Ind. J. Pharm. Edu. Res., 2026; 60(2):869-875.
https://www.ijper.org

Original Article

Validated HPTLC Method for Accurate Measurement of Quercetin in *Ficus benghalensis* Linn. Bark Extract

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ABSTRACT

Background: Quercetin is a key bioactive compound found in many plants and trees, popularly known for its ability to act as a potent antioxidant, reduce inflammation, decreases the risk of cancer, heart disease, and neurological disorders, and even help with allergies and infections. The bark of *Ficus benghalensis* Linn. Commonly known as banyan tree is believed to be rich in quercetin, making its accurate measurement essential for understanding its health benefits. **Purpose:** This study focuses on determining the amount of quercetin in bark extract of *Ficus benghalensis* Linn. using a reliable High-Performance Thin Layer Chromatography (HPTLC) method. **Materials and Methods:** Chromatographic separation was performed on aluminum plates pre-coated with silica gel 60 F_{254} using a mobile phase of Toluene, Ethyl Acetate, Acetone, and Formic Acid in the ratio 5:2.5:7.5:0.5. The plates were scanned at 366 nm and 245 nm, providing a clear separation of Quercetin at a specific Retention Factor (R_f) A calibration curve was created, showing strong linearity with y = 8.6567x − 1357. The correlation coefficient (R^2) was found to be 0.9985. The method also demonstrated a Detection Limit (LOD) of 75.60 ng/spot and a Quantification Limit (LOQ) of 272.5 ng/spot. **Results:** The quercetin content in the bark was found to range from 1.99% to 24.48% w/w. The method proved to be sensitive as well as accurate and precise. Thus, it suitable for regular quality control. The developed method for high performance thin layer chromatography HPTLC was validated according to ICH guideline. **Conclusion:** This study successfully quantified quercetin in *Ficus benghalensis* Linn. bark using a validated HPTLC method. The approach is reliable for routine testing and highlights the potential of this medicinal plant for pharmaceutical and health applications.

Keywords: High-Performance Thin Layer Chromatography (HPTLC), Quercetin, Flavonoids, *Ficus benghalensis* Linn., Accuracy, LOD, LOQ.

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Received: 14-07-2025;
Revised: 05-09-2025;
Accepted: 28-11-2025.

INTRODUCTION

Quercetin is a natural compound found mainly in plants, known for its impressive health benefits and gentle effects on the body. Due to Affordable and plant-based, it has attracted lots of attention over the years for its ability to support both physical and mental health while also reducing the risk of viral infections. The name "Quercetin" comes from the Latin word Quercetum, meaning "oak forest." This is because Quercetin was first isolated from the bark of oak trees. The name highlights its connection to oak trees, where it was originally found. Today, Quercetin is known as a powerful antioxidant and anti-inflammatory compound found in various plants and foods.[1] $C_{15}H_{10}O_7$ is its molecular formula; and its chemical structure formula are illustrated in (Figure 1).

DOI: 10.5530/ijper.20261891

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Quercetin has abundant medicinal properties, that includes anti-inflammatory, anti-allergy, anticancereous properties, anti-tumor properties, cardiovascular protection effect, anti-viral effect, immune-modulatory property, anti-hypertensive effect, gastroprotective effects and anti-diabetic effect. Quercetin is a crystalline insoluble solid substance having yellow in appereance.[2]

Ficus benghalensis L., also known as the Indian banyan tree, Vata vriksch, Indian fig tree, & Vada vriksch. It is a large, evergreen tree native to India and other tropical regions. It comes under the Moraceae family (mulberry group).[3,4] It is evergreen tree with many pharmacological properties. Banyan Tree was studied for its medicinal properties such as antistress, antihelmintic, anti-inflammatory, antioxidant, antimicrobial activity, antidiarrheal activity, antitumor activity, anti-allergic, antistress.[5]

Ficus benghalensis Linn. holds a prominent place in traditional systems of medicine such as Ayurveda and Unani. Its bark has been traditionally used for managing a range of ailments including diabetes, dysentery, wounds, and inflammatory conditions. The therapeutic efficacy of the plant is attributed to its rich phytoconstituent profile, especially its flavonoid

content, which plays a critical role in exerting antioxidant, anti-inflammatory, and metabolic-modulating effects.[6] Among the flavonoids, quercetin is a major bioactive compound that has drawn considerable attention due to its well-documented biological activities, including inhibition of lipid peroxidation, reduction of pro-inflammatory cytokines, and improvement in insulin sensitivity.[7] The presence of quercetin and other flavonoids supports the traditional claims of the bark's therapeutic use and highlights the importance of its quantification for standardization and validation in modern phytopharmacology.

High-Performance Thin Layer Chromatography (HPTLC) is an advanced version of traditional Thin Layer Chromatography (TLC) that has gained popularity for quickly analyzing complex mixtures. It is known for its high efficiency, reliability, and cost-effectiveness. In HPTLC, compounds are separated as they move through a stationary phase with the help of a mobile phase, much like in TLC. However, HPTLC provides better separation and lower detection limits because it uses high-performance adsorbent layers, such as silica gel with finer, more uniform particles (2.5 µm) compared to the coarser particles used in traditional TLC (12 µm).[8]

The present design focuses on the HPTLC fingerprinting of methanolic extracts of *Ficus benghalensis* Linn. bark, aiming to quantify quercetin and evaluate its stability. Through this research, we hope to contribute valuable insights into the therapeutic potential of *Ficus benghalensis* Linn., furthering its application in traditional medicine and enhancing our understanding of its bioactive components.

MATERIALS AND METHODS

Collection and Extraction of Bark Material

The *Ficus benghalensis Linn.* stem bark, authenticated by the Botanical Survey of India (Reference: BSI/PLANT CHEM/0003-2022), was confirmed through HPLC fingerprinting studies. The coarse powder of the bark underwent a defatting process with petroleum ether, followed by cold maceration in methanol. About 200 g of the powdered material was macerated in a 2-Liter flask for one week at room temperature with occasional shaking. The extract was filtered using syringe filter, concentrated under reduced pressure by means of a rotatory evaporator and air-dried before being stored for further analysis. [9]

Instrumentation

Chromatographic analysis was executed using the HPTLC system (Camag, Switzerland), which included 100 µL capacity syringe (Hamilton), dual wavelength UV lamps containing UV Cabinet, a twin-trough developing chamber of dimension (10×10 cm), a TLC Scanner IV of Camag, Switzerland, and a semi-automatic spotting device, Linomat V.

Chemicals and reagents

All the chemicals used are of analytical grade. Solvent used are of HPLC Grade.

Preparation of solutions

Preparation of standard stock solution

A standard stock solution of Quercetin of strength 1000 µg/mL was prepared (5 mg of Quercetin was dissolved in a 5 mL HPLC-grade methanol). From this stock solution, 1 mL was taken and diluted to 10 mL with HPLC-grade methanol to make a solution of strength 100 µg/mL. Finally, 1 mL of this solution was diluted again in a 10 mL volumetric flask with HPLC-grade methanol to prepare a working standard solution with a concentration of 10 µg/mL.

Preparation of Sample

A sample of 30 mg was dissolved in methanol. It was vortexed and ultra-centrifuged at 2500 rpm for 20 min. The clear supernatant obtained was stored for analysis.

Optimized Chromatographic conditions

Aluminum Plates (HPTLC Silica gel 60 F_{254} (merck)) was prior washed with methanol and heated in Hot Air Oven to 60ºC for 15 min to activate it for the experiment. Using a device Linomat V spotter, small amounts of the sample were placed on the plate in neat, narrow bands. Each band was 6 mm wide, spaced 15-20 mm apart, and positioned 8 mm from the bottom edge. The sample was applied at a steady rate of 150 nL per second, with a small starting dose of 0.20 µL. The plate was placed in a chamber which having a specific mix of solvents (toluene, ethyl acetate, acetone, and formic acid in a 5:2.5:7.5:0.5 ratio). The chamber was left to saturate with the solvent vapor for 20 min before adding the plate. The solvents moved up the plate, carrying the compounds with them over a distance of 75 mm. After chamber development, the plate was kept for drying for 10 min to remove any remaining solvents. The dried plate was scanned using a TLC scanner connected to specialized software. The scanner used specific settings to measure the compounds, focusing on wavelengths of 254 nm and 366 nm to identify and quantify the substances present.

Method Validation

In accordance to ICH (the International Council for Harmonization) guideline, the above developed method was validated[10] for, linearity, specificity, accuracy, precision, LOQ and LOD. The method demonstrated a linear range of 400-900 ng per band. Accuracy was assessed using the standard addition method, with percentage recovery calculated at three concentration levels: 75%, 95%, and 110%, each tested in triplicate. Precision was evaluated by analyzing three replicate samples of the same concentration, calculating the % Relative Standard Deviation

(% RSD) from the peak area. Similarly, intra-day and inter-day precision were determined by recording chromatograms at different times within the same day and across different days. Specificity was tested by comparing chromatograms of standard Quercetin with those of the *Ficus benghalensis* Linn extract. The final chromatograms of both the standard and the extract were analyzed and compared (Figure 3) to confirm the method's ability to distinguish Quercetin from other components. Robustness was calculated by deliberate changes in detection wavelength and saturation time. From the calibration curve at a lower concentration, the Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated.[11-13]

RESULTS

Optimization of the Chromatography

By adjusting key parameters such as wavelength, mobile phase ratios, and solvent chamber saturation time, extensive initial testing was conducted to achieve the best separation and resolution for the selected analytes. Using traditional TLC methods, after several trials the optimal mobile phase was finalized. The mobile phase chosen Toluene: Ethyl Acetate: Acetone: Formic Acid in the ratio 5:2.5:7.5:0.5. The overlay spectra of Quercetin are shown in Figure 2. Silica gel 60 F_{254} was used as the stationary phase, with a chamber saturation time of 20 min. Using these optimized parameters, of a well-resolved Quercetin peak with an R_f value of 0.67 was achieved (Figure 3).

Method Validation

Specificity

As each peak (as shown in Figure 4) is separated from each other without any kind of interference and well resolved, so it was found to be specific. When an analytical method can reliably measure the target analyte in the absence of any confounding variables, we say that it is specific.

Linearity and range

Linearity in an analytical method means how well the results correspond to the analyte's concentration. To check the linearity of the developed method, standard responses were measured three times for accuracy. A calibration curve was created to determine the range and linearity of Quercetin (Figure 5). Using the regression equation from the calibration curve, the method was confirmed to be excellent linear. Quercetin showed a clear and consistent linear relationship between 300-900 ng/spot. The R^2 value (correlation coefficient) was obtained 0.9985 and linear regression equation was found to be: $y = 8.6567x-1357$.

Accuracy

Accuracy refers to how close the test results are to the true value. To determine accuracy in this study, the standard addition method was used. This involves adding known amounts of standard Quercetin to pre-analyzed samples at different concentrations (300, 400, and 500 ng/mL). The amount of Quercetin in the sample was then calculated by comparing the peak areas from the chromatogram to the regression equation of the calibration curve. The recovery rate, which shows how well the method detected the added Quercetin, ranged from 97.04% to 98.80%, indicating that the method is highly accurate.

Precision

Precision refers to how consistently a method gives the same results when repeated. In this study, the same sample was applied three times on the same plate, without changing its position, using an automatic spotter. The same syringe was used for each application, and the spot was scanned multiple times to check how consistent the HPTLC instrument is.

To measure precision, the experiment was done in two ways: inter-day precision (performing the test on three different days) and intra-day precision (repeating the test three times on the same day). The solutions of Quercetin, at concentrations of 300, 400, and 500 ng/spot, were used for this. The variation in results was calculated as the percentage Relative Standard Deviation

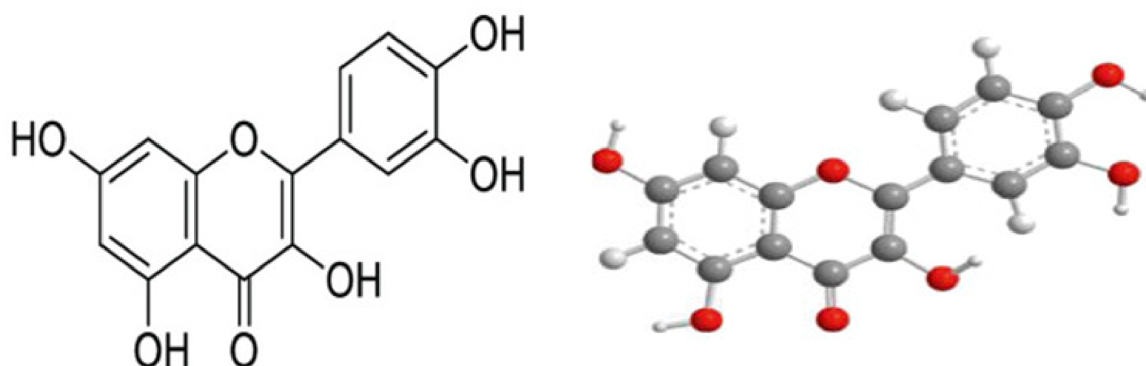

Figure 1: Structure of Quercetin.

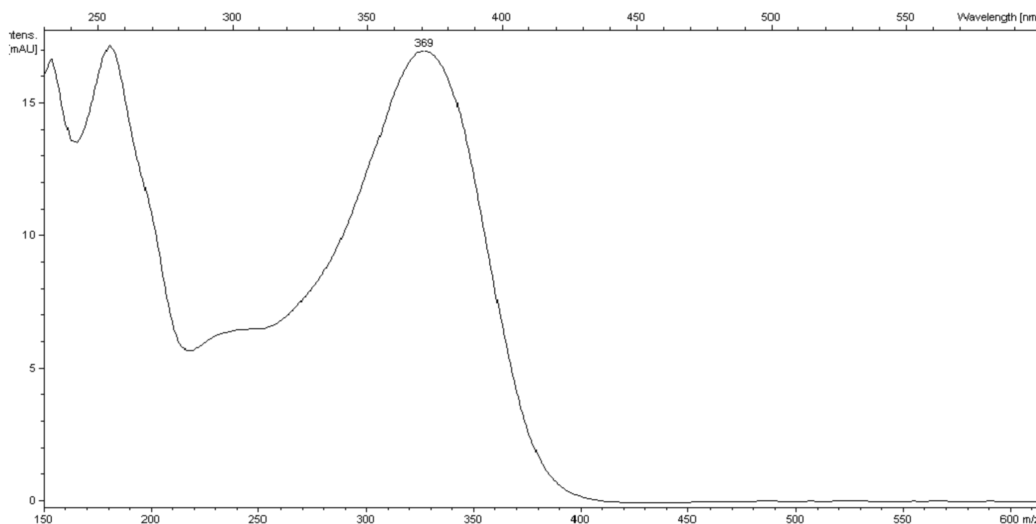

Figure 2: UV overlying spectra of Quercetin.

(%RSD), which shows how much the measurements differ from each other (Table 1).

Limit of Quantification and Limit of Detection

The value of LOD and LOQ was calculated from the equation (LOQ = 10× σ/S, LOD = 3.3× σ/S) Based on these calculations, the LOD was determined to be 75.60 ng/spot, meaning the method can reliably detect the presence of Quercetin at this low concentration. The LOQ was found to be 272.5 ng/spot (summarized in Table 2), which indicates the lowest concentration of Quercetin that can be accurately measured and quantified using this method. These values demonstrate that the method is highly sensitive and capable of detecting and quantifying even small amounts of Quercetin in the sample.

Quercetin is a notable flavonoid recognized for its antioxidant properties and a variety of potential health benefits, including anti-obesity effects. It is commonly found in numerous plant sources, including *Ficus benghalensis*. The congruence in R_f values observed in the HPTLC fingerprinting provides compelling evidence of quercetin's presence in the bark extract, underscoring its significance as a valuable source of this beneficial compound.

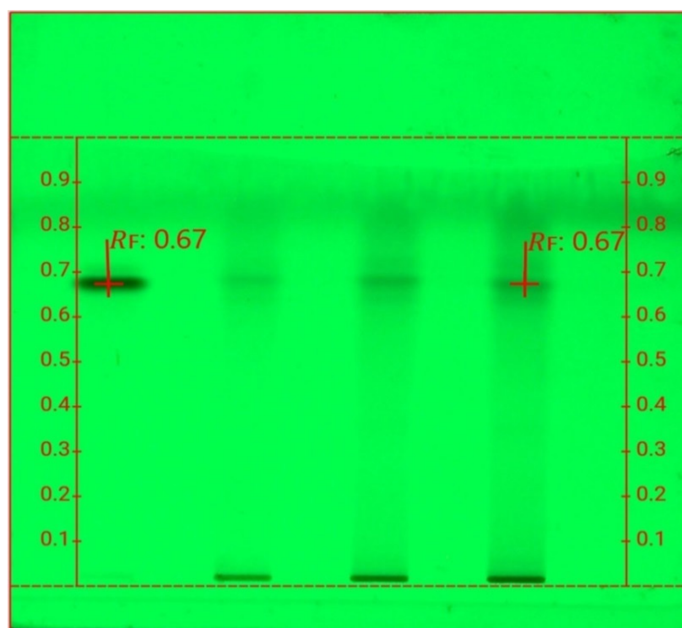

Figure 3: HPTLC profiling of *Ficus benghalensis* Linn.

DISCUSSION

This method for measuring the analyte works really well, as it shows strong accuracy and reliability. The method is very good at detecting only the target substance, without interference from other materials. Linearity and Range was found to be (300-900 ng/spot). It can accurately measure concentrations of the analyte in this range, meaning it's useful for a variety of sample types and concentrations. The relationship between the analyte concentration and the measured response is clear and predictable, allowing for accurate quantification. As the limit of Detection was found to be 75.60 ng/spot. It can detect very small amounts of the analyte, which is crucial for sensitive measurements. As the Limit of Quantification was found to be (LOQ = 272.5 ng/spot): It can reliably measure and quantify the analyte starting from this amount onward, ensuring accuracy. Robustness (% RSD = 0.67). The method is very stable, meaning small changes in conditions (like temperature or equipment) don't affect the results much. Overall, this method is precise, sensitive, and reliable, making it ideal for routine testing where accurate measurements are needed across a range of concentrations.

The developed method shows strong potential for routine analysis due to its high accuracy, sensitivity, and robustness. One of the key strengths is its ability to detect and quantify the analyte across a wide concentration range (300-900 ng/spot). This range is suitable for different types of samples and ensures

that the method can be applied to both low and high analyte concentrations without compromising performance.

The linearity of the method across this range is consistent and reliable. The clear relationship between the analyte concentration and the measured response supports its suitability for quantitative analysis. According to ICH guidelines, linearity is a crucial part of method validation, and this method meets those expectations well.[14]

Sensitivity is another strong point. With a Limit of Detection (LOD) of 75.60 ng/spot and a Limit of Quantification (LOQ) of 272.5 ng/spot, the method can detect even very small amounts of the analyte. This is particularly important in situations where trace-level detection is necessary, such as pharmaceutical quality control or environmental analysis. The low LOD and LOQ values confirm that the method is capable of handling sensitive measurements effectively.

Specificity was also well demonstrated, as the method selectively measured the target analyte without interference from other components. This is especially important when working with complex mixtures, where overlapping signals or background noise can be a challenge. The ability to isolate and measure just the analyte indicates strong selectivity and makes the method more reliable in real-world applications.

The robustness of the method was confirmed by the low %RSD (0.67), even when small, deliberate changes were made to experimental conditions. This shows that the method is stable and consistent, even under slightly varying conditions. In practical terms, this means that different analysts or slight changes in equipment or temperature won't significantly affect the results. Robustness like this is essential for methods intended for routine use in labs.

Although detailed data on accuracy and precision weren't included here, the method's overall performance strongly suggests that it meets the standard expectations for both. Low variability, good sensitivity, and consistent linearity all point toward reliable and accurate results.

In summary, this method is precise, sensitive, and stable. It meets key validation parameters and shows great potential for routine analysis in various fields. Whether used in a pharmaceutical lab or for research purposes, the method is dependable and effective.

Table 1: Precision of quercetin (*n*=3).

	Concentration (ng/band)	Area	%RSD
Interday precision	300	1299	0.38
	400	1976	
	500	2850	
Intraday precision	300	1298	0.39
	400	1980	
	500	2910	

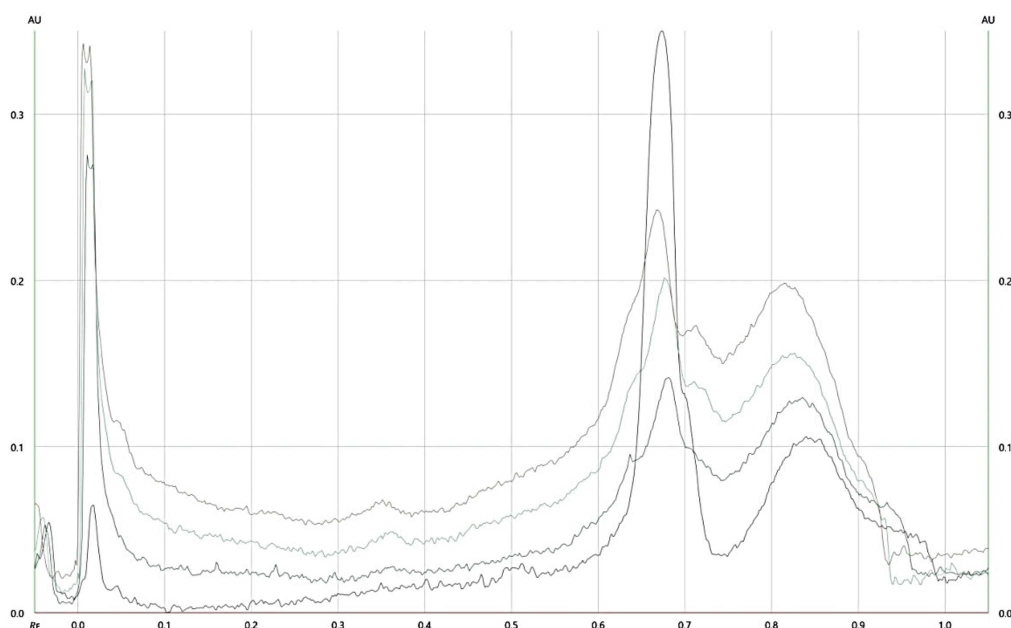

Figure 4: Overlay Chromatogram of standard Quercetin and Extract of *Ficus benghalensis Linn.*

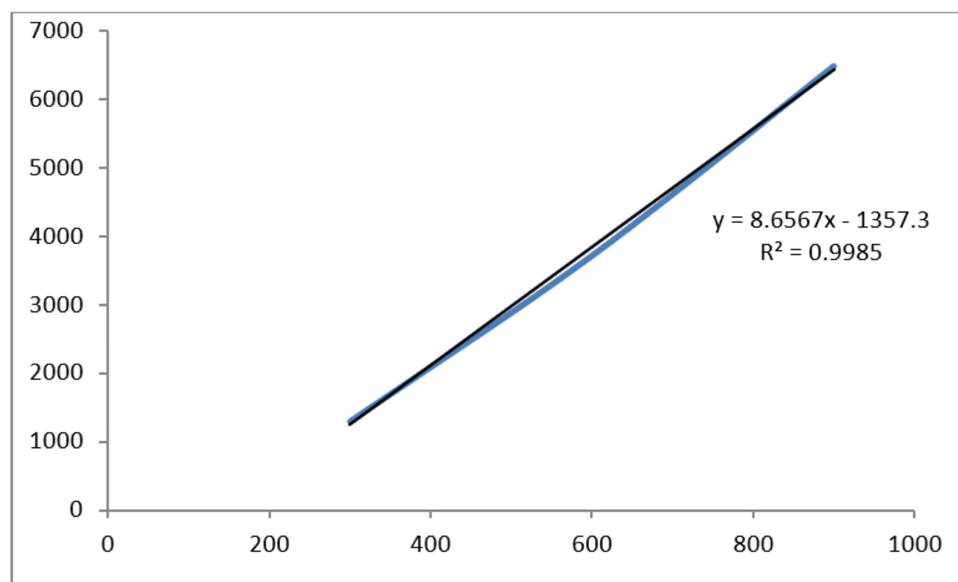

Figure 5: Calibration Curve of Quercetin standard.

Table 2: Summarized method validation parameter.

Validation parameters	Result
Specificity	Specific
Linearity and range	300-900 ng/spot
Regression equation	y = 8.6567x-1357.3,
Coefficient of correlation	0.9985
LOD	75.60 ng/spot
LOQ	272.5 ng/spot
Robustness (% RSD)	0.67

CONCLUSION

This study successfully quantified quercetin in the bark of *Ficus benghalensis* Linn. using a validated HPTLC method. The method demonstrated excellent sensitivity, accuracy, and precision, with a wide range of quercetin content found in the bark extract. The results support the potential of *Ficus benghalensis* as a valuable source of quercetin, a compound with numerous health benefits. This reliable analytical approach can be used for routine quality control, ensuring the consistency and efficacy of products derived from this medicinal plant. The findings contribute to the growing understanding of *Ficus benghalensis* in pharmaceutical and health applications.

The current study confirmed the presence of quercetin in the methanolic extract of *Ficus benghalensis* using HPTLC analysis. Quercetin, a polyphenolic bioflavonoid with the chemical name 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one, is commonly found in foods like grapes, onions, broccoli, berries, cherries, and citrus fruits. This plant compound is known for its wide range of health benefits, including strong antioxidant effects, anti-allergic, anti-inflammatory, antihypertensive, and anti-diabetic properties. It also has the ability to scavenge free radicals, act as a vasodilator, and provide chemo-preventive effects

Overall, the findings from this investigation lay a solid foundation for future research aimed at elucidating the efficacy and mechanisms of action of the identified bioactive compounds. This research not only validates the traditional uses of *Ficus benghalensis* but also encourages the exploration of its applications in the development of natural health products and therapeutic agents.

ACKNOWLEDGEMENT

We express our sincere gratitude to the CIF (Central Instrumentation Facility), Birla Institute of Technology, Mesra, Ranchi for providing the instrumental support required for this research. The access to advanced equipment and technical assistance was crucial in ensuring the successful completion of our work.

ABBREVIATIONS

CIF: Central Instrumentation Facility; **HPTLC:** High Performance thin layer chromatography; **LOD:** Limit of detection; **LOQ:** Limit of quantification; **Ng:** Nanogram.

DECLARATION

The author has no relevant financial or non-financial interest to disclose.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

DATA AVAILABILITY DECLARATION

All data supporting the findings of this study are available within the paper and its Supplementary Information.

AUTHOR CONTRIBUTION

Both The authors equally contributed to the manuscript. S.K., was responsible for conceptualization, data curation, validation of resources, and manuscript writing. M.P.C provided guidance, reviewed, and edited it. Both authors read and approved the finalized the manuscript.

SUMMARY

The HPTLC method developed for quantifying Quercetin in *Ficus benghalensis* Linn. bark is highly reliable, showing strong specificity, accuracy, precision, and sensitivity. The method demonstrates a clear linear range, low detection and quantification limits, and excellent robustness, making it ideal for routine quality control. It also holds great potential for use in pharmaceutical and health-related research, offering a reliable way to assess Quercetin content in the bark.

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Cite this article: Kumari S, Chopra MP. Validated HPTLC Method for Accurate Measurement of Quercetin in *Ficus benghalensis* Linn. Bark Extract. Indian J of Pharmaceutical Education and Research. 2026;60(2):869-75.