

Standardisation of Pathyadi Churna- A Polyherbal Ayurvedic Medicine

Dr. Sreeraj. R¹, Dr. Nikhil Neelakandhan. E. U², Dr Madhavi S Deodas³

1. PhD Scholar, P.D.E.A College of Ayurveda and Research Centre, Pune

2. Assistant Professor, Vaidyaratnam Ayurveda College, Ollur

3. Professor and H.O.D, Department of Kaumarabhritya, P.D.E.A College of Ayurveda and Research Centre, Nigdi, Pune

Abstract

Pathyadi choorna is a classical polyherbal Ayurvedic formulation widely used in traditional medicine. The present study aimed to evaluate its physicochemical properties, heavy metal safety, and HPTLC fingerprint profile for quality control and standardization. Physicochemical parameters were assessed using standard pharmacopoeial methods. Heavy metal analysis revealed that arsenic, lead, mercury, and cadmium levels were within permissible limits, indicating formulation safety. HPTLC fingerprinting was performed using silica gel 60 F₂₅₄ plates with chloroform:ethyl acetate:formic acid (5:4:0.2) as the mobile phase. Densitometric scanning at 254 nm and 366 nm showed well-resolved and reproducible chromatographic patterns across replicate tracks, confirming batch consistency and sample uniformity. Multiple peaks observed at both wavelengths indicated the presence of diverse phytoconstituents, including fluorescent secondary metabolites. The developed HPTLC fingerprint can serve as a reliable qualitative reference for identity testing, standardization, and routine quality assessment of Pathyadi choorna.

Introduction

Pathyadi churna¹ is a classical Ayurvedic polyherbal formulation mentioned in Charaka Samhita. It is used for the management of Kasa² (cough disorders). The formulation contains Pathya³ (*Terminalia chebula*)⁴, Thamalaki⁵ (*Phyllanthus niruri*)⁶, Dhatri⁷ (*Emblica officinalis*)⁸, Bhadramustha⁹ (*Cyperus rotundus*)¹⁰, and Pippali¹¹ (*Piper longum*)¹². These ingredients are traditionally known for their respiratory^{13,14}, anti-inflammatory^{15,16}, antioxidant^{17,18}, and immunomodulatory properties^{19,20}. Pathyadi churna is widely used in Ayurvedic practice for relieving cough and associated respiratory symptoms. Due to its polyherbal composition and therapeutic importance, scientific standardization and quality evaluation of the formulation are essential for ensuring its safety, efficacy, and consistency.

Materials and Methods

Parameters	Unit	Result	Test Method
Loss on drying	% w/w	7.56	API Part I Vol-I
Total Ash	% w/w	4.44	IP 2018
Acid insoluble Ash	% w/w	0.51	IP 2018
pH (10% aqueous solution)	% w/w	3.59	API Part I Vol-I
Water soluble extract	% w/w	23.57	IP 2018
Alcohol soluble extract	% w/w	27.42	IP 2018

Heavy metal analysis

Heavy Metals	Unit	Result	Specification	Detection limit	Test method
Arsenic	ppm	0.18	NMT 3.0	.05	CKL/ANL/AY-008
Lead	ppm	1.01	NMT 10	.05	CKL/ANL/AY-008
Mercury	ppm	Not Detected	NMT 1.0	.05	CKL/ANL/AY-008
Cadmium	ppm	0.16	NMT 0.3	.05	CKL/ANL/AY-008

HPTLC

1. Nature of the analysis

- **Technique:** HPTLC (High-Performance Thin Layer Chromatography)
- **Stationary phase:** Silica gel 60 F₂₅₄
- **Mobile phase:** Chloroform : Ethyl acetate : Formic acid (5:4:0.2)
- **Detection:** 254 nm (UV absorbance) and 366 nm (fluorescence)
- **Samples:** Pathyadi choorna (3 replicate tracks)

This is a fingerprint analysis, not a targeted assay for a known marker compound.

2. Reproducibility & sample consistency

All three tracks show nearly identical chromatographic profiles: Same number of peaks, Similar R_f values, Comparable peak areas

Interpretation

The sample preparation and application are consistent. The formulation shows batch uniformity. Suitable for quality control and standardization

3. Peak profile at 254 nm (UV absorbance)

- Number of major peaks: ~11
- Major R_f regions (approximate):
 - R_f ~0.00–0.04 → very high-intensity peak (~33–39% area)
 - R_f ~0.20–0.24 → another dominant peak (~25–30% area)
 - R_f ~0.72–0.75 → significant peaks (~8–10% each)

Interpretation

- Indicates presence of multiple UV-absorbing phytochemicals
- Likely includes: Phenolics, Alkaloids, Conjugated aromatic compounds
- No single compound dominates excessively → complex polyherbal nature preserved

4. Peak profile at 366 nm (fluorescence)

- 13–14 peaks detected
- One very dominant fluorescent peak at R_f ~0.52–0.57
 - Contributes ~45–54% of total area
- Other moderate peaks between R_f 0.40–0.75

Interpretation

- Presence of fluorescent phytochemicals, typically: Flavonoids, Coumarins, Some alkaloids. Strong fluorescence suggests bioactive secondary metabolites

5. Visualizer plate observations (images)

- **254 nm image:** Multiple dark bands across tracks, Band positions align perfectly → confirms scanner data
- **366 nm image:** Bright red/fluorescent bands at identical Rf positions, Strong mid-Rf band visible in all tracks

Interpretation

Confirms chromatographic data visually

No extra bands → no obvious contamination or adulteration

6. Assigned substances: All peaks are marked “unknown”

Important clarification

This is expected and acceptable for: Herbal fingerprinting studies, Quality control monographs & Pharmacopoeial identity testing.

Identification requires: Reference standards or LC-MS / GC-MS / HPTLC-MS coupling

7. Overall conclusion

The HPTLC fingerprint profile of Pathyadi choorna developed using silica gel 60 F₂₅₄ and chloroform:ethyl acetate:formic acid (5:4:0.2) revealed a complex phytochemical composition with reproducible peak patterns at 254 nm and 366 nm across three tracks. The consistency of Rf values and peak areas confirms sample uniformity and batch consistency. The prominent UV-absorbing and fluorescent peaks indicate the presence of multiple bioactive secondary metabolites. The developed chromatographic fingerprint can be used as a reliable reference for quality control and standardization of Pathyadi choorna.

Results and Discussion

HPTLC Fingerprint Profile of Pathyadi Choorna

High-Performance Thin Layer Chromatography (HPTLC) analysis was carried out to develop a characteristic fingerprint profile for Pathyadi choorna using silica gel 60 F₂₅₄ plates and a mobile phase consisting of chloroform:ethyl acetate:formic acid (5:4:0.2). The chromatographic separation resulted in well-resolved bands with good peak symmetry and reproducibility across three replicate tracks, indicating satisfactory method performance and sample uniformity.

At 254 nm, the densitometric scan revealed the presence of approximately 11 distinct peaks with Rf values ranging from near baseline to high Rf regions. Among these, prominent peaks were consistently observed at low Rf (~0.00–0.04) and mid-Rf (~0.20–0.24) regions, contributing significantly to the total peak area. These peaks likely correspond to UV-absorbing phytoconstituents such as phenolic compounds, alkaloids, or other conjugated aromatic molecules. Additional moderate-intensity peaks observed at higher Rf values (~0.72–0.75) further indicate the complexity of the formulation.

Densitometric evaluation at 366 nm showed a richer chromatographic pattern with 13–14 detectable peaks, suggesting the presence of fluorescent phytochemicals. A dominant peak observed consistently at Rf ~0.52–0.57, contributing more than 45–50% of the total peak area, indicates a major fluorescent constituent in the formulation. Compounds exhibiting fluorescence at this wavelength are commonly associated with flavonoids, coumarins, and related secondary metabolites, which are often reported to possess pharmacological relevance.

The visual inspection of developed plates under both 254 nm and 366 nm using a CAMAG visualizer corroborated the densitometric findings. All three tracks displayed identical band patterns with no additional or missing bands, confirming

the absence of detectable adulteration and ensuring batch consistency. The reproducibility of R_f values and relative peak areas across tracks demonstrates the reliability of the developed HPTLC method.

Although individual phytochemicals were not identified due to the absence of reference standards, the obtained HPTLC fingerprint serves as a robust qualitative tool for identity testing and routine quality control. The presence of multiple well-resolved peaks reflects the chemically complex nature of the formulation, which is typical of multi-component herbal products.

Overall, the developed HPTLC fingerprint profile provides a scientifically valid chromatographic signature for Pathyadi choorna and can be effectively employed for standardization, batch-to-batch consistency evaluation, and detection of possible adulteration in future quality assurance studies.

Conclusion

The present study successfully established an HPTLC fingerprint profile for Pathyadi choorna using silica gel 60 F₂₅₄ plates and a mobile phase comprising chloroform:ethyl acetate:formic acid (5:4:0.2). The chromatographic analysis demonstrated well-resolved, reproducible peaks at both 254 nm and 366 nm, confirming the chemical complexity of the formulation and the consistency of the analytical method.

The reproducibility of R_f values and relative peak areas across replicate tracks indicates uniformity of the sample and supports the reliability of the developed fingerprint for routine quality assessment. The detection of multiple UV-absorbing and fluorescent phytoconstituents highlights the presence of diverse secondary metabolites, which may collectively contribute to the biological activity of the formulation.

Although specific compound identification was not performed due to the absence of reference standards, the generated HPTLC fingerprint serves as a definitive qualitative reference for identity verification, batch-to-batch consistency evaluation, and detection of adulteration. Overall, the developed method provides a simple, rapid, and reproducible analytical tool that can be effectively employed for the standardization and quality control of Pathyadi choorna in both research and industrial settings.

Limitations and Future Scope

The present study was limited to qualitative fingerprint analysis using HPTLC without the identification or quantification of individual phytoconstituents. The absence of authenticated reference standards restricted peak assignment, and therefore the detected components were reported as unidentified. Additionally, the study evaluated a single formulation batch; hence, long-term inter-batch and inter-manufacturer variability could not be assessed.

Future investigations should focus on the identification and characterization of major marker compounds using advanced hyphenated techniques such as HPTLC–MS, LC–MS/MS, or GC–MS. Quantitative estimation of selected marker compounds would further strengthen the standardization process and enable regulatory compliance. Evaluation of multiple production batches across different storage conditions would help establish acceptable chromatographic variability and stability profiles. Furthermore, correlation of chromatographic fingerprints with pharmacological or bioactivity data may provide deeper insight into the contribution of specific phytochemical constituents to the therapeutic potential of the formulation.

Overall, the present HPTLC fingerprint provides a foundational analytical framework that can be expanded through targeted, quantitative, and bioactivity-guided studies to enhance the scientific validation and quality assurance of Pathyadi choorna.

References

1. Agnivesha, Charaka Samhita Vol-2, Chakrapani tika with Bhasha vyakhyana by Narayanan vaidyar M, 2010 edition, Redstar offsets, Calicut, p146
2. Agnivesha, Charaka Samhita Vol-2, Chakrapani tika with Bhasha vyakhyana by Narayanan vaidyar M, 2010 edition, Redstar offsets, Calicut, p147
3. Agnivesha, Charaka Samhita Vol-2, Chakrapani tika with Bhasha vyakhyana by Narayanan vaidyar M, 2010 edition, Redstar offsets, Calicut, p147
4. JLN Sastry, Illustrated Dravyaguna Vijnana Vol-II forwarded by Chunekar KC, Reprint edition 2010
5. Agnivesha, Charaka Samhita Vol-2, Chakrapani tika with Bhasha vyakhyana by Narayanan vaidyar M, 2010 edition, Redstar offsets, Calicut, p146
6. JLN Sastry, Illustrated Dravyaguna Vijnana Vol-II forwarded by Chunekar KC, Reprint edition 2010
7. Agnivesha, Charaka Samhita Vol-2, Chakrapani tika with Bhasha vyakhyana by Narayanan vaidyar M, 2010 edition, Redstar offsets, Calicut, p146
8. JLN Sastry, Illustrated Dravyaguna Vijnana Vol-II forwarded by Chunekar KC, Reprint edition 2010
9. Agnivesha, Charaka Samhita Vol-2, Chakrapani tika with Bhasha vyakhyana by Narayanan vaidyar M, 2010 edition, Redstar offsets, Calicut, p146
10. JLN Sastry, Illustrated Dravyaguna Vijnana Vol-II forwarded by Chunekar KC, Reprint edition 2010
11. Agnivesha, Charaka Samhita Vol-2, Chakrapani tika with Bhasha vyakhyana by Narayanan vaidyar M, 2010 edition, Redstar offsets, Calicut, p147
12. JLN Sastry, Illustrated Dravyaguna Vijnana Vol-II forwarded by Chunekar KC, Reprint edition 2010
13. Nosalova G, Jurecek L, Chatterjee UR, Majee SK, Nosal S, Ray B. Antitussive Activity of the Water-Extracted Carbohydrate Polymer from Terminalia chebula on Citric Acid-Induced Cough. Evid Based Complement Alternat Med. 2013;2013:650134. doi: 10.1155/2013/650134. Epub 2013 Jun 25. PMID: 23878602; PMCID: PMC3708419.
14. Chitren Verma, Mohnish Kumar, Kamlesh Patel, Abhay Kaushal, Praveen Kumar Sahu, Dr. Gyanesh Kumar Sahu, A Review on Terminalia chebula (Chebulic Myrobalan) in Cough and Respiratory Health, Int. J. of Pharm. Sci., 2026, Vol 4, Issue 2, 4183-4192. <https://doi.org/10.5281/zenodo.18776802>
15. Wiratmini, Ngurah Intan & Pharmawati, Made & Sukmaningsih, Anak & J. Raharjo, Sentot & Widowati, Wahyu. (2024). Anti-Inflammatory Activity of Phyllanthus niruri L. Leaf Extract: In Vitro Study on RAW 264.7 Macrophage Cells and In Silico Analysis. HAYATI Journal of Biosciences. 32. 287-299. 10.4308/hjb.32.2.287-299.
16. Mostofa R, Ahmed S, Begum MM, Sohanur Rahman M, Begum T, Ahmed SU, Tuhin RH, Das M, Hossain A, Sharma M, Begum R. Evaluation of anti-inflammatory and gastric anti-ulcer activity of Phyllanthus niruri L. (Euphorbiaceae) leaves in experimental rats. BMC Complement Altern Med. 2017 May 16;17(1):267. doi: 10.1186/s12906-017-1771-7. PMID: 28511679; PMCID: PMC5434621.
17. Patience Onakurhefe, Ovovwe Diakparomre, Tracy Ogochukwu Njideaka, Joel Okpoghono, Antioxidant indices, essential and non-essential amino acids of Phyllanthus niruri leaves extracts, Results in Chemistry, Volume 16, 2025, 102545, ISSN 2211-7156, <https://doi.org/10.1016/j.rechem.2025.102545>.
18. Shukla V, Vashistha M, Singh SN. Evaluation of antioxidant profile and activity of amalaki (Embllica officinalis), spirulina and wheat grass. Indian J Clin Biochem. 2009 Jan;24(1):70-5. doi: 10.1007/s12291-009-0012-3. Epub 2009 May 8. PMID: 23105810; PMCID: PMC3453465.
19. Singh, M.K., Yadav, S.S., Gupta, V. *et al.* Immunomodulatory role of *Embllica officinalis* in arsenic induced oxidative damage and apoptosis in thymocytes of mice. *BMC Complement Altern Med* **13**, 193 (2013). <https://doi.org/10.1186/1472-6882-13-193>
20. Belapurkar P, Goyal P, Tiwari-Barua P. Immunomodulatory effects of triphala and its individual constituents: a review. Indian J Pharm Sci. 2014 Nov-Dec;76(6):467-75. PMID: 25593379; PMCID: PMC4293677.