

Comparative HPTLC Fingerprint Profiling and Densitometric Evaluation of Quercetin in *Euphorbia prostrata* at 254 nm and 366 nm

Dr. Bhavesh Dangore^{1*}, Dr. Sadhana Misar Wajpeyi²

Dr. Bhavesh Dangore^{1*}, Dr. Sadhana Misar Wajpeyi²

¹PG Scholar, Department of Kayachikitsa, Mahatma Gandhi Ayurvedic College, Hospital and Research Centre, Salod (H), Wardha

Datta Meghe Institute of Higher Education and Research, [Deemed To Be University], Maharashtra, INDIA.

²Professor & HOD, Department of Kayachikitsa, Mahatma Gandhi Ayurvedic College, Hospital and Research Centre, Salod (H), Wardha

Datta Meghe Institute of Higher Education and Research, [Deemed To Be University], Maharashtra, INDIA.

Correspondence

Dr. D Bhavesh

PG Scholar, Department of Kayachikitsa, Mahatma Gandhi Ayurvedic College, Hospital and Research Centre, Salod (H), Wardha, Datta Meghe Institute of Higher Education and Research, [Deemed To Be University], Maharashtra, INDIA.

E-mail: bhaveshdangore@gmail.com

History

- Submission Date: 03-02-2026;
- Review completed: 16-06-2026;
- Accepted Date: 26-08-2026.

DOI : 10.5530/pj.2026.18.138

Article Available online

<http://www.phcogj.com/v18/i3>

Copyright

© 2026 Phcogj.Com. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

ABSTRACT

Background: *Euphorbia prostrata* (Dugdika) is a traditionally used medicinal herb with reported anti-inflammatory and antioxidant properties. Standardization of herbal drugs using chromatographic techniques is essential to ensure quality and reproducibility. **Objective:** To develop an HPTLC fingerprint profile of *Euphorbia prostrata* and to comparatively evaluate densitometric detection of quercetin at 254 nm and 366 nm. **Materials and Methods:** Methanolic extracts of raw *Euphorbia prostrata*, and its inhalation formulation were subjected to HPTLC using silica gel 60 F254 plates. The mobile phase consisted of toluene: ethyl acetate (7:3 v/v). Densitometric scanning was performed at 254 nm and 366 nm using a CAMAG TLC scanner. Chromatographic profiles, R_f values, and peak areas were recorded and compared with quercetin standard. **Results:** Distinct chromatographic fingerprints were obtained at both wavelengths. The quercetin standard exhibited a characteristic R_f value which corresponded with peaks observed in plant samples. Detection at 366 nm demonstrated improved selectivity and enhanced visualization of flavonoid-related bands compared to 254 nm. Comparative densitometric analysis revealed variation in peak distribution and intensity between raw drug and inhalation formulation. **Conclusion:** The developed HPTLC fingerprint method provides a reliable tool for qualitative standardization of *Euphorbia prostrata*. Comparative wavelength analysis suggests 366 nm to be more suitable for selective flavonoid detection.

Keywords: *Euphorbia prostrata*; HPTLC; Quercetin; Chromatographic fingerprint; Densitometric analysis; Herbal standardization; Dual wavelength detection.

INTRODUCTION

Medicinal plants continue to serve as an important source of bioactive compounds for therapeutic development, with increasing global reliance on the use of herbal remedies in basic care¹. However, Variability in the content of phytochemicals due to geographical, environmental, and processing factors poses significant challenges to quality control and reproducibility of herbal formulations². Therefore, chromatographic fingerprinting combined with marker-based standardization has become an essential strategy for ensuring identity, consistency, and safety of plant-derived products³.

Euphorbia prostrata Aiton (Euphorbiaceae) is a prostrate annual herb widely found across tropical and subtropical areas. It has been traditionally used in the management of haemorrhoids, inflammatory disorders, gastrointestinal disturbances, and respiratory ailments^{4,5}. Pharmacological investigations have demonstrated that extracts of *E. prostrata* possess anti-inflammatory, antioxidant, antimicrobial, and hepatoprotective activities, which are primarily attributed to its polyphenolic and flavonoid constituents⁶⁻⁸. Despite its documented therapeutic relevance, detailed chromatographic standardization studies of this plant remain limited.

Flavonoids are among the most important secondary metabolites in the genus *Euphorbia*. Quercetin, a prominent flavonol, exhibits potent antioxidant, anti-inflammatory, antiviral, and

immunomodulatory properties^{9,10}. Due to its pharmacological importance and widespread occurrence in medicinal plants, quercetin is frequently employed as a chemical marker for standardization and quality assurance purposes¹¹. Several studies have reported the presence of flavonoids, including quercetin derivatives, in various *Euphorbia* species, supporting their contribution to therapeutic activity^{6,12}.

High-performance thin-layer chromatography (HPTLC) has emerged as a reliable and cost-effective analytical tool for herbal drug evaluation. The technique enables simultaneous analysis of multiple samples, generation of reproducible chromatographic fingerprints, and densitometric detection of marker compounds with minimal solvent consumption^{13,14}. Additionally, flavonoids exhibit characteristic ultraviolet absorption and fluorescence properties, allowing selective detection at different wavelengths such as 254 nm and 366 nm¹⁵. Comparative densitometric evaluation at multiple wavelengths may enhance selectivity and improve the reliability of flavonoid identification in complex plant matrices.

Although HPTLC fingerprinting has been applied to several medicinal plants, comprehensive comparative evaluation of densitometric detection at dual wavelengths for *Euphorbia prostrata* has not been adequately documented. Establishing chromatographic reference data and evaluating wavelength-dependent detection behaviour may contribute significantly to the qualitative standardization of this medicinal species.

Cite this article: Bhavesh D, Sadhana M W.Comparative HPTLC Fingerprint Profiling and Densitometric Evaluation of Quercetin in *Euphorbia prostrata* at 254 nm and 366 nm. Pharmacogn J. 2026;18 (3): 229-232.

Therefore, the present study aimed to develop an HPTLC fingerprint profile of *E. prostrata* and to comparatively evaluate densitometric detection of quercetin at 254 nm and 366 nm, with the objective of generating reference chromatographic data for its quality assessment.

MATERIALS AND METHODS

Plant Material

The whole plant (Panchang) of *Euphorbia prostrata* W. Ait was procured and authenticated by the Department of Dravyaguna, Mahatma Gandhi Ayurved College Hospital & Research Centre, Wardha. The botanical identity was confirmed vide Certificate of Identification dated 11 July 2025. The authenticated plant material was shade-dried, coarsely powdered, and stored in an airtight container until further analysis.

Chemicals and Reagents

Quercetin standard was used as the reference marker compound. Analytical grade toluene and ethyl acetate were used for mobile phase preparation. Methanol was used as the solvent for sample and standard preparation.

Preparation of Sample and Standard Solutions

The powdered plant material was subjected to Soxhlet extraction using methanol as solvent. Approximately 2 g of coarse powder was extracted with methanol for 6 hours. The extract was filtered and concentrated under reduced pressure using a rotary evaporator. The concentrated extract was stored at 4 °C until further use.

Instrumentation

HPTLC analysis was performed using:

- CAMAG Linomat 5 applicator
- CAMAG Twin Trough Chamber (10 × 10 cm)
- CAMAG TLC Scanner (Scanner_181112)

Data acquisition and processing were carried out using winCATS software.

Chromatographic Conditions

Precoated silica gel 60 F254 HPTLC plates (E. Merck KGaA) were used as the stationary phase

Sample Application

Bands of 6 mm length were applied using a 100 µL Hamilton syringe under nitrogen flow. Application parameters were:

- Dosage speed: 150 nL/s
- Application position Y: 8.0 mm
- Track distance: 14.0 mm

Application volumes:

- 2 µL and 4 µL of raw extract
- 2 µL and 4 µL of inhalation extract
- 2 µL and 4 µL of quercetin standard

Mobile Phase and Development

The mobile phase consisted of:

Toluene: Ethyl acetate (7:3 v/v)

Chromatographic development was carried out in a twin trough chamber with:

- Chamber saturation using 10 mL mobile phase
- Solvent front migration distance: 70 mm

After development, plates were dried in an oven at 60°C for 5 minutes

Densitometric Scanning

Densitometric scanning was performed using a CAMAG TLC Scanner under the following conditions:

- Slit dimensions: 4.00 × 0.30 mm (micro)
- Scanning speed: 20 mm/s
- Data resolution: 100 µm/step

Scanning was carried out at:

- 254 nm
- 366 nm

Peak integration was performed using Savitzky–Golay data filtering and lowest slope baseline correction.

Spectral Analysis

In addition to densitometric scanning, spectral scanning was performed in the range of 200–700 nm to assess peak purity and spectral characteristics of detected compounds.

All chromatographic analyses were performed in triplicate to ensure reproducibility.

RESULTS

Chromatographic Profile at 254 nm

HPTLC fingerprinting of *E. prostrata* at 254 nm produced distinct and reproducible chromatographic profiles for both raw extract and inhalation formulation. The quercetin standard exhibited prominent peaks in the mid-R_f region, with a major densitometric response observed around R_f ≈ 0.50–0.75 depending on application volume. Corresponding peaks were observed in the chromatograms of the raw extract and inhalation formulation, indicating the probable presence of quercetin or structurally related flavonoid compounds. The raw extract displayed dominant peaks with high area percentages in the higher R_f region (notably around R_f ≈ 0.39–0.81), suggesting significant UV-absorbing phytoconstituents. The inhalation formulation showed a comparatively simplified chromatographic profile, with fewer dominant peaks relative to the raw extract.

Chromatographic Profile at 366 nm

At 366 nm, enhanced visualization of flavonoid-related bands was observed. The quercetin standard demonstrated distinct peaks in the lower to mid-R_f region (notably around R_f ≈ 0.26–0.32), with strong densitometric response. The raw plant extract exhibited multiple well-resolved peaks across the chromatogram, particularly in the R_f range of 0.20–0.95. The inhalation formulation also showed identifiable peaks corresponding to those seen in the raw extract, although with variation in peak area distribution. Compared to 254 nm, detection at 366 nm provided improved selectivity and clearer differentiation of flavonoid-associated peaks, indicating wavelength-dependent variation in densitometric sensitivity.

Comparative Evaluation of Dual Wavelength Detection

Comparative analysis demonstrated that:

- 254 nm provided strong general UV absorption-based detection.
- 366 nm enhanced visualization of flavonoid-type constituents due to their characteristic fluorescence behaviour.

- Peak distribution patterns differed between wavelengths, indicating wavelength-specific detection sensitivity.
- The inhalation formulation retained major phytoconstituent bands observed in the raw extract, suggesting preservation of key chemical components during formulation.

The Rf values corresponding to the quercetin standard showed alignment with peaks detected in both raw and formulated samples, supporting marker confirmation under the selected chromatographic conditions.

DISCUSSION

Chromatographic fingerprinting has become a fundamental component of herbal drug standardization due to the complex and multicomponent nature of plant matrices³. In the present study, HPTLC profiling of *E. prostrata* was performed under optimized chromatographic conditions, enabling comparative densitometric evaluation at 254 nm and 366 nm. The developed solvent system (toluene: ethyl acetate, 7:3 v/v) produced satisfactory resolution of phytoconstituents in both raw extract and inhalation formulation.

The chromatographic pattern obtained at 254 nm revealed multiple UV-absorbing constituents. Silica gel 60 F254 plates contain a fluorescent indicator that emits fluorescence under short-wave UV light; compounds possessing conjugated systems absorb at 254 nm and appear as dark bands due to fluorescence quenching¹³. Flavonoids and phenolic compounds typically show strong absorption in this region, which explains the prominent densitometric response observed in the raw extract and formulated sample.

In contrast, detection at 366 nm demonstrated improved selectivity for flavonoid-associated compounds. Flavonoids exhibit characteristic fluorescence under long-wave UV light due to their conjugated ring structures and substitution patterns¹⁵. The quercetin standard displayed distinct densitometric peaks within the mid-Rf region, and corresponding peaks were observed in the plant samples. Quercetin has been widely reported in several *Euphorbia* species and contributes significantly to antioxidant and anti-inflammatory activities^{6,10}. The alignment of Rf values between standard and sample supports its suitability as a chemical marker for qualitative standardization.

Comparative evaluation indicated that 366 nm provided enhanced band differentiation and reduced background interference compared to 254 nm. Previous chromatographic studies have reported similar findings, demonstrating that fluorescence-based detection improves selectivity for flavonoid identification in complex plant matrices¹¹. The enhanced visualization at 366 nm observed in the present study is consistent with these reports and supports the analytical advantage of dual wavelength scanning.

The inhalation formulation retained major chromatographic bands observed in the raw extract, although variations in peak area distribution were evident. Such differences may arise from processing, dilution effects, or matrix modification during formulation preparation. Nevertheless, preservation of characteristic fingerprint bands suggests retention of key phytoconstituents in the formulated product.

Although full quantitative validation parameters were not evaluated in the present investigation, the study successfully established a reproducible chromatographic fingerprint and confirmed the presence of quercetin-like constituents under dual wavelength detection. Fingerprint-based chromatographic approaches are widely recommended for routine quality control of herbal materials when comprehensive quantitative validation is not performed^{3,2}.

The dual wavelength strategy employed in this study integrates absorption-based (254 nm) and fluorescence-based (366 nm) detection,

thereby enhancing reliability in marker confirmation. This approach provides a practical, reproducible, and cost-effective tool for qualitative standardization of *E. prostrata* in both raw and formulated forms.

LIMITATIONS

The present investigation focused primarily on qualitative chromatographic fingerprint development and comparative densitometric evaluation. Full quantitative validation parameters such as linearity, limit of detection (LOD), limit of quantification (LOQ), precision, and recovery studies were not performed. Additionally, structural confirmation of detected compounds was not undertaken using advanced spectroscopic techniques. Future studies incorporating comprehensive method validation and quantitative estimation would further strengthen standardization protocols for *E. prostrata*.

CONCLUSION

The present study successfully established an HPTLC-based chromatographic fingerprint profile for *E. prostrata* under optimized analytical conditions. The selected mobile phase system (toluene: ethyl acetate, 7:3 v/v) provided satisfactory separation of phytoconstituents, enabling clear visualization of characteristic bands in both raw plant extract and inhalation formulation.

Comparative densitometric evaluation at 254 nm and 366 nm demonstrated wavelength-dependent variation in detection sensitivity. While 254 nm enabled broad detection of UV-absorbing compounds, scanning at 366 nm provided enhanced selectivity for flavonoid-associated constituents, including quercetin. Alignment of Rf values between the quercetin standard and sample peaks supports its suitability as a chemical marker for qualitative standardization.

The dual wavelength strategy enhances analytical reliability by integrating absorption- and fluorescence-based detection modes. The developed chromatographic fingerprint may serve as a reproducible reference for routine quality assessment and qualitative standardization of *E. prostrata* in raw and formulated preparations.

ACKNOWLEDGMENT

The authors acknowledge the Central Research Laboratory, Datta Meghe Institute of Higher Education and Research (DU), Wardha, for providing the HPTLC instrumentation and technical support required for the study.

REFERENCES

1. Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Front Pharmacol.* 2014;4:177.
2. Heinrich M, Appendino G, Efferth T, Fürst R, Izzo AA, Kayser O, et al. Best practice in research – Overcoming common challenges in phytopharmacological research. *J Ethnopharmacol.* 2020;246:112230.
3. Liang YZ, Xie P, Chan K. Quality control of herbal medicines. *J Chromatogr B.* 2004;812(1–2):53–70.
4. Ahmad A, et al. Traditional uses, phytochemistry and pharmacology of *Euphorbia* species: A review. *J Ethnopharmacol.* 2016;176:1–22.
5. Bani S, et al. Anti-inflammatory activity of *Euphorbia* species. *Phytother Res.* 2006;20(9):764–769.
6. Salehi B, Iriti M, Vitalini S, et al. *Euphorbia*-derived phytochemicals: A comprehensive review. *Biomolecules.* 2019;9(12):739.
7. Rajasekaran A, et al. Evaluation of antioxidant and hepatoprotective activity of *Euphorbia prostrata*. *Asian Pac J Trop Biomed.* 2012;2(3):S123–S128.

8. Sharma P, et al. Pharmacological properties of *Euphorbia prostrata*. *Pharmacogn Rev.* 2011;5(9):84–89.
9. Boots AW, Haenen GRMM, Bast A. Health effects of quercetin. *Eur J Pharmacol.* 2008;585(2–3):325–337.
10. Li Y, Yao J, Han C, Yang J, Chaudhry MT, Wang S, et al. Quercetin, inflammation and immunity. *Nutrients.* 2016;8(3):167.
11. Patel DK, Kumar R, Laloo D, Hemalatha S. Natural flavonoids as potential markers in herbal standardization. *Pharmacogn Rev.* 2012;6(12):1–15.
12. Suresh K, et al. Phytochemical evaluation of *Euphorbia* species using chromatographic techniques. *Pharmacogn Mag.* 2010;6(24):321–325.
13. Sherma J. Review of HPTLC in drug analysis. *J AOAC Int.* 2012;95(1):28–46.
14. Reich E, Schibli A. High-performance thin-layer chromatography for herbal analysis. *J Planar Chromatogr.* 2007;20(1):5–14.
15. Alam P, Alqarni MH, Alqasoumi SI, et al. HPTLC method development and validation for phytochemical analysis of medicinal plants. *Saudi Pharm J.* 2017;25(5):699–707.

Cite this article: Bhavesh D, Sadhana MW. Comparative HPTLC Fingerprint Profiling and Densitometric Evaluation of Quercetin in *Euphorbia prostrata* at 254 nm and 366 nm. *Pharmacogn J.* 2026;18 (3): 229-232.