RESEARCH PAPER

Exploring The Potential of HPTLC as a Method for Measuring Quercetin and Confirming Its Efficacy

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Received: 15th Sep, 2025; Revised: 24th Oct 2025; Accepted: 10th Nov, 2025; Available Online: 1st December, 2025

ABSTRACT
Cinnamomum tamala has historically been utilized for the treatment of diabetes, metabolic diseases, and renal issues. This paper introduces a validated HPTLC technique for the precise measurement of quercetin in a 70:30 methanolic leaf extract. The optimal separation was achieved using a solvent mixture of toluene, ethyl acetate, and diethyl amine at a ratio of 8.0:2.5:0.5 (v/v/v) on silica gel plates, followed by a densitometric scan at 365 nm. The approach yielded a distinct quercetin peak at Rf 0.65, with limits of detection (LOD) and quantitation (LOQ) of 9.32 ng/band and 83.92 ng/spot, respectively. The extract contained 7.6% w/w quercetin. Validation in accordance with ICH standards demonstrated that the results were accurate and exact. This enhanced HPTLC technique is a rapid, sensitive, and reproducible approach for standardizing C. tamala extracts, ensuring dependable quality control.

Keywords: *Cinnamomum tamala, HPTLC, Quercetin, method validation, Standardization.*
How to cite this article: Singh R, Madan S, Walia R, Chandra A, Exploring the Potential of HPTLC as a Method for Measuring Quercetin and Confirming Its Efficacy. Int J Drug Deliv Technol. 2026;16(1): 100-104. DOI: 10.25258/ijddt.16.1.10
Source of support: Nil.
Conflict of interest: None

INTRODUCTION

Cinnamomum tamala, sometimes referred to as Indian bay leaf or tej patta, is a tree that is indigenous to India and a member of the Lauraceae family. Around 55 genera and more than 2000 species, predominantly from tropical climates, are members of this family. In addition to its usual use as a spice, tej patta has been shown in a few studies to possess a variety of pharmacological qualities, such as the capability to scavenge free radicals and have anti-diabetic, anti-oxidant effect, among others. Tej Patta's extra properties shield the central nervous system. The plant's extract protects against kidney toxicity and reduces oxidative damage. Numerous conditions, including anal and rectal disorders, flatulence, cancer, coryza (inflammatory mucous membranes), anorexia, abnormalities of the liver and spleen, and cardiovascular issues, can be treated with it[1,3]. It consists of various chemical constituents such as Quercetin ($C_{15}H_{10}O_7$, molecular weight 302.23 g/mol), (Fig.1) Kaempferol, Myricetin etc.

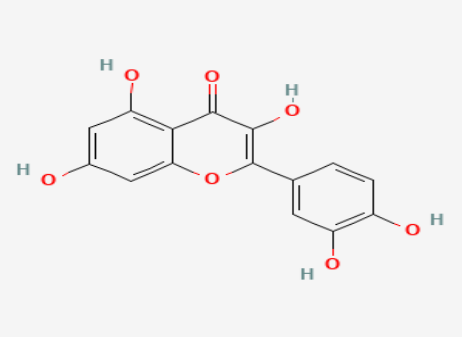

Figure 1 The chemical structure of Quercetin

Medicinal plants are very important in medicine research and are becoming more popular in the US and throughout the world[4]. To maintain the optimal quality of herbal medicines, it is essential to verify the phytoconstituents to assure the reliability and accuracy of pharmacological and quantifiable studies into their bioactivities and potential adverse effects[5,6]. The WHO praised the use of both qualitative and quantitative methods to standardize herbal medicine formulations for quality assurance. The phytoconstituents (biomarkers or chemical markers) in the plant must be measured in conjunction with the chemical and activity profile of the products using analytical methods [7,8]. High-performance thin-layer chromatography (HPTLC) is the most advanced variant of planar chromatography,

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providing a cost-effective and efficient approach for sample analysis. HPTLC is a methodical process in which sample application, chromatogram generation, and detection are clearly delineated from one another. It is often utilized to separate chemicals, standardize a procedure, and validate its accuracy[9,10,11,12]. It appears that HPTLC assessment patterns are better for fingerprinting plant extracts[13].

The objective of this research is to devise and enhance a straightforward and precise HPTLC method for distinguishing the various components of Cinnamomum tamala. Based on information from early preparation tests that helped us choose the developing system, we were able to figure out the most important factors that affect how well the recommended HPTLC method separates things in this work.

2. MATERIAL

2.1 Instrumentation

The HPTLC experiment employed alumina HPTLC plates of 20 cm by 10 cm, with 200 mm layers of pre-coated silica gel. A sample applicator fitted with a 100 µL syringe and bands measuring 5 mm in width and 10 mm in spacing was employed to deliver the quercetin samples. A mobile phase consisting of toluene, ethyl acetate, and diethyl amine in a volumetric ratio of 8:2.5:0.5 was used in a CAMAG glass twin-trough chamber, which had been saturated with mobile phase vapor for five minutes at 110°C. This was performed to identify compact bands as the chromatogram progressed to a distance of 158 mm. We utilized a drier to dehydrate the plate after development and subsequently scanned it at 365 nm using a CAMAG TLC Scanner 4 connected to visionCATS 3.0 software.

2.2 Chemicals and reagents

Merck in India sold the typical Quercetin. The utilized chemicals and solvents were exclusively of chromatography and analytical reagent grades. Merck in India sold us HPTLC plates composed of precoated silica gel.

2.3 Plant material

Taxonomists from the Raw Materials Herbarium and Museum in Delhi (RHMD), a department of the CSIR-National Institute of Science Communication and Policy Research, collected and validated fresh leaves of *Cinnamomum tamala*. The authentication no of the certificate is NIScPR/RHMD/Consult/2023/4415-16-1. We cleaned the samples and rinsed them with tap water. Subsequently, we dehydrated the leaves in sunlight and ground them into a coarse powder using a clean, dry grinder (Maharaja Mixer Grinder). The coarse powdered crude pharmaceutical compound was enclosed in a sealed container for further analysis.

2.4 Extraction of plant material

The extraction of 50 g of dried leaf powder using 300 mL of 70% methanol by reflux distillation necessitated 48 hours at 70°C. Whatman No. 1 filter paper was utilized to filter the extract. This was done three times, and all the extracts were put together. It was determined that the extract had a yield of 7.6% w/w. The analysis employed an extract at a concentration of 10 mg/mL.

2.5 Preparation of standard stock solution

A stock solution containing 1 mg/mL of quercetin was produced in 10 mL of HPLC-grade methanol using a volumetric flask. Before HPTLC analysis, the standard stock solution was filtered using 0.45 mm syringe filters (12).

2.6 Chromatographic parameters

The investigation was performed at a temperature of 25 ± 2°C and a humidity of 60 ± 4%. The slit dimensions were 6 × 0.45 mm, and the scanning velocity was 100 mm/s. We administered 5µL of C. tamala leaf extract and 2, 4, 6, 8, and 10µL of quercetin onto a TLC alumina plate pre-coated with silica gel. The plate was prepared using 20 mL of a mobile phase composed of toluene, ethyl acetate, and diethyl amine in a volumetric ratio of 8:2.5:0.5.

2.7 Calibration curve of Quercetin

A stock solution of standard quercetin (100 µg/mL) was formulated in methanol. A semi-automatic sample applicator was utilized to dispense different quantities of the stock solution (2, 4, 6, 8, and 10 µL) onto the HPTLC plate. This produced concentrations of 200, 400, 600, 800, and 1000 ng/spot of quercetin, respectively. Figure 2 illustrates the calibration curve for standard quercetin.

Table 1 Linearity study by HPTLC

S. No.	Conc µg/ml	Area-I	Area-II	Mean
1	2	179.8	182.4	181.1
2	4	332.5	334.3	333.4
3	6	506.8	508.7	507.75
4	8	689.9	693.8	691.85
5	10	883.2	889.4	886.3

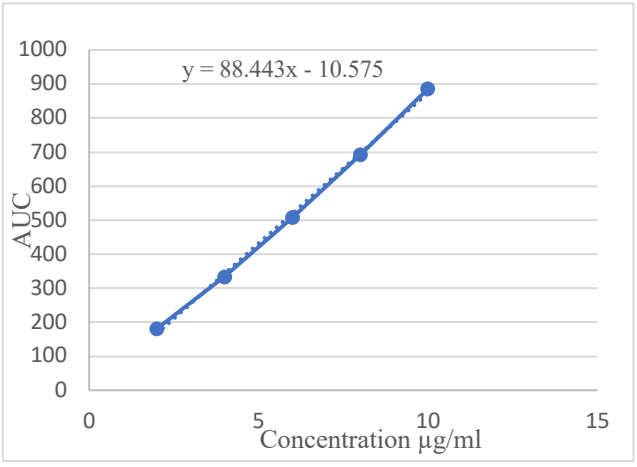

Figure 2 A calibration plot illustrating the peak area obtained through HPTLC at various concentration levels of standard quercetin

2.8 Method Validation

2.8.1 Precision

The ICH criteria were used to check the analytical methods' accuracy, precision, and ability to be repeated. We utilized six copies (n = 6) of the same amount of quercetin to find out how precise the procedure is. We utilized freshly prepared quercetin solutions at a consistent concentration (200–1000 ng/band) on the same day throughout six distinct intervals (n = 6) to evaluate intra-day accuracy. We assessed the precision of the data on a daily basis by applying the same concentration (200–1000 ng/band) of the quercetin solution six times over three separate days.

2.8.2 Specificity

The maximum purity level of the standard (quercetin) in the extract remained consistent at several stages, including the onset, peak, and conclusion. We confirmed the isolated band standard (quercetin) with the extract by examining the RF values in their scanning densitometric chromatograms.

2.8.3 Repeatability

The method's reproducibility was confirmed by analyzing 400 ng of quercetin on TLC plates (n = 6).

2.8.4 Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) and the limit of quantification (LOQ) were evaluated using different concentrations of a quercetin standard solution, with methanol as the blank, and the signal-to-noise (S/N) ratio was computed. The signal-to-noise ratios for the limit of detection (LOD) and the limit of quantification (LOQ) were 3:1 and 10:1, respectively.

2.8.5 Robustness

To identify the impact of the outcome, minor adjustments were made to the mobile phase's composition, volume, and saturation time. The investigation was completed within a robustness estimation range of ± 10%.

2.8.6 Recovery studies

The method's accuracy was confirmed by recovery trials performed using the extract at three different levels utilizing standard addition procedures.

2.8.7 System suitability

To verify the experiment's resolution and reproducibility, system suitability tests were conducted. Densitograms were obtained by applying a freshly made standard solution of quercetin (n = 6) to the plate under comparable chromatographic conditions, scanning, and obtaining a concentration of 400 ng/band.

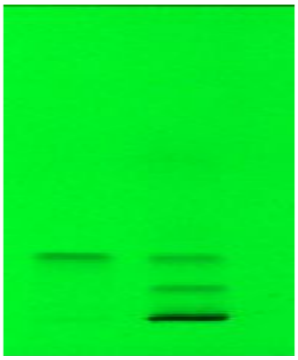

Figure 3 Chromatograms of (a) standard quercetin and (b) methanolic extract of Cinnamomum tamala

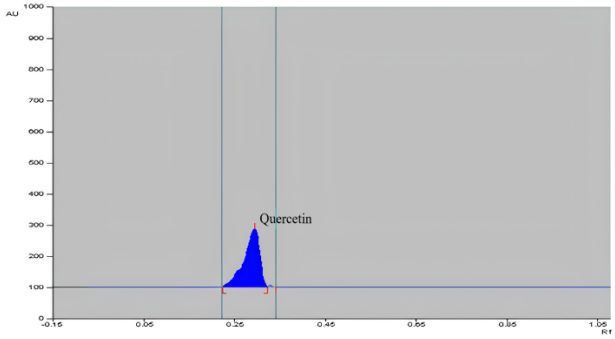

Figure 4 Chromatogram of Quercetin (Standard)

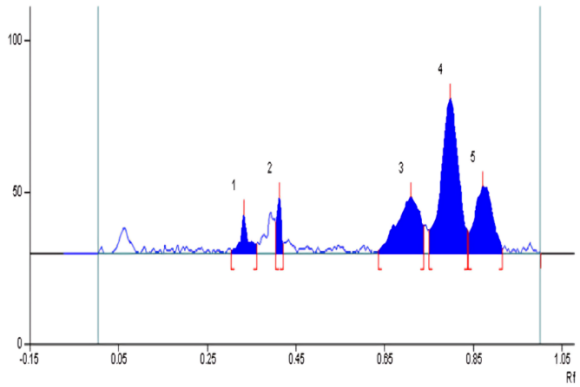

Figure 5 HPTLC chromatogram of methanolic extract of Cinnamomum tamala at 365nm

Table 2 Validation parameter outcomes for the quantification of Quercetin in the methanolic extract of Cinnamomum tamala leaves

Parameters	Quercetin
Wavelength (nm)	365 nm
Retardation factor	0.65
Correlation coefficient (*r*2)	0.9997
Limit of detection (ng/spot)	9.32

Limit of quantification (ng/spot)	83.92
Specificity Specific	Specificity Specific
Robustness Robust	Robustness Robust

Table 3 Intra-day and inter-day precision of Quercetin

Amount (ng/spot)	Inter-day precision		Intra-day precision	
	Mean peak area ± SD	%RSD	Mean peak area ± SD	%RSD
2000	2476.08 ± 47.61	1.923175	2461.35 ± 51.07	2.07
3000	3474.35 ± 17.14	0.49335	3459.60 ± 26.23	0.75
4000	4876.58 ± 70.17	1.438938	4887.93 ± 52.84	1.08

RSD: Regressed Standard Deviation.

Table 4 Accuracy of Quercetin (mean ± SD) using the HPTLC technique (n = 6).

% Standard injected into the sample	Theoretical content (ng)	Amount of drug recovered ng ± SD	% of drug recovered	% RSD
0	2500	2539.35 ± 19.24	101.57	0.75
50	3750	3746.03 ± 24.18	99.89	0.64
100	5000	5106.14 ± 98.66	102.12	1.93
150	6250	6172.22 ± 65.99	98.75	1.06

3. RESULTS AND DISCUSSION

High-Performance Thin Layer Chromatography (HPTLC) has several benefits, including dependability, cost-efficiency, adaptability, reduced solvent consumption, and suitability for herbal analysis, making it widely employed in the quality monitoring of herbal medicines[15,16]. This work created a validated HPTLC technique for the identification and quantification of quercetin in C. tamala leaf extract. Previous studies have used HPTLC to measure quercetin in various plant extracts and formulations, generally using mobile phases of toluene, ethyl acetate, and diethylamine in different proportions to achieve excellent separation[17]. The composition of the mobile phase is crucial for ensuring the effective separation of phytoconstituents. We experimented with several solvent combinations and achieved effective separation using toluene: ethyl acetate: diethylamine (8:2.5:0.5 v/v/v). The twin-trough chamber was saturated with mobile phase vapours for 20 minutes before the examination. Quercetin was identified at an Rf value of 0.65 as a prominent, well-defined peak (Fig. 3–5).

The polynomial regression employed to generate the calibration curve yielded the equation Y = 88.443x – 10.575, with a regression coefficient (r^2) of 0.999789 ± 0.20%, indicating a very linear relationship.

• Specificity: The reference standard was tested with C. tamala extract, revealing the presence of quercetin at 365 nm. The spectra at the peak apex, commencement, and conclusion exhibited a robust association ($r^2 > 0.9997$).

• LOD and LOQ: The approach exhibited high sensitivity, with a limit of detection (LOD) of 9.32 ng/band and a limit of quantification (LOQ) of 89.32 ng/band.

• Precision: Analyses of inter-day and intra-day variance revealed %RSD values below 1% (Table 3), indicating the reproducibility of the results.

The average recovery values (Table 2, 4) demonstrated accuracy, with quercetin recovery being consistent across all hydroalcoholic extracts.

• Robustness: Deliberate modifications in chromatographic settings resulted in %RSD <1%, indicating the method's reliability.

The leaves of C. tamala contained 7.6% w/w quercetin. Table 2 presents a comprehensive enumeration of all validation parameters. The proposed HPTLC technique is user-friendly, precise, accurate, specific, and repeatable for quantifying quercetin in C. tamala leaves. HPTLC possesses several benefits compared to other published techniques, such as HPLC and UV-spectrophotometry. These benefits encompass reduced solvent usage, lower operational costs, and the capability to examine many samples simultaneously. However, HPLC often exhibits superior sensitivity and selectivity. HPTLC serves as an effective option for the routine assessment of the quality of herbal medicines containing quercetin.

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REFERENCE

1. B. Aggarwal B, Prasad S, Reuter S, Kannappan R, R. Yadav V, Park B, et al. Identification of Novel Anti-inflammatory Agents from Ayurvedic Medicine for Prevention of Chronic Diseases: “Reverse Pharmacology” and “Bedside to Bench” Approach. Current Drug Targets. 2011 Oct 1;12(11):1595–653.
2. Siddhuraju P, Becker K. Antioxidant Properties of Various Solvent Extracts of Total Phenolic Constituents from Three Different Agroclimatic Origins of Drumstick Tree Leaves. Journal of Agricultural and Food Chemistry. 2003 Apr 1;51(8):2144–55.
3. Bhatia L, Sharma A, Kalra R, Sunita. Phytochemical And Antioxidant Activity Of Cinnamomum Tamala Leaf Extract. Journal of Advanced Zoology. 2023 Nov 8;44(S2):3603–11.

4. Mukherjee PK, Banerjee S, Katiyar CK, Sharma S, Chattopadhyay N. Traditional Medical System (TMS) for Sustainable Healthcare in India. In: Dikshit M, editor. Drug Discovery and Drug Development: The Indian Narrative [Internet]. Singapore: Springer; 2021 [cited 2025 Aug 11]. p. 1–36. Available from: https://doi.org/10.1007/978-981-15-8002-4_1
5. Mukherjee PK, Bahadur S, Chaudhary SK, Kar A, Mukherjee K. Chapter 1 - Quality Related Safety Issue-Evidence-Based Validation of Herbal Medicine Farm to Pharma. In: Mukherjee PK, editor. Evidence-Based Validation of Herbal Medicine [Internet]. Boston: Elsevier; 2015 [cited 2025 Aug 11]. p. 1–28. Available from: https://www.sciencedirect.com/science/article/pii/B9780128008744000015
6. Champati BB, Das PK, Sahoo C, Ray A, Jena S, Sahoo A, et al. Chemical fingerprinting and multicomponent quantitative analysis for quality control of Cinnamomum tamala collected from Western Himalaya by HPLC-DAD. Heliyon. 2024 May 15;10(9):e30361.
7. Sharma A, Katiyar CK, Banerjee S, Chanda J, Kar A, Biswas S, et al. RP-HPLC and HPTLC Methods for Analysis of Selected Herbs Used as Complexion Promoters in Ayurveda and Unani Systems of Medicine. Journal of AOAC International. 2020 June 1;103(3):692–8.
8. Carvalho D, Pinho C, Oliveira R, Moreira F, Oliveira AI. Chromatographic Methods Developed for the Quantification of Quercetin Extracted from Natural Sources: Systematic Review of Published Studies from 2018 to 2022. Molecules. 2023 Nov 22;28(23):7714.
9. Mukherjee PK. Chapter 4 - Qualitative Analysis for Evaluation of Herbal Drugs. In: Mukherjee PK, editor. Quality Control and Evaluation of Herbal Drugs [Internet]. Elsevier; 2019 [cited 2025 Aug 11]. p. 79–149. Available from: https://www.sciencedirect.com/science/article/pii/B9780128133743000041
10. Mansour FR, Abdallah IA, Bedair A, Hamed M. Analytical Methods for the Determination of Quercetin and Quercetin Glycosides in Pharmaceuticals and Biological Samples. Critical Reviews in Analytical Chemistry. 2025 Jan 2;55(1):187–212.
11. Zhao T, Jiang X, Yu L, Zhang W, Zhan H, Wang Q, et al. Comparison of nutritional status between migrant and nonmigrant school-age children in Kunming, Yunnan Province, China. Curr Top Nutraceutical Res. 2024;22(2):617–623. https://doi.org/10.37290/ctnr2641-452X.22:617-623
12. Singh S, Agrawal K, Lalpekkimi A, Marak DC, Singh D, Rana D, et al. Heavy metals as environmental carcinogens: Implications for lung cancer in humans. J Exp Biol Agric Sci. 2025;13(5):648–656. https://doi.org/10.18006/2025.13(5).648.656
13. Srinivas A, Nehra S. Development of HPTLC method for simultaneous determination of quercetin and kaempferol in leaf extract of Hibiscus mutabilis. Journal of Chromatography B. 2024 Oct 1;1246:124277.
14. Yelwantge V, Chauhan V. Phytochemical Characterization, In-Vitro Antioxidant Activity, and Molecular Docking of Quercetin, Rutin and Apigenin. Journal of Drug Delivery and Therapeutics. 2025 Apr 15;15:59–71
15. Das B, Saha P, Maity N. Quantitative Estimation of Quercetin of Some Selected Edible Plants of West Bengal by High-performance Thin-layer Chromatography Densitometry Method. Asian Journal of Pharmaceutical Research and Health Care AJPRHC. 2024 Mar 31;15:254–61.
16. Verma A, Patel P, Sharma A, Kurmi BD. Quercetin in ayurvedic formulations: High-performance thin-layer chromatography based rapid fingerprinting profiling. Phytomedicine Plus. 2023 Feb 1;3(1):100416.
17. Khan AD, Singh MK, Lavhale PM, Kaushik R. Phytochemical Screening and HPTLC Analysis of Bio-active Markers of Ethanol Extract of Indian Bay Leaves. Journal of Herbs Spices and Medicinal Plants. 2023 Apr 3;29(2):156–67.

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