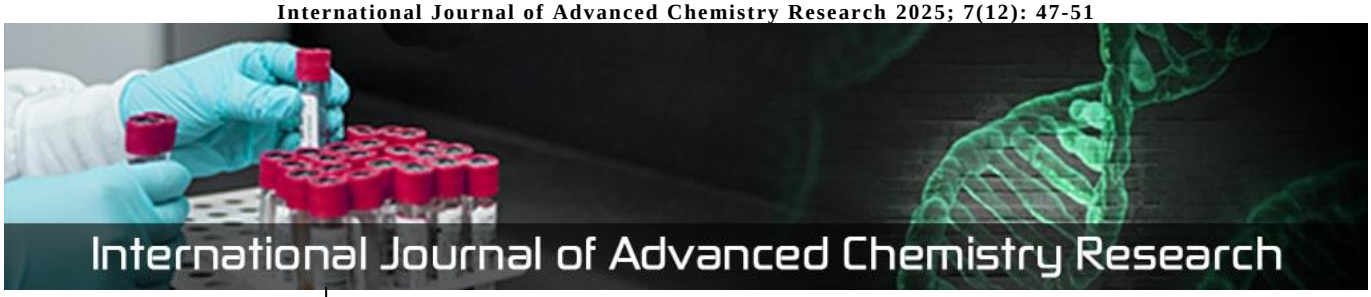

ISSN Print: 2664-6781
ISSN Online: 2664-679X
NAAS Rating (2025): 4.77
IJACR 2025; 7(12): 47-51
www.chemistryjournals.net
Received: 06-11-2025
Accepted: 08-12-2025

Madhupriya S
Department of Veterinary Pharmacology and Toxicology, Veterinary College and Research Institute, Orathanadu, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India

Akshay Durga Prasad M
Research scholar, Department of Veterinary Pharmacology and Toxicology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India

Kannan K
Assistant Professor, Department of Veterinary Pharmacology and Toxicology, Veterinary College and Research Institute, Orathanadu, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India

Elamaran A
Assistant Professor, Department of Veterinary Pharmacology and Toxicology, Veterinary College and Research Institute, Orathanadu, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India

Kesavan G
Research scholar, Department of Veterinary Pharmacology and Toxicology, Veterinary College and Research Institute, Orathanadu, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India

Corresponding Author:
Kesavan G
Research scholar, Department of Veterinary Pharmacology and Toxicology, Veterinary College and Research Institute, Orathanadu, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India

HPTLC-based quantitative estimation of quercetin and rutin in *Euphorbia hirta* L. for phytochemical standardization

Madhupriya S, Akshay Durga Prasad M, Kannan K, Elamaran A and Kesavan G

DOI: https://www.doi.org/10.33545/26646781.2025.v7.i12a.346

Abstract
Euphorbia hirta L., commonly known as the asthma plant, is a widely used medicinal herb in traditional systems of medicine and is valued for its rich phytochemical composition and diverse pharmacological properties. Among its bioactive constituents, the flavonoids quercetin and rutin are of particular importance due to their well-documented antioxidant, anti-inflammatory and antimicrobial activities. The present study aimed to develop and apply simple, rapid and reliable High-Performance Thin Layer Chromatography (HPTLC) methods for the quantitative estimation of quercetin and rutin in the whole plant of *Euphorbia hirta*. The ethanolic extract of the plant was prepared by cold maceration and subjected to preliminary phytochemical screening, which confirmed the presence of flavonoids and other secondary metabolites. HPTLC analysis was carried out on silica gel 60 F_{254} plates using optimized mobile phases for each analyte, followed by densitometric scanning at 366 nm for quercetin and 254 nm for rutin. Identification of quercetin and rutin in the extract was achieved by comparing their retardation factor (Rf) values with those of the corresponding standards. The methods exhibited good linearity and reproducibility, enabling accurate quantification of both compounds. The quercetin and rutin contents in the whole plant extract of *Euphorbia hirta* were found to be 0.226% (w/w) and 0.29% (w/w), respectively. The developed HPTLC methods provide a cost-effective and robust approach for quality control and standardization of *Euphorbia hirta* and herbal formulations containing these flavonoids as marker constituents.

Keywords: *Euphorbia hirta*, Quercetin, Rutin, HPTLC, Herbal standardization

Introduction
Euphorbia hirta L. (Family: Euphorbiaceae), commonly referred to as the “asthma plant,” is a widely distributed medicinal herb found abundantly along roadsides, grasslands and wastelands across tropical and subtropical regions, including India, Southeast Asia and Africa. The plant has been extensively used in traditional systems of medicine for the treatment of respiratory ailments such as bronchial asthma, cough and bronchitis, as well as gastrointestinal disorders, skin diseases and inflammatory conditions [1]. Owing to its broad ethnomedicinal relevance and ease of availability, *E. hirta* continues to attract scientific interest as a source of bioactive phytoconstituents with therapeutic potential.

Phytochemical investigations of *E. hirta* have revealed the presence of diverse secondary metabolites, including flavonoids, phenolic acids, tannins, terpenoids, alkaloids and glycosides, which collectively contribute to its pharmacological activities [1]. Among these, flavonoids have gained particular attention due to their well-documented antioxidant, anti-inflammatory, antimicrobial and cytoprotective properties. Two flavonoids of notable importance in *E. hirta* are quercetin and rutin, both of which are widely recognized for their biological efficacy and relevance in human and veterinary medicine.

Quercetin is a polyphenolic flavonol known for its strong free radical scavenging activity, anti-inflammatory effects, modulation of oxidative stress pathways and potential protective roles in cardiovascular and metabolic disorders.

Rutin, a glycosylated derivative of quercetin, exhibits complementary pharmacological properties including vascular protection, antioxidant activity, anti-inflammatory effects and antimicrobial action. The presence of these flavonoids in medicinal plants enhances their therapeutic value and supports their traditional use in inflammatory and infectious conditions [2, 3]. Consequently, accurate identification and quantification of quercetin and rutin are essential for standardization, quality control and validation of herbal raw materials and formulations derived from *E. hirta*.

Despite the growing interest in herbal medicines, variability in phytochemical composition remains a major challenge in ensuring consistent efficacy and safety. Factors such as geographical location, cultivation conditions, harvesting stage and extraction method can significantly influence the concentration of active constituents in medicinal plants. Therefore, the development of reliable analytical techniques for quantitative estimation of marker compounds is critical for herbal drug standardization and regulatory compliance.

High-Performance Thin Layer Chromatography (HPTLC) has emerged as a robust and cost-effective analytical tool for the qualitative and quantitative analysis of phytoconstituents in complex herbal matrices. Compared to conventional TLC, HPTLC offers enhanced resolution, improved sensitivity, densitometric scanning and the ability to analyze multiple samples simultaneously under identical experimental conditions. These advantages make HPTLC particularly suitable for routine quality assessment of medicinal plants and herbal formulations [4]. Several studies have demonstrated the successful application of HPTLC for the estimation of flavonoids such as quercetin and rutin in different plant matrices, highlighting its reliability and reproducibility [2, 3].

Although *E. hirta* is well recognized for its medicinal importance, systematic quantitative data on key flavonoids using validated chromatographic techniques remain limited. Establishing a standardized HPTLC method for simultaneous estimation of quercetin and rutin would not only strengthen the phytochemical profiling of *E. hirta* but also facilitate its rational utilization in herbal and pharmaceutical preparations. In this context, the present study was undertaken to develop and apply HPTLC-based densitometric methods for the quantitative estimation of quercetin and rutin in the ethanolic extract of whole plant of *Euphorbia hirta*, thereby contributing to its scientific validation and quality standardization.

Materials and Methods

Collection and authentication of plant material

The whole plant of *Euphorbia hirta* L. was collected from the Herbal Garden of the Veterinary College and Research Institute, Orathanadu, Tamil Nadu, India. The plant material was thoroughly washed with distilled water to remove extraneous matter, shade-dried at room temperature (25-30 °C) to preserve heat-sensitive phytoconstituents, and subsequently pulverized into coarse powder using a mechanical grinder. The powdered material was stored in airtight containers until further analysis.

Preparation of plant extract and percentage yield

The powdered whole plant material was subjected to extraction by the cold maceration technique using ethanol as the solvent. Briefly, a known quantity of the powdered plant material was soaked in ethanol and allowed to macerate with intermittent stirring for 72 h at room temperature. The extract was then filtered, and the filtrate was concentrated under reduced pressure using a rotary vacuum evaporator to obtain a dried extract. The percentage yield of the extract was calculated using the formula:

$$\text{Percentage yield} = \frac{\text{Weight of dried extract}}{\text{Weight of initial plant material}} \times 100$$

Qualitative phytochemical screening

Preliminary qualitative phytochemical analysis of the ethanolic extract of *Euphorbia hirta* was carried out using standard chemical tests to detect the presence of major phytoconstituents. The extract was screened for alkaloids, glycosides, flavonoids, phenolic compounds, tannins, terpenoids, carbohydrates, proteins and amino acids according to established methods described by Trease and Evans [5] and Sahu *et al*. [6]. These tests provide an initial understanding of the phytochemical composition of the plant and support further chromatographic analysis.

Preparation of standard and sample solutions

Standard solutions of quercetin and rutin were prepared separately by dissolving accurately weighed quantities of the respective reference standards in methanol to obtain concentrations of 100 µg/ml for quercetin and 1 mg/ml for rutin. The ethanolic extract of *E. hirta* was prepared at a concentration of 100 mg/ml by dissolving 1 g of dried extract in 100 ml of methanol. The solution was sonicated and centrifuged at 2500 rpm for 10 min, and the clear supernatant was used for HPTLC analysis.

HPTLC instrumentation and chromatographic conditions

HPTLC analysis was performed on precoated silica gel 60 F_{254} aluminium plates (20 × 10 cm, Merck). Sample and standard solutions were applied as bands using a CAMAG Linomat V automatic sample applicator equipped with a 100 µl Hamilton syringe. Chromatographic development was carried out in a CAMAG twin-trough glass chamber under linear ascending conditions.

For quantification of quercetin, the mobile phase consisted of toluene: Ethyl acetate: Formic acid (10.9: 8.7: 0.4, v/v/v), while for rutin estimation the mobile phase used was ethyl acetate: formic acid: acetic acid: water (13: 1.5: 1.5: 3.5, v/v/v/v), as optimized for effective separation. After development, the plates were air-dried and derivatized using p-anisaldehyde–sulphuric acid reagent.

Densitometric scanning was performed using a CAMAG TLC Scanner at 366 nm for quercetin and 254 nm for rutin, with a slit dimension of 6 × 0.45 mm, scanning speed of 20 mm/s and data resolution of 100 µm/step. All chromatographic data were processed using VisionCATS software.

Calibration curve and quantification

Calibration curves for quercetin and rutin were constructed by applying increasing volumes of the respective standard solutions to the HPTLC plates. Peak area and height were plotted against the corresponding concentrations to obtain regression equations. Quantification of quercetin and rutin in the plant extract was carried out by comparing the peak areas of sample tracks with those of the standards, and the

results were expressed as percentage (% w/w) of dry plant material.

Results

The whole plant of *Euphorbia hirta* was subjected to extraction by the cold maceration technique using ethanol as the solvent. The percentage yield of the ethanolic extract obtained by this method was found to be 4.3%, indicating satisfactory extraction efficiency for polar phytoconstituents.

Qualitative phytochemical screening

Preliminary phytochemical analysis of the ethanolic extract of *E. hirta* revealed the presence of several important secondary metabolites. The qualitative tests confirmed the presence of alkaloids, glycosides, flavonoids, phenolic compounds, tannins, terpenoids, carbohydrates, proteins and amino acids. The detection of flavonoids and phenolic compounds supported further chromatographic analysis aimed at quantifying the major flavonoid constituents, quercetin and rutin.

HPTLC fingerprinting and identification of quercetin and rutin

HPTLC analysis was successfully developed for the separation and identification of quercetin and rutin in the ethanolic extract of *E. hirta*. Under the optimized chromatographic conditions, well-resolved and compact bands were obtained for both standard compounds and plant extract samples.

For quercetin, chromatographic development using the mobile phase toluene: ethyl acetate: formic acid (10.9: 8.7: 0.4, v/v/v) resulted in a distinct band with an Rf value of 0.49, which was consistently observed in both the standard and the plant extract tracks when scanned at 366 nm. The matching Rf values of the standard and sample confirmed the presence of quercetin in the *E. hirta* extract.

Similarly, rutin was effectively separated using the mobile phase ethyl acetate: formic acid: acetic acid: water (13.5: 1.5: 1.5: 3.5, v/v/v/v). The rutin standard and the extract produced prominent bands at an Rf value of 0.58 when scanned at 254 nm, confirming the identity of rutin in the plant extract.

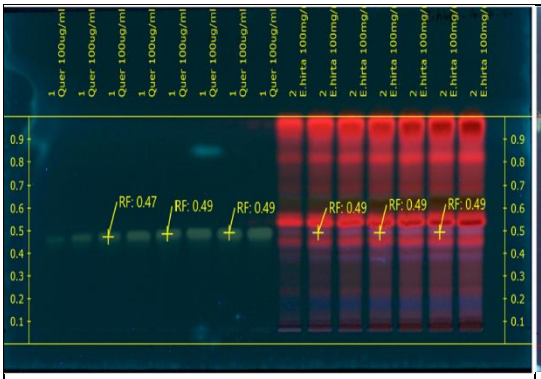

Fig 1a: TLC plates of Quercetin at 366nm

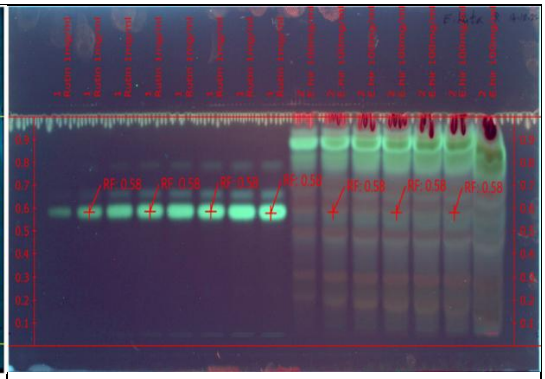

Fig 1b: TLC plates of Rutin at 254nm

Calibration curves and linearity

Calibration curves for both quercetin and rutin were constructed by applying increasing concentrations of their respective standard solutions onto the HPTLC plates. Linear relationships were observed between the concentration applied and the corresponding peak height/area values generated using Vision CATS software. The linearity of the calibration curves demonstrated the suitability of the developed HPTLC methods for quantitative estimation of both flavonoids.

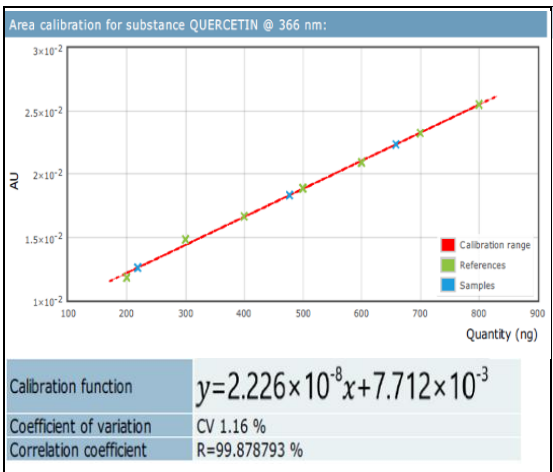

Fig 2a: Calibration curve of quercetin

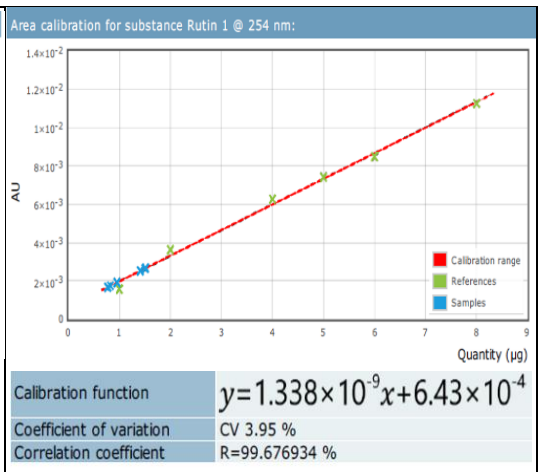

Fig 2b: Calibration curve of rutin

Quantification of quercetin and rutin

Quantitative estimation revealed that the quercetin content in the whole plant extract of *Euphorbia hirta* was 0.226% (w/w), while the rutin content was found to be 0.29% (w/w) on a dry weight basis. The quantification was carried out by comparing the peak areas of the sample tracks with those of the corresponding standards using the regression equations derived from the calibration curves.

The consistent Rf values, clear resolution of bands, and reproducible densitometric responses observed across multiple applications demonstrate the reliability of the developed HPTLC methods for the estimation of quercetin and rutin in *E. hirta*.

Discussion

High-Performance Thin Layer Chromatography (HPTLC) has emerged as a widely accepted analytical technique for the qualitative and quantitative evaluation of phytoconstituents in medicinal plants. Its advantages, including simplicity, cost-effectiveness, minimal solvent consumption and the ability to analyze multiple samples simultaneously, make it particularly suitable for routine analysis and standardization of herbal drugs [4]. In the present study, HPTLC was employed to quantify two pharmacologically important flavonoids, quercetin and rutin, from the whole plant of *Euphorbia hirta*, a medicinal herb extensively used in traditional medicine.

The optimized chromatographic conditions adopted in this study enabled effective separation of quercetin and rutin from the complex phytochemical matrix of *E. hirta*. The Rf values obtained for quercetin (0.49) and rutin (0.58) in the plant extract closely matched those of the corresponding reference standards, confirming the specificity and selectivity of the developed methods. Matching Rf values between standards and samples is a critical parameter in planar chromatography, as it validates the identity of analytes and rules out interference from co-eluting compounds [7]. The distinct and well-resolved bands observed under UV detection further demonstrate the suitability of the selected mobile phases for flavonoid separation.

Calibration curve analysis showed good linearity for both quercetin and rutin over the tested concentration ranges, indicating a strong correlation between applied concentration and densitometric response. Linearity is a key indicator of method reliability and accuracy in quantitative chromatographic analysis [8]. The consistent densitometric responses obtained using VisionCATS software confirm that the HPTLC methods developed in this study are robust and reproducible, making them suitable for routine quantitative applications.

Quantitative estimation revealed that the whole plant of *Euphorbia hirta* contains 0.226% (w/w) quercetin and 0.29% (w/w) rutin. These findings substantiate earlier reports highlighting *E. hirta* as a rich source of flavonoids and phenolic compounds responsible for its antioxidant, anti-inflammatory and antimicrobial properties [1]. The presence of both quercetin and rutin in appreciable amounts supports the ethnomedicinal use of the plant in treating inflammatory and respiratory conditions, where oxidative stress and microbial involvement play significant roles.

Comparative evaluation with previously published studies indicates that the flavonoid content of medicinal plants can vary considerably depending on several intrinsic and extrinsic factors. Doshi and Une [2] reported variability in quercetin and rutin content across different plant species and plant parts, emphasizing the influence of botanical source and extraction conditions. Similarly, Rodríguez-Valdovinos *et al*. [3] demonstrated that phenolic-rich extracts exhibit significant quantitative differences even within the same genus, attributable to environmental and methodological variations. The quercetin and rutin levels observed in the present study fall within the range reported for flavonoid-rich medicinal herbs, reinforcing the pharmacological relevance of *E. hirta*.

Extraction technique plays a crucial role in determining phytochemical yield and composition. The cold maceration method employed in this study yielded 4.3% extract, which is consistent with previous reports using polar solvents for flavonoid extraction. Cold maceration is known to preserve thermolabile compounds and prevent degradation of sensitive phenolics, thereby enhancing the recovery of bioactive constituents [9]. The use of ethanol as the extraction solvent further contributes to efficient solubilization of flavonoids while maintaining compatibility with chromatographic analysis.

Variations in phytochemical composition may also arise due to factors such as geographical location, soil type, climatic conditions, harvesting stage and post-harvest processing. *E. hirta* is a widely distributed species, and such environmental factors are known to influence secondary metabolite biosynthesis [10]. Therefore, quantitative profiling using validated analytical methods is essential to ensure consistency and reproducibility in herbal drug development and quality control.

From a standardization perspective, the developed HPTLC methods provide a reliable analytical platform for routine estimation of quercetin and rutin in *E. hirta*. Unlike more complex techniques such as HPLC or LC–MS, HPTLC offers a balanced combination of analytical performance and operational simplicity, making it particularly suitable for laboratories engaged in herbal quality assessment [4,7]. The ability to simultaneously quantify multiple bioactive markers further enhances its applicability in standardizing herbal raw materials and formulations.

Overall, the results of the present study confirm that *Euphorbia hirta* is a valuable source of quercetin and rutin and that HPTLC densitometry is an effective tool for their quantitative estimation. The generated data contribute to the scientific validation of this traditionally important medicinal plant and support its potential use in standardized herbal and pharmaceutical preparations.

Conclusion

The present study successfully established simple, rapid and reliable HPTLC methods for the quantitative estimation of quercetin and rutin in the whole plant of *Euphorbia hirta*. The developed methods demonstrated good specificity, reproducibility and suitability for routine analysis, enabling effective identification and quantification of these key flavonoids. The findings support the application of HPTLC as a practical tool for quality control and standardization of *E. hirta* and herbal formulations containing quercetin and rutin as marker phytoconstituents.

References

1. Kumar S, Malhotra R, Kumar D. Euphorbia hirta: Its chemistry, traditional and medicinal uses, and pharmacological activities. Pharmacognosy reviews. 2010 Jan;4(7):58.
2. Doshi GM, Une HD. Quantification of quercetin and rutin from *Benincasa hispida* seeds and *Carissa congesta* roots by high-performance thin layer chromatography and high-performance liquid chromatography. Pharmacognosy Research. 2016 Jan;8(1):37.

3. Valdovinos RKY, Garciglia SR, Sánchez VM, Bernal AD, Zamudio OE, *et al*. Quantitative analysis of rutin by HPTLC and *in vitro* antioxidant and antibacterial activities of phenolic-rich extracts from *Verbesina sphaerocephala*. Plants. 2021 Mar 3;10(3):475.
4. Dharmamoorthy G. An overview on high-performance thin-layer chromatography (HPTLC). Asian Journal of Pharmaceutics (AJP). 2023 Mar 15;17(1).
5. Trease GE, Evans WC. Pharmacognosy. 13th. ELBS/Bailliere Tindall, London. 1989:345-6.
6. Sahu M, Vermaand D, Harris KK. Phytochemical analysis of the leaf, stem and seed extracts of *Cajanus cajan* L (Dicotyledoneae: Fabaceae). World J Pharm Pharm Sci. 2014 May 12;3(8):694-733.
7. Sherma J, Fried B, editors. Handbook of thin-layer chromatography. CRC press; 2003 Apr 18.
8. Reich E, Schibli A. High-performance thin-layer chromatography for the analysis of medicinal plants. Thieme Verlag; 2007.
9. Azmir J, Zaidul IS, Rahman MM, Sharif KM, Mohamed A, Sahena F, *et al*. Techniques for extraction of bioactive compounds from plant materials: A review. Journal of food engineering. 2013 Aug 1;117(4):426-436.
10. Neto GL, Lopes NP. Medicinal plants: factors of influence on the content of secondary metabolites. Química Nova. 2007;30:374-381.