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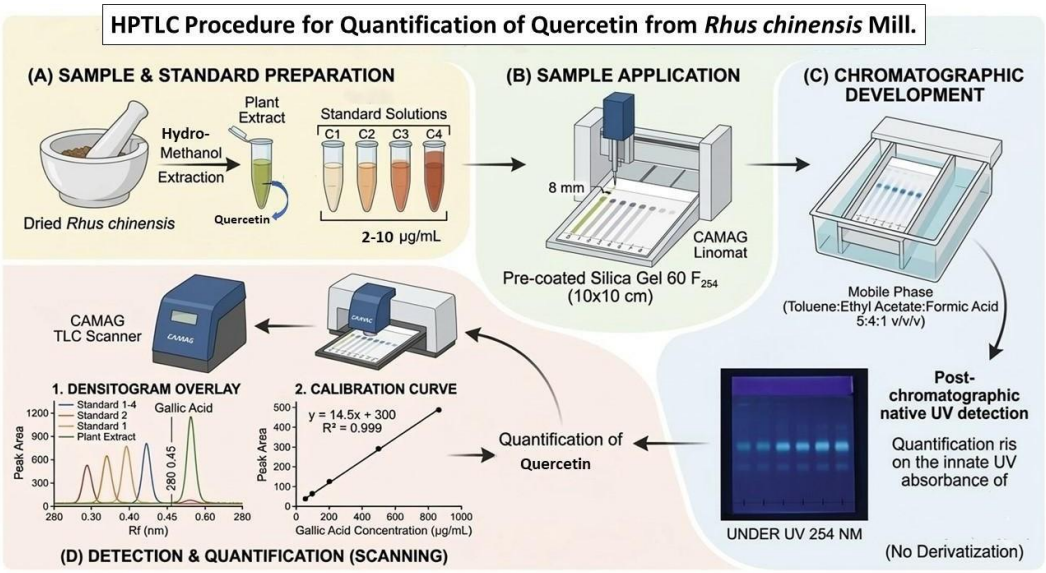
Quantitative Determination of Quercetin in Hydroalcoholic extract of *Rhus chinensis* Mill. using a Validated HPTLC Method

Thokchom Biona, Kalpana Pravin Rahate*

Department of Pharmacy, School of Medical and Allied Sciences, Galgotias University, Greater Noida, Gautam Buddh Nagar, Uttar Pradesh 203201
*Corresponding author: Dr. Kalpana Pravin Rahate
E-mail address: rahatekalpana@gmail.com

ABSTRACT: *Rhus chinensis* Mill. is a medicinal plant widely used traditionally due to its rich phytochemical profile, particularly the flavonoids. The objective of our study was to develop a validated simple, precise, and accurate HPTLC method for the quantitative determination of quercetin in hydroalcoholic extract of *Rhus chinensis* Mill. For the HPTLC analysis, silica gel 60 F_{254} plates was used as stationary phase while Toluene: ethyl acetate: formic acid (5:4:1 v/v/v) was used as the mobile phase and the densitometric scanning was performed at 254 nm. The method was validated as per the guidelines of ICH Q2(R2). Well-resolved bands for quercetin (Rf 0.73) were obtained and the method exhibited good linearity (R^2 > 0.988), precision (%RSD < 2%), and recovery (98-100.2%). The content of quercetin in hydroalcoholic extract of *R. chinensis* was found to be 42.45± 0.6% w/w. The developed HPTLC method was validated, reliable and can be used for routine quality control and standardization of *R. chinensis*.

GRAPHICAL ABSTRACT:



1. INTRODUCTION

Flavonoids represent a diverse group of polyphenolic compounds which is widely distributed in plants and have gained significant attention due to their potential pharmacological activities, including anti-oxidant, anti-inflammatory, and antidiabetic properties. Among them, quercetin is one of the most abundant and extensively studied flavonols[1-3]. It exhibits

antidiabetic properties through multiple mechanisms, including the inhibition of α-glucosidase and α-amylase enzymes, the enhancement of insulin secretion, and the promotion of glucose uptake in peripheral tissues[4,5]. Moreover, quercetin has been shown to modulate oxidative stress and inflammatory pathways that plays a crucial role in the development of diabetes and its complications[6].

Rhus chinensis Mill. or locally known as "Heimang" in Manipur, North-east India is a medicinal plant used extensively in traditional systems of medicine in Asia. *R. chinensis* is a good source of bioactive constituents such as flavonoids, tannins and phenolic acids responsible for its various therapeutic potentials including antimicrobial, antioxidant, hepatoprotective and antidiabetic activities[7]. The presence of quercetin and its derivatives make *R. chinensis* a potential candidate for phytochemical and pharmacological studies. However, the variation of phytochemical constituents depending on environmental and processing factors requires the establishment of consistent and reliable analytical methods for the quality control and standardization.

Thin layer chromatography of high performance (HPTLC) has emerged as an efficient, rapid and cost-effective analytical method for qualitative and quantitative estimation of the phytoconstituents in phytochemicals research. HPTLC has the advantages over other chromatographic techniques such as minimal sample preparation, simultaneous running of multiple samples and high reproducibility[8-11]. This technique is particularly suitable for routine quality control of plant extracts and has been widely employed to determine flavonoids, including quercetin, in various plants.

The present study is to perform the quantitative determination of quercetin in the hydroalcoholic extract of *R. chinensis* Mill. using a validated HPTLC method. Method validation is carried out according to the guidelines of International Council for Harmonisation (ICH) to ensure accuracy, precision, specificity, and robustness of the analytical procedure[12]. The development of a validated HPTLC method will not only facilitate the standardization of *Rhus chinensis* extracts but also contribute to the quality assurance of plant-based formulations containing this plant.

MATERIALS AND METHODS:

Collection of Plant Materials

The ripe fruits of the plant were collected from Ukhrul district of Manipur. India (GPS of collection site: Lat N 24°04'12.1'' Long. 094°21'09.8'' during October-November, 2025 and was later authenticated and identified with assigned Herberium no. IBSD/M-277. A voucher specimen was deposited for reference. The name of the plant was also verified from https://www.worldfloraonline.org/ and https://powo.science.kew.org/

Preparation of Extract:

The dried powdered fruits of *R. chinensis* was extracted using hydro alcoholic maceration extraction method using 70% methanol and 30% water. The extract was filtered and subsequently the filtrate was concentrated under reduced pressure in the rotary evaporator set at 45°C. The semi-solid mass is collected and store in a screw capped bottle at 4ºC for further use.

Quantitative analysis by HPTLC

Quercetin standard solution

Stock solutions of quercetin (1 mg/ml) were prepared using HPLC grade methanol and then further diluted to obtain working concentrations.

Instrumentation

Thin layer chromatography (TLC) was performed utilizing various mobile phases with different solvent ratios in the screening process (Table 1). Samples were spotted in band formations by utilizing CAMAG microliter syringe on a precoated silica gel 60 F_{254} plates, facilitated by the CAMAG Linomat 5, an automatic sample spotter with a bandwidth of 8 mm. Following the application, the plates were developed using a solvent system with CAMAG glass twin trough chamber that had been saturated with the solvent for 20 mins and were air dried. The TLC plate was subsequently scanned using a CAMAG TLC scanner.

Method Validation

The validation of the optimized HPTLC technique was carried out according to the ICH Harmonized Tripartite Guideline Q2 (R2)[12]. The primary aim of this assessment is to evaluate the following parameters: linearity, specificity, limit of quantification (LOQ), limit of detection (LOD), precision, accuracy, and robustness.

Linearity

The linearity of the prepared dilutions of the standard was assessed by constructing a calibration curve with five distinct volumes (0.1 mg/mL) in the range of (2–10 µg/band). The calibration curve was generated by plotting with standard concentrations against the corresponding mean peak area or height.

Specificity

The specificity of the method was evaluated by analyzing the standard reference and sample extract. The presence of quercetin in the hydroalcoholic extract of *R. chinensis* was confirmed by comparing the Rf values and spectra of the spot detected with that of the standard reference. Additionally, the peak purity of the quercetin was evaluated by analyzing the spectra at three different positions: the peak start (S), peak apex (M), and peak end (E) positions of the spot.

LOD and LOQ

To determine the Limit of Detection (LOD) and Limit of Quantification (LOQ), the following formulas were used i.e., LOD = 3.3σ/S and LOQ = 10σ/S were used, where σ is the standard deviation of the response and S is the slope of the obtained from the calibration curve.

Accuracy

The percentage recovery of standard quercetin was used to determine the accuracy, which was expressed as relative standard deviation (%RSD) from the mean recovery of the theoretical concentrations. Three different known concentrations were used to spike the sample dilutions in triplicate (0.2, 0.4 and 0.6 μg/band).

Precision and Robustness

The inter-day and intra-day precision of the optimized method was determined. Three concentrations (0.2, 0.4, and 0.6 µg/band) of the standard biomarkers were used to spike the sample in six replicates (n=6). Intra-day precision was analyzed with the three concentrations on a single day. Inter-day precision was assessed by conducting the intraday experiment for three consecutive days. Minor adjustments were made in the mobile phase composition, volume, duration of saturation time, and activation of the pre-washed TLC plates with methanol, and the effects on the results were examined. The robustness of the method was tested through triplicate testing (Table 2).

RESULT:

HPTLC Method Development and Optimization

A robust HPTLC method was successfully developed for the simultaneous separation and quantitative estimation of quercetin in hydroalcoholic extract of *R. chinensis*. Several solvent systems with varying polarities were initially evaluated to achieve optimal resolution. The mobile phase ratio comprising of toluene: ethyl acetate: formic acid (5:4:1, v/v/v) resulted in a sharp, well-defined, and reproducible bands with minimal tailing. Densitometric scanning at 254 nm showed distinct peaks indicating good separation and specificity of the method.

The optimized chromatographic conditions ensured consistent migration behavior and peak symmetry, which are essential for accurate quantification. No interference from other phytoconstituents was observed or detected at the corresponding Rf values as shown in Figure 1 A, B and C, thereby confirming the selectivity of the method employed.

Linearity

The method demonstrated good linearity and the regression equations obtained were $y = 0.0013x + 0.0015$ with correlation coefficients (R^2) of 0.988, respectively. These values suggest a strong linear relationship between area and concentration.

Precision

Precision was evaluated through analysis of repeatability and intermediate precision. The %RSD values for quercetin were found to be less than 2%, reflecting high precision and reproducibility of the analytical method used.

Accuracy

The accuracy of the method was determined by recovery studies at three distinct levels (80%, 100% and 120%). The percentage recoveries were found to be in the range from 99.02% to 100.2% indicating the accuracy of the method and the assurance of quality as shown in Table 3.

Sensitivity (LOD and LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) were 1.9 and 5.8 ng/spot, respectively. This low value result indicates high sensitivity of the method which also allows detection of trace levels of the analytes.

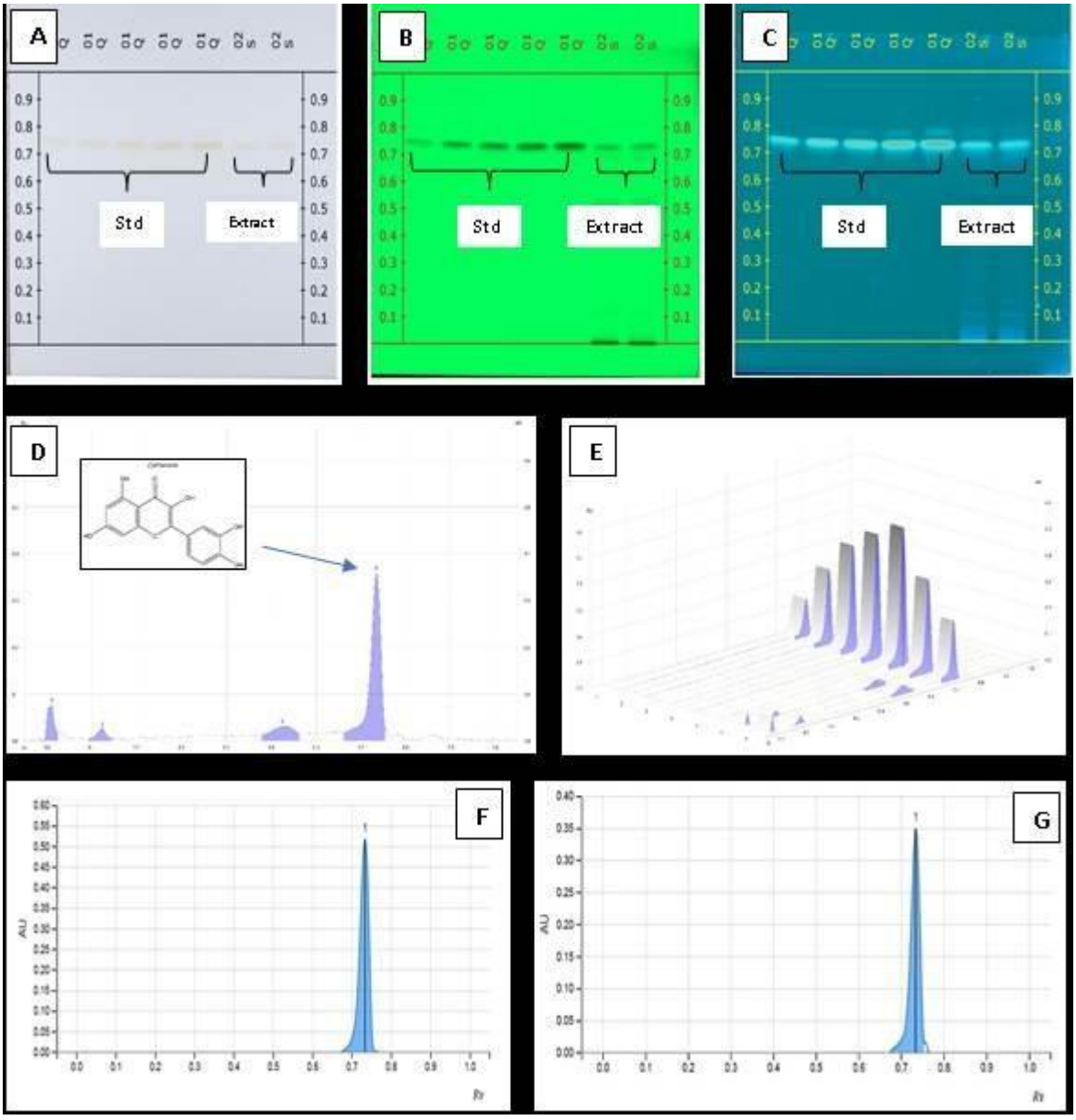


Figure 1: A) Visualization of quercetin in R White. B) Visualization of quercetin in R 254 C) Visualization of quercetin in R 366. D) Quercetin in hydroalcoholic extract of *R. chinensis*. E) 3D visualization of quercetin in both the standard and extract.

F) Chromatogram of standard quercetin G) Chromatogram of quercetin in hydroalcoholic extract of *R. chinensis*.

Robustness and Specificity

The robustness of the method was confirmed by the fact that small variations in chromatographic conditions had no significant influence on the results. Moreover, good specificity was confirmed by the absence of interfering peaks at the corresponding Rf values (0.73).

Quantification of Quercetin in Extract

The validated method was successfully applied for the quantification of quercetin in *R. chinensis* hydroalcoholic extract. The content of quercetin was found to be 42.45±0.6% w/w. The presence of quercetin suggests its major contribution to the pharmacological activities of the plant. Flavonoids are well-documented for their antioxidant and α-glucosidase inhibitory activities, supporting the traditional use of *R. chinensis* in managing metabolic disorders.

Table 1. Optimized Chromatographic Conditions for Quantification of Quercetin

Plate layout	
Stationary phase	Merck, HPTLC Silica gel 60 F_{254}
Plate format	100 x 100 mm
Application type	Band
Sample Solvent type	Methanol
Dosage speed	150 nL/s
Pre dosage volume	0.20 µL
Saturation time	20min
Application	
Tank	TTC 20x10
Volume front through	10 mL
Volume rear through	20 mL
Drying time	5 min
Drying temperature	Room temperature
TLC Visualizer	
Quality	Enhanced
RT White	Auto capture, Auto, level 85% Band
R 254	Auto capture, Auto, level 85% Band
R 366	Auto capture, Auto, level 85% Band
Scan developed plate	
Scanner type	Multiple λ
Optimization for	Resolution
Measurement mode	Absorbance
Scanning speed	20 mm/s
Data resolution	100 µm/step
Slit	5 x 0.2 mm, micro
Lamp	Deuterium
Wavelength (s)	254 nm

DISCUSSION:

The present study effectively established a simple, precise, and reliable HPTLC method for quantifying quercetin in *R. chinensis*. The mobile phase system provided obtained an optimal resolution and reproducibility, which is consistent with previous studies focused on flavonoid separation using similar solvent systems. The optimized chromatographic conditions enabled clear resolution of this flavonoid marker with well-defined peaks and no interference from other constituents, demonstrating the specificity of the method. The high linearity and low %RSD values showed that the method is appropriate for quantitative analysis. This validated method complies with ICH guidelines, exhibiting excellent linearity ($R^2 > 0.988$), high precision (%RSD < 2%), satisfactory accuracy (99.02-100.2% recovery), and good sensitivity with low LOD and LOQ values. The sensitivity parameters both LOD and LOQ underscores the method's ability to detect low concentrations of analytes, making it suitable for quality control. The significant levels of this bioactive marker further support its potential use in the development of antidiabetic and antioxidant formulations. Overall, the developed HPTLC method serves as a reliable analytical tool for the standardization and quality assessment of *R. chinensis* and its associated products.

Table 2. Robustness of the method

Optimization Conditions	**Quercetin (%RSD)**
Toluene, ethyl acetate, and formic acid 5:4:1, 6:3:1 v/v/v, and 6.5:3:0.5 v/v/v	0.08
Mobile phase 10, 15, and 20 ml	0.19
Time duration for saturation 10, 20, and 30 min	0.21
TLC plate activation 2, 5, and 7 min	0.24
Spotting time 10, 20, and 30 min	0.26
Time from chromatography to scanning 10, 20, and 30 min	0.15

Table 3. Validation Parameters for gallic acid estimation

Linear regression parameters (2–10 µg/band)	**Quercetin**
Equation	y = 0.0013x + 0.0015
Correlation coefficient (R^2)	0.988
Slope	0.0013
Intra-day	0.2-0.4
Inter-day	0.1-1.7
LOD	1.9 ng/spot
LOQ	5.8 ng/spot
Specificity	Specific
Accuracy (%)	(99.02%-100.2%)

CONCLUSION:

The successful quantification of quercetin highlights the significant presence of biologically active flavonoids. The method developed is cost-effective, efficient, rapid execution, and suitable for routine quality control and standardization of phytochemicals. Additionally, the presence of significant level of quercetin underscores the pharmacological significance of the plant, particularly in relation to its antioxidant and potential antidiabetic activities. This study provides a scientific foundation for the quality assurance and future phytopharmaceutical development involving *Rhus chinensis* Mill.

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ABBREVIATIONS

HPTLC: High-Performance Thin-Layer Chromatography**; ICH:** International Council for Harmonisation**; *R. chinensis*:** *Rhus chinensis***; Rf:** Retention Factor**; RSD:** Relative Standard Deviation**; TLC:** Thin layer chromatography**; LOQ:** Limit of Quantification**; LOD:** Limit of Detection**; S:** Peak Start**; M:** peak apex**; E:** peak end.

AUTHOR CONTRIBUTIONS

Thokchom Biona formally analysed the study and performed the investigation, methodology, validation and written the original manuscript. Kalpana Pravin Rahate supervised and edited the final manuscript. All authors reviewed and approved the final version of the manuscript.

CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this investigation**.**

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