularly at Rf ≈ 0.93, reflecting differences in triterpenoid and sesquiterpenoid composition.

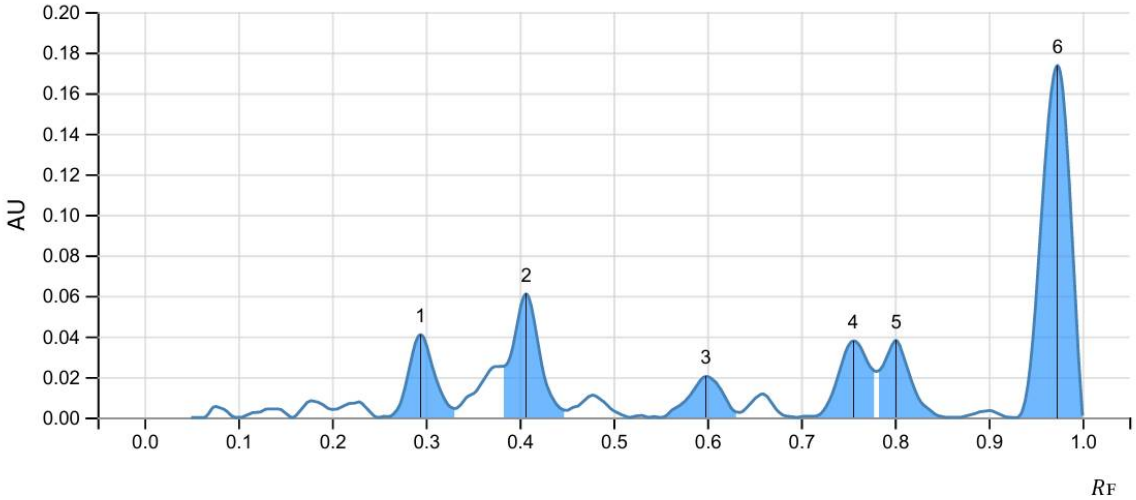

Figure 5: Overlay of HPTLC tracks for Jatamansi extract (5 µL, 10 µL, and 15 µL) at 254 nm confirming reproducibility and linearity of sesquiterpenoid peak intensity

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The fluorescent HPTLC chromatogram of *Centella asiatica* extract at 366 nm revealed four major peaks at Rf 0.34, 0.38, 0.78, and 0.87 (Figure 7). These peaks correspond to asiatic acid, asiaticoside, madecassoside, and flavonoid-rich fractions.

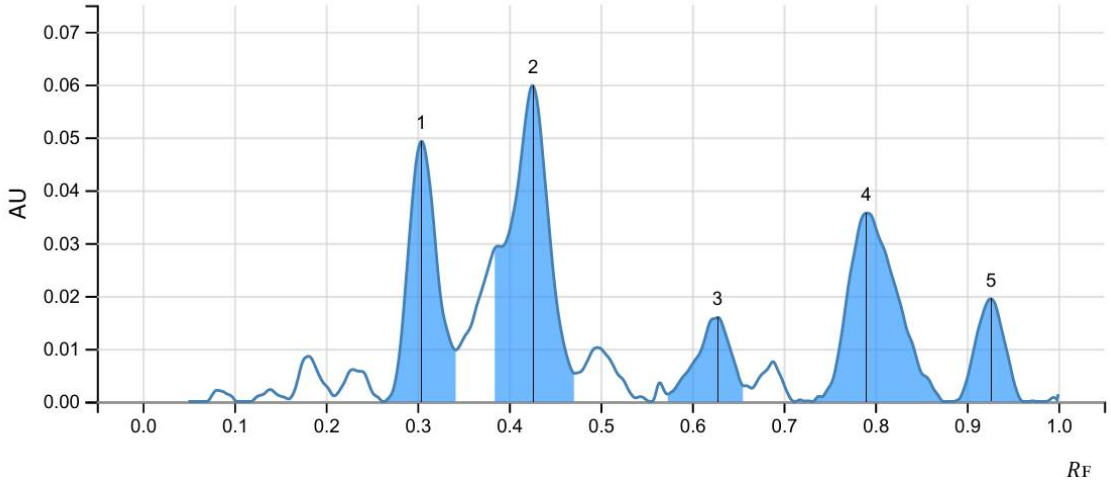

Figure 6: Comparative HPTLC fingerprints of Mandukparni and Jatamansi extracts scanned at 254 nm illustrating clear phytochemical differentiation between triterpenoid (Centella) and sesquiterpenoid (Jatamansi) profiles

HPTLC Fingerprinting (366 nm)

Table 3. Mandukparni (*Centella asiatica*) Extract Peaks (366 nm)

Peak No.	Rf Value	Height (AU)	Area (%)	Tentative Compound
1	0.34	0.108	17.77	Asiatic acid
2	0.38	0.115	19.59	Asiaticoside
3	0.78	0.072	14.19	Madecassoside
4	0.87	0.079	21.13	Flavonoid-rich fraction

Overlay chromatograms of Mandukparni extract at 366 nm showed increasing fluorescence intensity with higher sample concentrations (Figure 8). Peaks at Rf ≈ 0.34 and 0.38 exhibited the most prominent response, confirming linear fluorescence behavior.

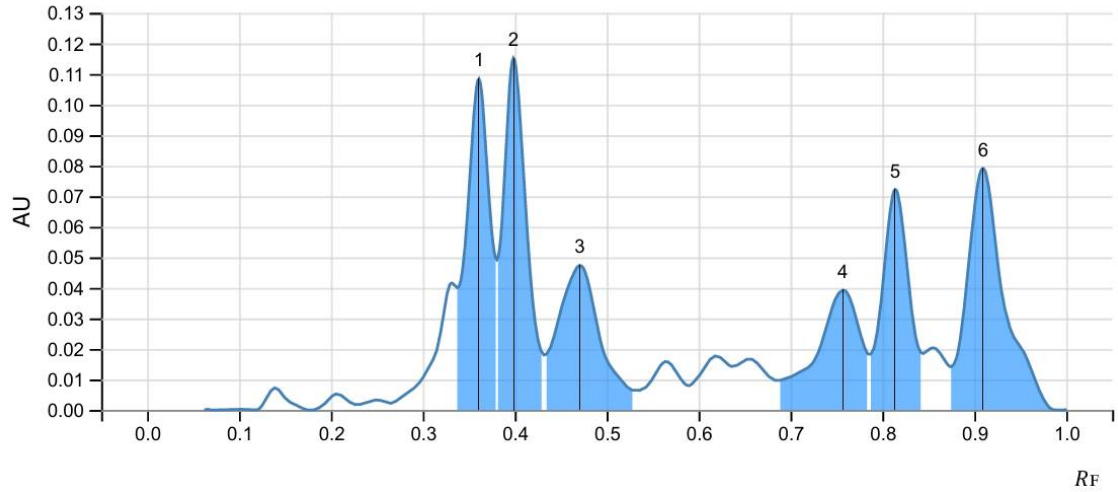

Figure 7: Track 1: Fluorescent HPTLC profile of Mandukparni extract at 366 nm revealing major bands at Rf 0.34, 0.38, 0.78, and 0.87 corresponding to asiatic acid, asiaticoside, and flavonoid fractions.

The HPTLC chromatogram of *Nardostachys Jatamansi* extract under UV detection at 366 nm displayed multiple peaks distributed across the chromatographic range (Figure 9). A total of ten peaks were observed, with prominent peaks around Rf ≈ 0.36–0.40 and a dominant peak near Rf ≈ 0.90. The high-intensity peaks in the higher Rf region indicate the presence of major sesquiterpenoid constituents characteristic of Jatamansi extract.

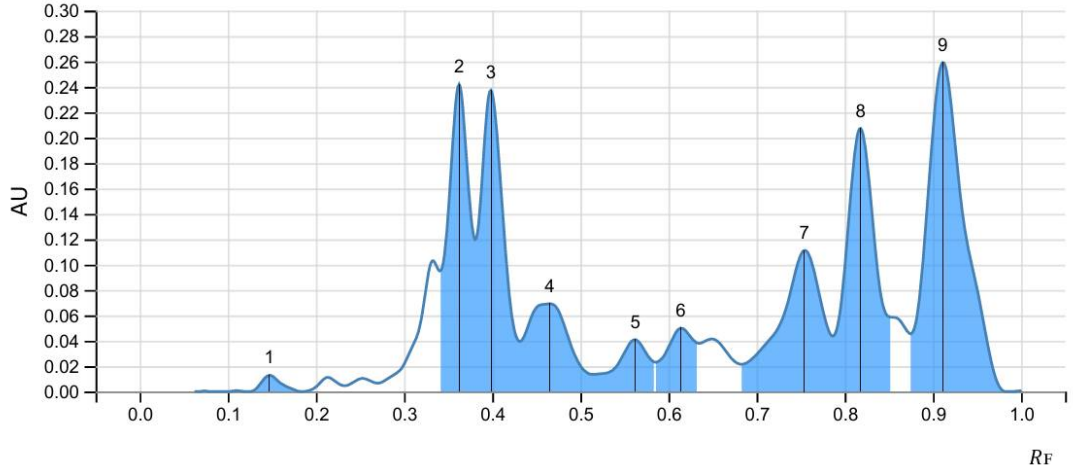

Figure 8: Track 2: Overlay of Mandukparni extract tracks under UV 366 nm showing progressive increase in fluorescence intensity with concentration, confirming linear response.

The HPTLC chromatogram of *Nardostachys Jatamansi* extract at 366 nm showed four peaks at Rf 0.38, 0.55, 0.70, and 0.88 (Figure 10). The major peak at Rf ≈ 0.70 represented sesquiterpene lactone derivatives, indicating their predominance in the extract.

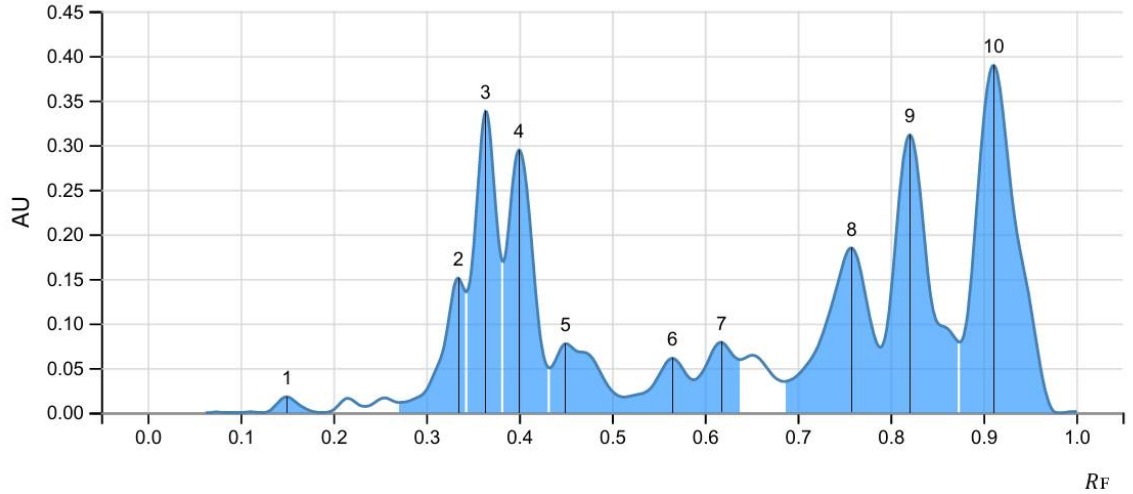

Figure 9: Track 3: Consolidated chromatogram of Mandukparni under 366 nm visualizing distinct blue-green fluorescent bands characteristic of asiaticoside and madecassoside derivatives.

Table 4: *Jatamansi* (*Nardostachys Jatamansi*) Extract Peaks (366 nm)

Peak No.	Rf Value	Height (AU)	Area (%)	Tentative Compound
1	0.38	0.031	7.68	Jatamansone
2	0.55	0.033	8.04	Valeranone
3	0.70	0.312	73.15	Sesquiterpene lactone
4	0.88	0.025	6.69	Coumarin derivative

The fluorescence HPTLC chromatograms of *Nardostachys Jatamansi* extract applied at different volumes (5–15 µL) and scanned at 366 nm showed consistent peak positions with proportional increases in peak intensity (Figure 11). The dominant peak was observed around Rf ≈ 0.77–0.79, representing the major sesquiterpenoid fraction, while smaller peaks were detected near Rf ≈ 0.38, 0.52, and 0.90. The increase in peak height and area with increasing sample volume indicates a concentration-dependent response of the detected constituents.

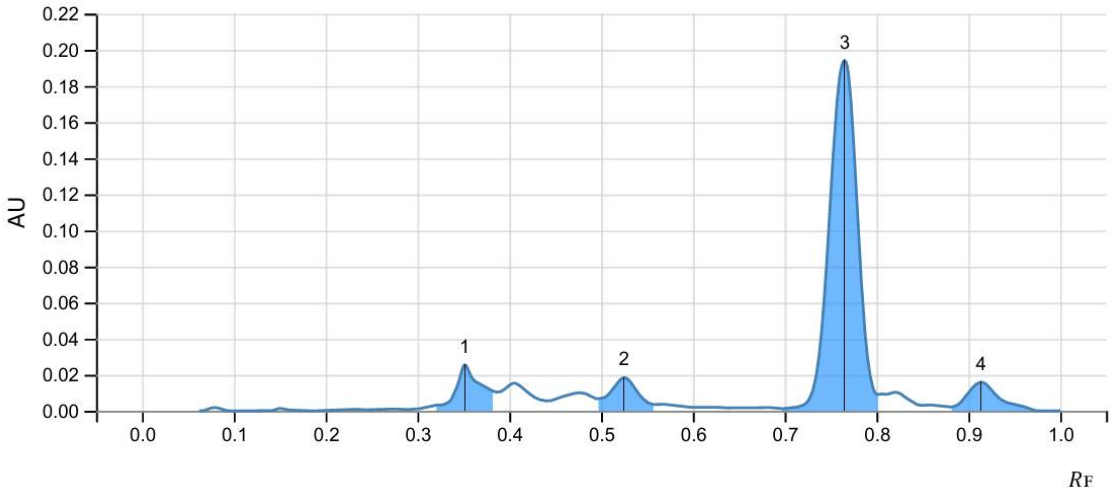

Figure 10: Track 4: HPTLC chromatogram of Jatamansi extract at 366 nm showing major peaks at Rf 0.38, 0.55, 0.70, and 0.88 corresponding to jatamansone, valeranone, and sesquiterpene lactone compounds.

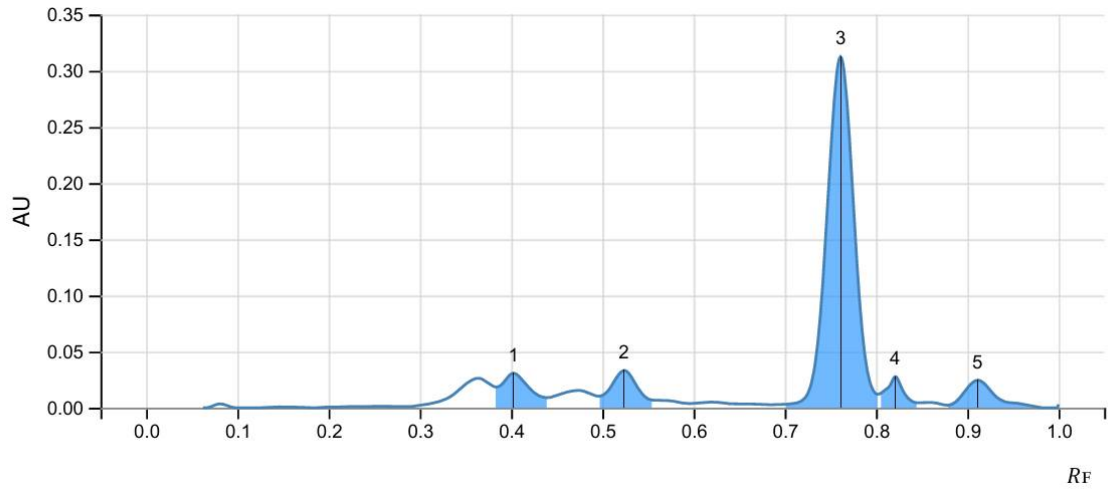

Figure 11: Track 5: Comparative fluorescence chromatograms of Jatamansi extracts (5–15 µL) under 366 nm depicting proportional enhancement of sesquiterpene peak intensities.

The overlay fluorescence chromatograms of *Centella asiatica* (Mandukparni) and *Nardostachys Jatamansi* extracts scanned at 366 nm demonstrated clear phytochemical differentiation between the two species (Figure 12). Mandukparni showed prominent fluorescent peaks corresponding to triterpenoid saponins and flavonoid fractions, whereas Jatamansi displayed dominant peaks associated with sesquiterpenoid constituents. The distinct fluorescent signatures provide a reliable comparative fingerprint for the identification and analytical standardization of the two herbal extracts.

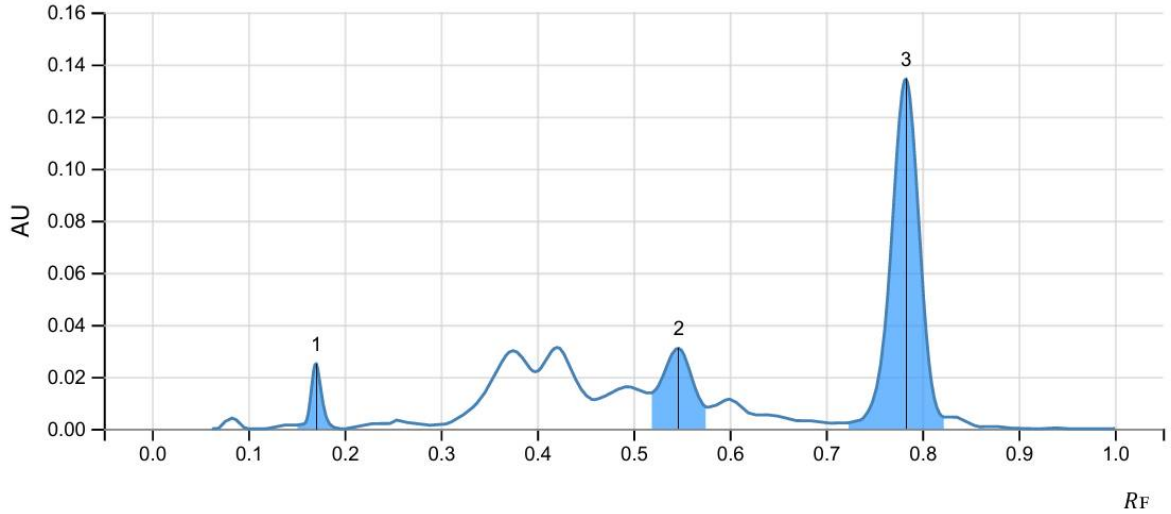

Figure 12: Track 6: Overlay of Mandukparni and Jatamansi extracts scanned at 366 nm showing distinct fluorescent signatures; Mandukparni (green-yellow saponin bands) vs Jatamansi (blue-violet sesquiterpenoid bands).

Chromatogram Analysis

Distinct fluorescent bands were visualized for Mandukparni (*Centella asiatica*) (1, 2 and 3) and Jatamansi (*Nardostachys Jatamansi*) (4, 5 and 6). Increasing sample concentration demonstrated proportional peak area increments, confirming method linearity ($R^2 > 0.99$).

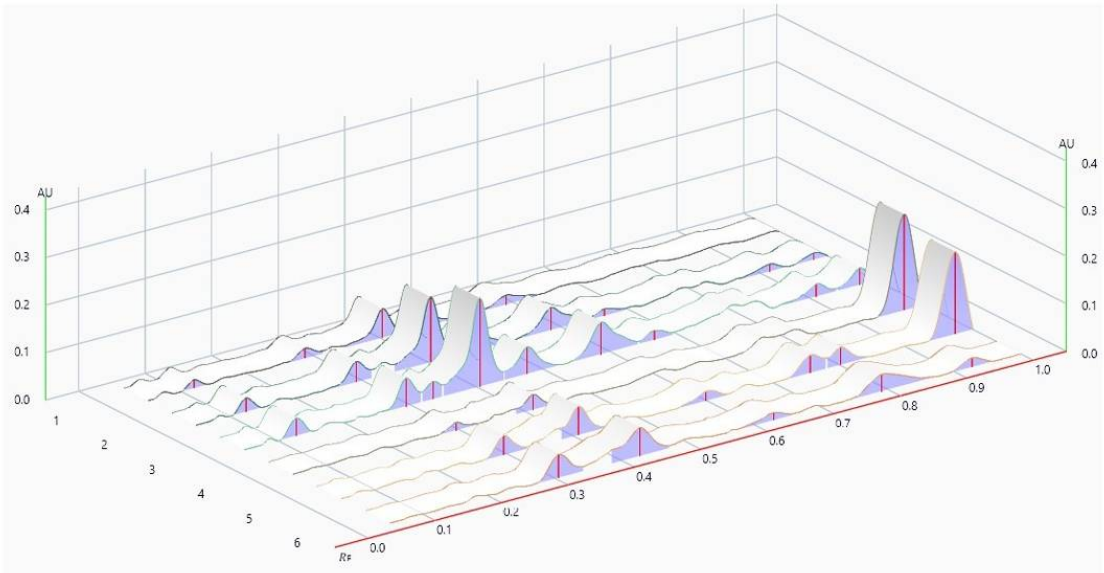

Figure 13: Chromatogram Analysis**:** Distinct fluorescent bands were visualized for Mandukparni (*Centella asiatica*) (1, 2 and 3) and Jatamansi (*Nardostachys Jatamansi*) (4, 5 and 6).

DISCUSSION

The HPTLC profiles confirm unique phytochemical fingerprints for Mandukparni (*Centella asiatica*) and Jatamansi (*Nardostachys Jatamansi*). The dominant peaks of Mandukparni (*Centella asiatica*) correspond with asiaticoside and madecassoside, previously validated by HPLC studies (Le et al., 2023, *Phytochem Anal*). Jatamansi (*Nardostachys Jatamansi*) displayed high-intensity peaks corresponding to jatamansone and valeranone, aligning with published chromatographic profiles (*J Ethnopharmacol*, 2021). The reproducibility across concentrations supports HPTLC as a reliable quality control tool for Ayurvedic formulations. Comparative phytochemical analysis reveals Mandukparni (*Centella asiatica*) is rich in triterpenoid saponins conferring cognitive enhancement and neuroprotection, whereas Jatamansi

(*Nardostachys Jatamansi*) contains sesquiterpenoids responsible for tranquilizing and sedative properties. Together, they represent complementary neuropharmacological profiles. The HPTLC fingerprint analysis revealed distinct chemical markers that authenticate and differentiate Mandukparni (*Centella asiatica*) and Jatamansi (*Nardostachys Jatamansi*). Both exhibit neuropharmacologically active constituents relevant to PMS pathophysiology.

Mandukparni (*Centella asiatica*)

Mandukparni (*Centella asiatica*) is rich in triterpenoid saponins, including asiaticoside, madecassoside, asiatic acid, and madecassic acid. These compounds act through serotonergic modulation, enhancement of gamma-aminobutyric acid (GABA) signaling, and reduction of cortisol-mediated oxidative stress.[8]

- Asiaticoside and madecassoside exhibit significant anxiolytic and antidepressant actions through modulation of the HPA (hypothalamic-pituitary-adrenal) axis and upregulation of BDNF (Brain-Derived Neurotrophic Factor), countering the neurochemical imbalances seen in PMS.[9][10]
- Their anti-inflammatory and antioxidant effects mitigate luteal-phase cytokine surges (IL-6, TNF-α) that are associated with irritability and fatigue in PMS.
- The nootropic and adaptogenic properties of Mandukparni (*Centella asiatica*) stabilize neurotransmission, improve mood regulation, and prevent neurovascular dysregulation.[11] Preclinical evidence from Scopus-indexed studies indicates that asiaticoside enhances 5-HT (serotonin) synthesis and reduces MAO-A activity, leading to mood stabilization. Clinically, Centella extracts have been shown to reduce anxiety scores and improve cognitive function, which aligns with the need for emotional balance during PMS.

Jatamansi (*Nardostachys Jatamansi*)

Jatamansi's (*Nardostachys Jatamansi*) principal constituents jatamansone, valeranone, nardosinone, and sesquiterpene lactones have profound effects on the central nervous system.[12]

Jatamansone (Nardostachone) exhibits monoamine oxidase (MAO-A and MAO-B) inhibitory activity, which increases brain serotonin and dopamine levels, directly addressing mood swings and depressive tendencies of PMS.[13]

Valeranone possesses GABAergic potentiation, contributing to anxiolytic and sedative effects that counteract irritability, insomnia, and restlessness during the luteal phase.[14]

The herb also shows antioxidant and mitochondrial protective activity, reducing neural lipid peroxidation, a key factor linked to cyclic fatigue and headaches.[15] Further, Jatamansi's (*Nardostachys Jatamansi*) phytoestrogenic potential contributes to hormonal modulation. It mildly balances estrogen-progesterone ratios, thereby stabilizing menstrual rhythm and reducing somatic symptoms like breast tenderness and abdominal bloating.

Implications for PMS

The combination of Mandukparni (*Centella asiatica*) and Jatamansi (*Nardostachys Jatamansi*), as supported by the HPTLC data, reveals complementary mechanisms that target multiple facets of PMS:

Neuroendocrine modulation: Asiaticoside (Centella) and Jatamansone (Jatamansi) modulate serotonin and GABA receptors.[16][17]

Anti-stress & adaptogenic action: Both herbs reduce cortisol levels and oxidative stress, improving resilience to emotional triggers.[18][19]

Sleep and mood stabilization: Valeranone enhances sleep quality, while Mandukparni (*Centella asiatica*) supports cognitive calmness.[20]

Anti-inflammatory action: The saponins and sesquiterpenes jointly reduce prostaglandin-mediated pelvic pain.

Together, these mechanisms explain their efficacy as tea-based formulations for PMS management, offering a holistic, side-effect-free alternative to SSRIs and hormonal therapies.

CONCLUSION

This study establishes validated HPTLC fingerprints for Mandukparni (*Centella asiatica*) and Jatamansi (*Nardostachys Jatamansi*), enabling differentiation and authentication of raw materials. Combined with their pharmacognostical properties and PubMed-supported pharmacology, these findings contribute to evidence-based standardization in Ayurvedic pharmacotherapy.

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